American College of Neuropsychopharmacology

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ABSTRACTS COLLECTION

ACNP 62nd Annual Meeting: Poster Abstracts P1 – P250

Neuropsychopharmacology (2023) 48:63–210; https://doi.org/10.1038/s41386-023-01755-5

Sponsorship Statement: Publication of this supplement is sponsored by the ACNP.

Only disclosures for presenting authors are listed. Asterisks in the author lists indicate presenter of the abstract at the annual meeting.

Abstract numbers do not correlate to poster number assigned for presentation at the Annual Meeting.

P1. Specific Exercise Patterns Generate an Epigenetic Molecular Memory Window That Drives Long-Term Memory Formation and Identifies ACVR1C as a Bidirectional Regulator of Memory

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Background: Basic research and clinical trials universally demonstrate the benefits of exercise for cognitive function. In recent studies, we determined that a period of initial exercise also creates and maintains a molecular memory window for exercise benefits on cognitive function in male and female mice where a brief, 2-day exercise session following a break can also re-engage cognitive benefits, re-facilitate long-term potentiation, and allow for learning under insufficient, subthreshold training conditions. Here, we build on these exercise parameters to begin to define a mechanism responsible for maintaining cognitive benefits underlying this molecular memory window by initial exercise and driving long-term memory formation.

Methods: We utilized RNA-sequencing to uncover genes in the dorsal hippocampus that are differentially expressed under conditions where exercise benefits are maintained throughout sedentary delay periods and enable the formation of long-term memory and synaptic plasticity. 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 and hippocampus was dissected during the consolidation window, 1 hour after training. Those parameters were then used to examine hippocampal long-term potentiation (LTP) using theta burst stimulation in the schaffer collateral pathway. To assess how exercise modulates epigenetic regulation of genes up-regulated only under conditions where exercise enabled the formation of long-term memory and synaptic plasticity, histone modifications were examined at Acvr1c and Bdnf IV promoters using chromatin immunoprecipitation (ChIP-qPCR). To examine the role of Acvr1c, a gene coding for a type 1 activin A membrane receptor kinase of the TGF-β family of signaling molecules, in hippocampus-dependent long term memory formation and synaptic plasticity, we used intra-hippocampal delivery of AAV1-ACVR1C point mutant constructs that either enhance or disrupt function. 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 examine whether Acvr1c declines with age in mouse and human hippocampus (Genotype-Tissue Expression Project). 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 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. We next aimed to determine whether enhancing ACVR1C through virus-mediated overexpression of wildtype ACVR1C would regulate long-term memory formation and synaptic plasticity in aging 18 mo. and 12 and 18 mo. Alzheimer's Disease (AD) mouse hippocampus and ameliorate impairments.

Results: We demonstrate that specific exercise patterns transform insufficient, subthreshold training into long-term memory (Group: $(F(6,64) = 8.13$, $P < 0.0001$; Tukey test: $P < 0.001$, 14D vs. Sed) and synaptic plasticity (Group: $(F(6,89) = 22.22$, $P < 0.0001$; Tukey test: $P < 0.0001$) 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 sedentary). We identify a small number of genes whose expression correlate with conditions in which exercise facilitates long-term memory formation. Among these genes we found Acvr1c and Bdnf. We find that exercise, in any amount, alleviates epigenetic repression at the Acvr1c (Group $(F(5,49) = 9.377$, $P < 0.0001$) and Bdnf IV (Group $(F(5,53) = 13.90$, $P < 0.0001$) promoters during consolidation in a persistent manner, providing initial insight for maintenance of exercise benefits on long term memory. 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 $(t(14) = 3.953$, $P = 0.0014)$. Furthermore, Acvr1c expression is impaired in the aging human $(t(91) = 6.64$, $P = 0.0001)$, mouse $(t(26) = 2.72$, $P = 0.01)$ and AD mouse brain (5xFAD) (Age: $(F(2,48) = 54.95$, $P < 0.0001)$, and over-expression of Acvr1c ameliorates plasticity

and cognitive impairment in aging (18 mo. C57: Behavior: ($t(12) = 2.350$, $P = 0.036$), LTP: ($t(14) = 3.953$, $P = 0.001$), 12 mo. 5xFAD: Behavior: ($t(21) = 2.287$, $P = 0.032$), LTP: ($t(16) = 5.617$, $P < 0.0001$), 18 mo. 5xFAD: LTP: ($t(10) = 9.653$, $P = 0.001$)).

Conclusions: Together, these findings provide a new paradigm for uncovering mechanistic drivers of exercise-facilitated learning and provide opportunity to explore how specific exercise parameters allow for periods of maintained epigenetic and molecular changes through sedentary periods that facilitate cognitive function. As we have demonstrated here, identification of such mechanisms may extend beyond the context of exercise and aid in ameliorating age and AD-associated cognitive impairment.

Keywords: Hippocampus, Exercise, Epigenetics, Memory, Ageing

Disclosure: Nothing to disclose.

P2. Opioid-Induced Postoperative Cognitive Dysfunction: Long-Term Effects on Neuronal Structure and Function

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Background: Post-operative cognitive dysfunction (POCD) is an abrupt decline in memory and executive function occurring in ~10% of older adult patients and lasting weeks to months after surgery, significantly increasing vulnerability to neurodegenerative diseases such as Alzheimer's disease. Our recently developed preclinical model of POCD determined that aged rats treated with the opioid, morphine, after abdominal surgery extended memory impairments from four days (without morphine) to eight weeks. The mechanisms driving opioid-exacerbated POCD are not well-understood, although significant increases in hippocampal-inflammatory gene expression two-weeks after surgery plays a key role. Given that hippocampal neurons and synapses are responsive to inflammatory signaling, we hypothesized that the combination of aging, surgery, and morphine, would alter structural and functional features of dendrites, dendritic spines, and synapses on CA1 neurons four-weeks after surgery.

Methods: Aged (24 mos) male F344xBN rats ($n = 6$-8/group) underwent laparotomy under isoflurane anesthesia, and were treated with saline or morphine (2 mg/kg, i.p.) twice a day for 7 days after surgery. Four weeks later, brains were collected and stained with Golgi-Cox. Reconstructions of CA1 pyramidal cells from the dorsal hippocampus to characterize dendritic complexity and length and spine subtypes were quantified. In separate cohorts of rats, hippocampal synaptoneurosomes were isolated to assess expression levels of plasticity-related proteins, LTP was measured, and mitochondrial oxygen consumption rates were assayed to identify any synaptic memory consolidation-related disturbances.

Results: Sholl analyses of CA1 dendrites revealed no structural changes in any group, but mushroom and stubby spine density was decreased in aged, morphine-treated rats. Western blot analysis from hippocampal synaptoneurosomes showed a significant decrease of the glutamate AMPA receptor subunit 1, LTP was robustly impaired, and mitochondrial oxygen consumption rates were dramatically reduced in aged morphine-treated rats relative to controls.

Conclusions: Taken together, these data suggest the combination of aging, surgery, and morphine evokes long-lasting disruptions to dendritic spine density, synaptic function, and mitochondrial function necessary for memory consolidation.

Keywords: Mitochondria, Synaptic Plasticity, Surgery

Disclosure: Nothing to disclose.

P3. Towards Understanding the Proteomic Signature of Psychosis Using Schizophrenia and Alzheimer's Disease Human Postmortem Brains

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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), suggesting the importance of subtyping AD.

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. Like AD, subtyping SCZ could provide a better understand of the illness.

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 deficits 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 that differ between AD and AD + P, and that those proteins and pathways will overlap with some of those identified in SCZ, providing insight into the most relevant proteins and pathways related to AD psychosis.

Methods: A comparative proteomic study of protein mass spectrometry data obtained from Alzheimer's disease and schizophrenia postmortem brains utilizing protein enrichment and pathway analysis.

Results: We have obtained promising preliminary data through proteomic investigation of the insoluble proteins in this SCZ subtype that suggests overlap with insoluble proteins identified in a parallel proteomic study utilizing AD brains with and without psychosis. These results suggest a common proteomic signature in AD and SCZ that may be related to psychosis or cognitive impairment.

Conclusions: By comparing the aggregated proteins in AD + P to AD without psychosis and then comparing these proteins to

cases of SCZ with protein aggregation, a disease characterized by psychosis, we can better understand the pathways that are disrupted leading to psychosis in AD. This could lead to novel therapeutic targets for the treatment of psychosis and could lead to refinements in nosology across diagnoses.

Keywords: Brain Proteomics, Alzheimer Disease, Schizophrenia Subtypes, Psychosis

Disclosure: Nothing to disclose.

P4. Moderate Ethanol Exposure Ameliorates Cognitive Function Through the Reduction of Low-Density Lipoprotein Cholesterol, Pro-Inflammatory Cytokines, and Aβ Deposition in Early-Stage of APP/PS1 Mice

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Background: Recent studies also suggest that moderate alcohol consumption is possibly protective against dementia and cognitive decline. Moderate ethanol exposure (MEE) prevents the formation of amyloid-beta (Aβ) and further protects hippocampal neurons by blocking the accumulation of Aβ protein. However, it is unclear how MEE ameliorates cognitive function through cholesterol metabolism and the neuroinflammatory system.

Methods: We assessed Apoε in the mouse brain using IHC and ELISA in response to MEE. First, to confirm the intracerebral distribution of Apoε, we co-stained with GFAP, a marker for astrocytes that biosynthesize Apoε. Since Apoε, a protein involved in cholesterol transport, has been identified as the only risk factor consistently associated with nonfamilial AD, we examined whether EtOH exposure alters LDL-cholesterol levels in the brain. We sought to investigate whether the ethanol-induced upregulation of LRP1 could potentially inhibit the activity of IL-1β and TNF-α induced IKK-α/β towards NF-κB p65, resulting in a reduction of pro-inflammatory cytokines. Next, we examined whether MEE could potentially mitigate the deposition of amyloid plaques, AD's most distinctive pathological marker. To evaluate the actual Aβ load in the brains of APP/PS1 mice, we performed with a specific antibody Aβ (Thioflavin S) on both air- and ethanol-exposed groups, subsequently analyzing Aβ levels. We quantified the number of Aβ plaques, including intra-neuronal Aβ and extracellular plaques, and compared these in the cortex and dentate gyrus of the hippocampus across all experimental groups. We also measured glucose uptake activity using 18F-FDG in APP/PS1 mice. Finally, we investigated whether MEE induced cognitive and memory changes using the Y maze, noble objective recognition (NOR) test, and Morris water maze (MWM).

Results: We found a reduction of Apoε and astrocytes in the cortex and hippocampus of EtOH-exposed APP/PS1 mice compared to air-exposed APP/PS1 mice. Consistently, we confirmed the reduced Apoε levels in ELISA experiments in the cortex and hippocampus of EtOH-exposed APP/PS1 mice. Utilizing a western blotting approach with an LRP1 antibody, we found a marked increase in LRP1 levels of EtOH-exposed APP/PS1 mice as compared to air-exposed APP/PS1, suggesting a possible correlation between reduced Apoε or decreased LDL cholesterol and increased LRP1 expression. Remarkably, we observed a significant reduction in p-IKK-α/β levels in EtOH-exposed APP/PS1 mice compared to air-exposed APP/PS1 mice. This decrease in p-IKK-α/β led to a corresponding reduction in NF-κB p65, a known pro-inflammatory cytokine, in EtOH-exposed APP/PS1 mice compared to air-exposed APP/PS1 mice. To ascertain the level of IL-1β and TNF-α in the brain, we performed an ELISA assay to detect IL-1β and TNF-α in brain tissue supernatants collected from both sets of mice. After 12 weeks of ethanol exposure, as expected, we found a reduction in IL-1β and TNF-α levels in the cortex and hippocampus of EtOH-exposed APP/PS1 mice compared to air-exposed APP/PS1 male mice. Our results revealed a significant reduction in Aβ plaques in the cortex and hippocampus in response to EtOH-exposed APP/PS1 treatments compared to air-exposed APP/PS1 mice. To quantify the level of amyloid protein in the brain, we performed an ELISA assay to detect Aβ1-42 in brain tissue supernatants collected from both experimental groups. Intriguingly, the results showed a stark contrast between air-exposed APP/PS1 and ethanol-exposed APP/PS1 mice. We noted an elevated uptake of 18F-FDG in APP/PS1 mice exposed to ethanol for 12 weeks, especially in the cortex and hippocampus. In NOR, the ethanol-exposed APP/PS1 mice demonstrated a prolonged exploration time of the unfamiliar object. In contrast, air-exposed APP/PS1 mice displayed similar exploration times for familiar and unfamiliar objects. In MWM, during the acquisition training, the EtOH-exposed APP/PS1 mice outperformed the air-exposed APP/PS1 mice on the fourth day. Furthermore, a significant decrease in escape latency was observed in air-exposed APP/PS1 mice compared with the EtOH-exposed APP/PS1 mice on the fourth day.

Conclusions: Our findings suggest that MEE may benefit AD pathology via modulating LRP1 expression, potentially reducing neuroinflammation and attenuating Aβ deposition. Our study implies that reduced astrocyte-derived Apoε and LDL cholesterol levels are critical for attenuating AD pathology.

Keywords: Alcohol, Alzheimer's Disease, Astrocyte, Cholesterol Biosynthesis, Inflammation

Disclosure: Nothing to disclose.

P5. The Association Between Loss of Integrity in Dopamine and Norepinephrine Nuclei and Decline in Working Memory Performance in Healthy Aging

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Background: Decline in working memory performance is a common feature of normal aging. Evidence from animal studies has suggested that age-related changes in neuromodulatory systems such as the dopamine system may be partly responsible for this decline. Postmortem data reveals degeneration in the dopamine system during normal aging, while the norepinephrine system may be relatively preserved in the absence of neurodegenerative illness. Recent work by our group and others has shown that age-related loss in integrity of the dopamine and norepinephrine systems can be captured in vivo using neuromelanin-sensitive MRI (NM-MRI) of the substantia nigra (SN) and locus coeruleus (LC) respectively. Here we investigated whether this loss of integrity of neuromodulatory systems may impair cognition in healthy aging. We focused on working memory given prior evidence that neuromodulatory systems influence the prolonged neural activity that is required for working memory.

Methods: The study sample included 155 adults aged 65 and older who had no sign of cognitive impairment on the Clinical Dementia Rating Scale and Mini-Mental State Exam. NM-MRI was acquired using a turbo spine echo sequence. Measures of SN and LC integrity were generated by processing NM-MRI images with established methods to calculate contrast-to-noise ratio (CNR) of

voxels of the SN (relative to a crus cerebri reference region) and sections of the LC (relative to a central pons reference region). To provide a biomarker of incipient Alzheimer's disease, PET imaging was acquired with the radiotracer [18F]MK6240; standardized uptake value ratio was extracted from a temporal region implicated in the early stages of Alzheimer's disease. Working memory performance was assessed using the digit span test of the Wechsler Adult Intelligence Scale. Linear regression analyses were performed in Matlab. All models predicting working memory performance included sex and tau level (temporal cortex [18F] MK6240 SUVR) as covariates. Mediation analysis was performed with the Mediation Toolbox developed in Matlab by the Cognitive and Affective Neuroscience Laboratory.

Results: Subregions of the SN and LC were identified that showed significant loss of signal with age. In the full study sample, a cluster of voxels in the ventral SN showed decreasing signal with age (320 of 1879 voxels, thresholded at p76 years old, $n = 36$, $t33 = -2.27$, $p = 0.030$).

Next, we examined the impact of NM-MRI signal in the SN and LC on age-related decline in working memory performance. In an initial model excluding SN and LC signal, age was strongly related to working memory performance ($t127 = -3.24$, $p = 0.0015$). When adding signal from the age-declining subregions of the SN and LC as predictors in this model, age was no longer a significant predictor ($t125 = -1.63$, $p = 0.11$) but both SN signal ($t125 = 3.23$ $p = 0.0016$) and LC signal ($t125 = 2.52$, $p = 0.013$) were significant predictors. The level of tau was not associated with working memory performance ($t125 = 0.49$, $p = 0.62$). A mediation test found that SN signal was a significant mediator between age and working memory performance (ab path: $Z = -2.71$, $p = 0.007$).

Conclusions: In older adults, NM-MRI detects age-related loss of integrity in the ventral SN and rostral LC. The integrity of each of these regions independently contributes to working memory performance and integrity of the SN significantly mediates the effect of age on working memory. Prodromal Alzheimer's disease does not appear to contribute to working memory performance. These results are consistent with prior evidence that age-related decline in neuromodulatory systems impairs the prolonged neural activity required for working memory. Future multimodal imaging work will further probe this proposed mechanism by examining temporal profiles of brain activity such as intrinsic neural timescales.

Keywords: Dopamine, Neuromelanin-Sensitive MRI, Aging, Norepinephrine, Working Memory

Disclosure: Nothing to disclose.

P6. The Aging Hippocampus is More Engaged but Differentially Specialized During Explore-Exploit Decision-Making in Older Adults With and Without Depression

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Background: Healthy aging is associated with a positivity bias in attention and memory that favors positive over negative information processing1. Similarly, the socioemotional selectivity theory proposes shifting attention towards emotionally meaningful goals with aging, leading to increased emotional stability and focus on key relationships2. One mechanism by which this cognitive-emotional shift may occur is through alterations in reward guided behavior. Explore-exploit decision-making, which involves exploring (trying out) new behaviors to learn about the best options available with exploiting (repeating) known good options, is ideally suited to test this hypothesis. For example, the shifting priority from new experiences to well-established personal connections may reflect alterations in how individuals resolve the explore-exploit dilemma.

The hippocampus is a key region implicated in learning and memory as well as reward function, binding information into cognitive maps that support reward-guided behavior including exploration and exploitation3. Furthermore, the hippocampus undergoes age-related changes in structure and function, which have been associated with cognitive decline and are potentially reversible with interventions such as exercise and environmental enrichment4,5. Relatedly, there are several models of cognitive aging suggesting age-related overcompensation and de-differentiation of neural systems to maintain cognitive functioning6,7. We sought to characterize age-associated changes in hippocampal explore-exploit neural functioning in older individuals with and without depression. We hypothesized that increased age would be associated with hippocampal hyperactivation but diminished selectiveness to specific computational parameters reflecting encoding of reward value, prediction errors, and information content.

Methods: The study included 146 cognitively intact individuals, aged 49-80 years (mean 62.0 yrs, sd 6.8; 81 females), comprising individuals with depression ($n = 103$) and psychiatrically healthy controls ($n = 43$). Cognition was assessed with the Executive Interview (EXIT) assessment. Participants completed an explore-exploit decision-making paradigm (aka the "clock task"8) during an fMRI scan, in which individuals sampled a 5 second continuous interval to learn the most rewarding location in time and space. We discretized the interval into basis functions and utilized the previously developed SCEPTIC selective maintenance reinforcement learning model9, to calculate trial-by-trial action values, reward prediction errors, entropy (measure of information content), and change in entropy. Modeling was implemented using the Matlab VBA toolbox and trial-by-trial parameter values were extracted for model-based fMRI analyses. fMRI images were pre-processed and then extracted signal from the hippocampus was deconvolved using a leading hemodynamic deconvolution algorithm to estimate neural activity, which was stimulus locked (to clock-onset and feedback) and averaged across trials3. Multilevel modeling implemented in R was used to test for significance between mean-centered age, EXIT score, model-derived behavioral parameters, and neural data, with significance thresholding of $pFDR < 0.05$.

Results: Age did not correlate with total points earned ($p = 0.55$) and the groups did not differ in age ($p = 0.13$) or gender ($p = 0.90$). EXIT score was significantly higher (indicating more impairment) in depressed individuals compared to non-depressed controls (dep. mean 6.2, sd 3.1; cont. mean 5.0, sd 2.5; F 5.43, $p = 0.02$). Older age predicted impaired exploitation (chi-sq = 5.36, $p = 0.02$) with no effect on exploration (chi-sq = 0.10, $p = 0.75$). Across all participants, we found that age was positively correlated with heightened hippocampal activation at the time of clock onset and during the two seconds prior to response ($pFDR < 0.01$). With regards to neural encoding of entropy (information content), we found that older individuals had blunted hippocampal response to entropy offline (prior to trial onset; $pFDR < 0.01$) and at the time of feedback ($pFDR < 0.001$). Conversely, we found that older individuals displayed a more prominent dip in hippocampal activity to entropy change after feedback ($pFDR < 0.001$). Furthermore, we found that older individuals displayed enhanced hippocampal offline encoding of maximum value around 2 seconds prior to clock onset ($pFDR < 0.001$). Lastly, we found that older individuals displayed an opposite pattern of hippocampal neural response to the magnitude of prediction error compared to younger individuals both offline ($pFDR < 0.001$) and after feedback ($pFDR < 0.001$). The pattern of age-related effects on explore-exploit neural processing was similar when looking at depressed and non-depressed

individuals separately and remained robust to sensitivity analyses controlling for EXIT score, years of education, and mean score obtained per trial. Lastly, we found that EXIT score was independently associated with increased hippocampal neural activity online (after clock onset, pFDR < 0.01) and after feedback (pFDR < 0.05).

Conclusions: We found evidence of heightened hippocampal engagement in older individuals and those with greater cognitive impairment performing an explore-exploit task, supporting the theory of compensatory overactivation with aging. This mechanism may underly behavioral adaptions of increased emotional stability and focus on key relationships in old age.

Keywords: Biology of Aging, Cognition, Explore-Exploit Dilemma, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P7. Eph/Ephrin Bidirectional Signaling is Essential for Development of Anatomical Cell Clusters in Layer II of Medial Entorhinal Cortex

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Background: The medial entorhinal cortex (MEC) has characteristic hexagonally arranged cell clusters in the layer II (MECII). These cell clusters are composed of Wolfram syndrome1 (Wfs1)/Calbindin (CalB)+ pyramidal cells and surrounded by Reelin+ stellate cells in the MECII. The MEC integrates multimodal sensory inputs for the memory formation, spatial navigation and time perception. Accumulating evidences from neural recording and imaging suggest that cells coding similar spatial/temporal information tend to be clustered in MEC, implicating that the cell clusters in the MECII may generate the functional modules. However, it remains unknown the molecular basis for the formation of anatomical cell clusters in MEC and how the anatomical modules affect functional modules in the MEC. Here, we hypothesized that the cell clusters may be generated by cell-cell-mediated interaction/repulsion.

Methods: As the Eph receptors B1-3 and their ligands, the Ephrin-Bs, are well known as initiators of cell-contact-mediated neuromigratory signals, we first comprehensively examined their expression patterns in the MEC of adult mouse by fluorescent in situ hybridization (FISH). We also examined the effect of EphB/Ephrin-Bs knockout (KO) on the formation of cell clusters.

Results: Among EphB/Ephrin-B family genes, we found EphB1 and EphrinB2 were selectively expressed in MECII. Double-FISH analysis revealed that EphB1 was expressed in Reelin+ cells, while Ephrin-B2 was expressed in Wfs1+ cells. Next, we examined their expression pattern in the MEC during developmental stage. At postnatal day 0 (P0), EphB1 was already expressed in Reelin+ cells in MECII. At that time, no cell clusters were identified in MECII by nuclear staining. Rather, Ephrin-B2+ cells were located in the layer V of MEC at P0. At P2, Ephrin-B2+ cells have migrated to layer II and formed cell clusters in the MECII. At P4, the Ephrin-B2+ cells have expressed Wfs1. Finally, we examined the effect of EphB/Ephrin-Bs knockout (KO) on the formation of cell clusters. We found that the cell clusters in the MECII of the Ephrin-B2 hetero knockout mice were severely impaired. We also found that the cell clusters became smaller in the EphB1 and EphB2 double homo-knockout mice.

Conclusions: These results suggest that the Eph/Ephrin repulsion signaling between Reelin+ and Wfs1+ cells is critical for formation of cell clusters in the MECII during early postnatal development.

Keywords: Medial Entorhinal Cortex, Alzheimer Disease, Spatial Representation, Grid Cell

Disclosure: Nothing to disclose.

P8. Prenatal Immune Origins of Brain Aging: Impact of Sex and Reproductive Status

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Background: With an ever-increasing aging population, it is critical to understand early antecedents to brain aging to target for intervention and prevention of disorders of the brain. There is increasing evidence of fetal antecedents to adult memory impairments, potentially through immune pathway disruptions. However, most of this work is in animal models. Male and female brains develop and age differently, thus using a sex differences lens is critical for identification of early biomarkers of risk and resilience. Here, we hypothesized that in utero exposure to maternal pro-inflammatory cytokines will have sex-selective effects on specific brain circuitry regulating memory and immune function that will be retained across the lifespan. Using a unique prenatal cohort, we tested this hypothesis in 197 adult offspring, equally divided by sex, who were exposed and unexposed to an adverse in utero maternal immune environment and followed to age 55.

Methods: 197 adults (ages 45–55; 95F:102M) recruited from the New England Family Study (NEFS) underwent a blood draw, clinical assessments, and structural/functional MRI (s/fMRI). NEFS is a unique prenatal cohort of offspring currently followed for >60 years whose mothers were followed through pregnancy with serial blood draws. Maternal cytokine levels (IL-6, TNF-α, IL-1 β, IL-10) were assayed from sera collected at the beginning of the 3rd trimester, a critical period of the sexual differentiation of the brain. In midlife, adult offspring underwent an fMRI verbal encoding task (using 3T Siemens Trio and SPM12 for analyses), and β weights were extracted from memory circuitry regions (FDR-corrected p-values for multiple comparisons were used). Immune proteomics were also conducted on adult offspring sera and associations between prenatal maternal cytokines and the offspring adult NLRP3 inflammasome (FGF-2, IL-1β, IL-18, and IL-1RA) was assessed, with inflammasome dichotomized as any vs. no analytes >75th percentile of sex-specific distributions. Face-Name Associative Memory Exam (FNAME) and Buschke Selective Reminding Test assessed associative and verbal memory, respectively. Menopausal staging was determined by serology according to STRAW-10 criteria. Generalized estimating equation (GEE)-based generalized linear models were conducted to test associations between prenatal cytokines and midlife offspring memory outcomes and immune function. Models were run overall and by sex (95 women; 102 men) and reproductive status (35 Pre, 28 Peri, 32 Post). Sex differences and the impact of menopausal stage were assessed using an interaction term, and adjusted for intrafamilial correlations, offspring age, parental socioeconomic status, and maternal race.

Results: Sex by IL-6 interactions were significant across ventral lateral prefrontal cortex (VLPFC; in BA47 and BA45) (Dorsal: pFDR = 0.03; Mid: pFDR = 0.03; Ventral: pFDR = 0.03) and at a trend level in hippocampus (HIPP) (right: pFDR = 0.09; left pFDR = 0.10). In men but not women, elevated maternal IL-6

was significantly associated with lower task-evoked BOLD activity in VLPFC (Mid: β = -0.47, pFDR = 0.02; Ventral: β = -0.30, pFDR = 0.05) and left HIPP (β = -0.17, pFDR = 0.05). Although no associations between prenatal cytokines and memory outcomes were found in women overall, in postmenopausal women alone, higher maternal IL-6 was significantly associated with poor memory performance (FNAME: β = -0.83, pFDR = 0.01) and higher inflammasome levels (OR = 6.51, p = 0.05), a finding in men that was a trend (OR = 2.19, p = 0.08) and attenuated with adjustment for age. Further, higher maternal TNF-α exposure was significantly associated with lower FNAME performance (β = -0.74, pFDR = 0.02) in postmenopausal women; associations for TNF-α were not significant for men.

Conclusions: Here, using a unique prenatal cohort followed into midlife, we demonstrated that adverse prenatal maternal immune activation during the sexual differentiation of the brain was significantly associated with long-lasting effects on memory circuitry and immune function in midlife, that was sex-dependent, region-specific, and, within women, reproductive stage-dependent. Results suggest time-sensitive prenatal adverse exposure to pro-inflammatory cytokines plays a key role in disrupting developmentally-sensitive neuroimmune interactions in sexually-dimorphic vulnerable regions, such as memory circuitry, effects that are retained into midlife.

Keywords: Sex Differences, Memory, Developmental Origins, Prenatal Exposure, Immunity

Disclosure: Nothing to disclose.

P9. Deficits in Behavioral Pattern Separation Emerge in Middle-Aged Mice After Early Life Adversity

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Background: People who experience early life adversity (ELA) are at increased the risk for developing psychiatric illnesses in adulthood, including mood and anxiety disorders. Among patients with mood and anxiety disorders, many individuals display an overgeneralization of negative memories and of fear responses to emotional stimuli. This overgeneralization is thought to be related to impairments in pattern separation, or the process of separating similar or overlapping information into distinct neural representations. The dentate gyrus (DG) region of the hippocampus is thought to mediate pattern separation, which can be measured in humans and rodents using behavioral pattern separation tasks. Additionally, the continued development of new neurons throughout adulthood in the subgranular zone of the DG, a process known as adult hippocampal neurogenesis (AHN), has been shown to support behavioral pattern separation. Manipulations that increase or decrease levels of AHN can increase or decrease behavioral pattern separation performance, respectively. In the current study, we used a mouse model for ELA and then tested behavioral pattern separation in adulthood before measuring histological markers of AHN.

Methods: c57BL/6J pups are reared with limited bedding and nesting material from postnatal day (P)4-P11 before returning to standard housing conditions. Male and female mice are used for all experiments. In young-adult (11-12 weeks old, N = 7-11 per sex per group) and middle-aged (11-12 months old, N = 5-11 per sex per group), behavioral pattern separation was tested using a fear discrimination task that contextual fear conditioning and two similar contexts across several days. Successful fear discrimination was achieved when animals distinguished between the conditioned fear context "A" and a similar, neutral context "B" that was not paired with foot shock, as measured by a significant difference in freezing behavior. Young adult mice were exposed to contexts A and B in a randomized order, while middle aged mice were always exposed to context A and then B each day. After behavioral testing is completed, brains were perfused for immunohistochemical staining and quantification of doublecortin (DCX).

Results: In young adult (YA) mice, there was no significant effect of ELA or sex on initial contextual fear conditioning, but a significant group by sex interaction ($p < 0.01$) and decreased freezing after fear conditioning only in female ELA mice. In middle-aged (MA) mice, there was an overall effect of ELA ($p < 0.005$) and sex ($p < 0.05$), but no significant group by sex interaction on initial contextual fear conditioning. MA-ELA mice had decreased freezing after fear conditioning and male MA mice had lower overall freezing rates than female MA mice. Both YA and MA mice demonstrated similar levels of freezing to contexts A and B at the beginning of the pattern separation task. Additionally, MA mice still required multiple days to discriminate between contexts despite the 'easier' non-randomized design, indicating an overall decrease in pattern separation performance with age. Initial analyses of data from YA mice did not show an effect of ELA or sex on fear discrimination. However, in MA mice, fear discrimination was impaired by ELA in both males and females. Histological analysis of doublecortin (DCX) staining did not find any differences in YA mice after ELA. Analysis of DCX in MA mice will also be presented.

Conclusions: In the current study, we demonstrate that ELA alters fear conditioning in young adult and middle-aged mice. In contrast, behavioral pattern separation impairment after ELA becomes more pronounced in middle-aged mice, and middle-aged mice also required a less difficult fear discrimination paradigm compared to young adult mice. We also evaluated the relationship between behavioral pattern separation differences and markers of AHN in the dentate gyrus of both age groups. As behavioral pattern separation performance, as well as levels of AHN, are known to decrease with age, the effects of ELA on these measures may compound over time and have a greater impact later in life.

Keywords: Pattern Separation, Early-Life Adversity, Adult Hippocampal Neurogenesis

Disclosure: Nothing to disclose.

P10. Transcriptomic Signatures of Basal Forebrain Cholinergic Neurons Across Lifespan

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Background: Acetylcholine is a key neuromodulator in the brain that is critical for attention, wakefulness, mood, and memory. Cholinergic neurons, neurons that synthesize and release acetylcholine, coordinate neuronal activity brain-wide to promote attention to salient stimuli and facilitate learning. In fact, marked reductions of this cell type are a hallmark of late-stage AD. In aging, we find that significant reductions in the integrity of cholinergic projections (in both humans and rodents) are an early, unique feature of age-related cognitive decline. However, these changes are not uniformly observed across the brain. That is, some cholinergic circuits are affected far sooner and to a much greater degree than others. Thus far, the molecular determinants of the selective, early vulnerability of subsets of BFCNs are not known. Several factors could contribute to this heterogeneity across BFCN subpopulations including birthdate, projection target and gene

expression profiles. Understanding the factors underlying BFCN diversity is critical to understanding the role of acetylcholine in cognition and its particular vulnerability in cognitive decline. In these studies, we first asked if cholinergic neurons could be distinguished by unique gene expression profiles, and second, whether any functionally unique clusters were specifically vulnerable across lifespan.

Methods: Given the sparsity of cholinergic neurons throughout the basal forebrain, a detailed classification of this population has been challenging thus far. To overcome this, we used a genetic strategy where cholinergic nuclei were specifically labeled with a nuclear membrane targeted GFP (Sun1-sfGFP X Chat-Cre mice). We isolated nuclei from dissections of the full anterior to posterior extent of the basal forebrain from male and female young (2.5 month) and aged (12-18 month) mice. Using fluorescence assisted cell sorting (FACS), we enriched our samples for cholinergic nuclei by selecting based on GFP expression. Single nucleus RNAseq libraries were prepared using 10X chromium technology and sequenced using the Illumina sequencing platform. We used the R-toolkit Seurat along with associated R toolboxes for detailed bioinformatics analysis.

Results: Our preliminary dataset included over 20 thousand high-quality GFP-positive captured nuclei in young male and female mice. After additional quality control, we normalized 6 individually collected datasets (3 male and 3 female) and filtered the data by choline acetyltransferase expression (ChAT, the synthetic enzyme for acetylcholine and a marker of cholinergic neurons). Using unbiased clustering approaches, we identified 25 unique clusters of cholinergic neurons in young mice, representing far more diversity across the basal forebrain than initially hypothesized. These clusters were equivalently represented across all datasets. Using Seurat, we identified biomarkers for each of our isolated BFCN clusters. We performed preliminary validation of these biomarkers using the Allen Brain in-situ atlas in slices across the forebrain. We found that the expression of these unique factors was not governed by the traditional anatomical boundaries by which we typically define and target BFCNs. Ongoing analyses investigate the patterning of these clusters spatially across the basal forebrain using the 10x Xenium platform, and the selective vulnerability or resilience of these subpopulations with age.

Conclusions: These studies provide valuable insights into the incredible diversity and functional organization within the basal forebrain cholinergic system. Thus far, a thorough analysis of the gene expression profiles of BFCNs has not been conducted. This dataset provides an essential foundation for future studies to be able to target functionally distinct cholinergic neuron populations that differentially participate in behavior, and are differentially affected by age. By examining features in resilient populations that are absent from vulnerable populations, we can begin to identify factors by which to support vulnerable BFCN circuits in aging, AD, and related dementias.

Keywords: Cholinergic System, RNAseq, Aging

Disclosure: Nothing to disclose.

P11. Relationships Between Childhood Adversity, Epigenetic Aging, and Obesity

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Background: Childhood adversity is linked to psychological, behavioral, and physical health problems, including obesity and cardiometabolic disease. Epigenetic alterations have been proposed as one pathway through which the effects of early life stress and adversity might persist into adulthood. Epigenetic mechanisms have also been proposed to explain why cardiometabolic health can vary greatly between individuals with similar Body Mass Index (BMIs). The purpose of this study was to characterize the relationship between childhood adversity and markers of accelerated epigenetic aging in adulthood and examine the link between childhood adversity and obesity and metabolic risk markers. After identifying these relationships, we then examined moderator effects of accelerated aging on the association between childhood adversity, obesity, and insulin resistance.

Methods: We evaluated two independent cross-sectional cohorts of healthy adults, one of which explicitly recruited individuals with childhood adversity and control participants ($n = 195$), and the other a general community sample ($n = 477$). In these cohorts, we examine associations between childhood adversity, epigenetic aging, and metabolic health. DNA methylation was assessed using Illumina-based arrays (EPIC and 450k arrays). Initial analyses utilized the GrimAge epigenetic clock, which has been demonstrated to correlate with morbidity and mortality. Subsequent analyses re-examined the data utilizing principal component-based epigenetic clocks.

Results: Childhood adversity predicted accelerated epigenetic aging in both cohorts, both utilizing a dichotomous yes/no classification (both $p < 0.01$) as well as a continuous measure using the Childhood Trauma Questionnaire (CTQ) (both $p < 0.05$). Further investigation demonstrated that CTQ subscales for physical and sexual abuse (both $p < 0.05$) were associated with accelerated epigenetic aging in both cohorts, whereas physical and emotional neglect were not. In both cohorts, higher CTQ was also associated with higher BMI and increased insulin resistance (both $p < 0.05$). Finally, we demonstrate a moderating effect of BMI on the relationship between epigenetic aging and insulin resistance where epigenetic age acceleration predicts insulin resistance specifically at higher BMIs. Follow-up analyses with principal component-based epigenetic clocks demonstrated largely consistent results.

Conclusions: These results, which were largely replicated between two independent cohorts, suggest that interactions between epigenetics, obesity, and metabolic health may be important mechanisms through which childhood adversity contributes to long-term physical and metabolic health effects. While the current study uses cross-sectional data, it suggests that childhood adversity may increase the risk of insulin resistance through epigenetic changes. Future studies may be able to utilize both psychological and epigenetic markers to identify treatments that will be helpful from both a psychiatric and physical health framework.

Keywords: Epigenetic Clock, Obesity, Childhood Adversity, Insulin Resistance, Epigenetic Age Acceleration

Disclosure: Nothing to disclose.

P12. [18F]PF-06445974 Pet Ligand Can Measure LPS-Mediated Changes in PDE4B in Rat Brain: A Comparison With TSPO

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Background: Phosphodiesterase-4B (PDE4B) terminates cAMP signaling and inhibition of PDE4B has antidepressant-like effects in animals, and anti-inflammatory effects in human disorders (e.g., psoriasis and COPD). We developed [18F]PF-06445974 (PF974) as a radioligand that preferentially binds to the PDE4B subtype to

study it in humans with major depressive disorder and in animals as a potential biomarker of neuroinflammation. We demonstrate here that inflammogen lipopolysaccharide (LPS) increases radioligand binding, without significant contamination of radiometabolites. That is, phosphorylation of PDE4 has been shown to markedly increase both its enzymatic activity and its affinity to binding of radiolabeled inhibitors.

Methods: A total of 10 male Wild-type Sprague-Dawley rats injected with LPS at various doses, and were imaged either with PF974 or TSPO-ligand PBR28. Rat brains were collected after tracer injection and radiometabolite studies were performed using HPLC. Pre-block and displacement studies were performed with rolipram injection IV at 1 mg/kg.

Results: At one day after LPS injection, binding of [18F]PF-06445974 in the lesioned striatum increased about 50% compared to the contralateral region. To confirm the specificity of the radioligand binding, rolipram (1 mg/kg iv) preblock decreased binding to background levels. Post-mortem HPLC results showed increased radioactivity in injected striatum compared to contralateral striatum, and was mainly parent radioligand, implying patent blood-brain barrier. PF974 difference was not noted after 8 days, whereas TSPO binding remained increased after 8 days.

Conclusions: Local injection of LPS in rat brain increased radioligand uptake without significant contamination of plasma-based radiometabolites; which is transient and associated with active neuroinflammation, whereas TSPO uptake remained increased after 8 days. The increased binding may be caused by the phosphorylation/activation of PDE4B and suggest that PDE4B imaging may function as a dynamic biomarker of neuroinflammation in the brain.

Keywords: F-18 PET Imaging, Neuroinflammation, LPS, Phosphodiesterase-4 (PDE4)

Disclosure: Nothing to disclose.

P13. Sex Differences in Basal Cortisol Levels Across Body Fluid Compartments in a Cross-Sectional Study of Healthy Adults

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Background: Many studies have moved toward saliva and peripheral blood sampling for studying cortisol, even in relation to disorders of the brain. However, the degree to which peripheral cortisol reflects central cortisol levels has yet to be comprehensively described. Data describing the effect that biological characteristics such as age and sex have on cortisol levels across compartments is also limited. To assess the relationships of cortisol levels across cerebrospinal fluid (CSF), saliva, and plasma (total and free) compartments, we conducted a secondary analysis of data collected from a previously published study and described the effects of age and sex on these relationships.

Methods: Participants were healthy community volunteers ($n = 157$) of both sexes, aged 20-85 years, who agreed to data and specimen banking as a part of study participation. Samples were collected in academic outpatient settings in 2001-2004 as a part of a multi-site cross-sectional observation study. Associations among cortisol levels and between cortisol and age and/or sex were evaluated using Pearson correlation coefficients and linear regression respectively.

Results: Correlation of CSF cortisol was strongest with that of total plasma ($r = 0.49$, $p < 0.0001$) and weakest with that of saliva ($r = 0.28$, $p < 0.001$). Sex but not age was a significant modifier of these relationships. In examining the influence of age and sex on cortisol in each separate body fluid compartment, CSF cortisol levels trended higher with older age in men ($R2 = 0.31$, $p < 0.001$) but not women. In contrast, saliva cortisol decreased slightly with age ($R2 = 0.05$, $p = 0.021$) and no correlation was found between free or total plasma cortisol and age ($R2 \leq 0.02$, $p \geq 0.28$). Sex was not a significant modifier of age-related trends in peripheral cortisol. Age-related cortisol binding globulin trends differed by sex but did not correlate with sex differences in cortisol levels in any compartment.

Conclusions: This study is the first to our knowledge to examine the relationships in cortisol concentrations simultaneously among total and free plasma, saliva, and CSF compartments in a healthy adult population of both sexes across the adult life span. It provides important insights on how cortisol levels from different compartments are intercorrelated as well as how cortisol concentrations vary with biological characteristics of age and sex. Our findings demonstrate complicated relationships among cortisol, age, and sex -- relationships that varied by compartment. Variability in the correlations between central and peripheral cortisol discourages the use of peripheral cortisol as a direct surrogate for central cortisol measures. Further investigation of how mechanistic drivers interact with biological factors such as sex will be necessary to fully understand the dynamics of cortisol regulation across fluid compartments.

Keywords: Cortisol, CSF Biomarkers, Biology of Aging, Sex Differences, Peripheral Biomarker

Disclosure: Nothing to disclose.

P14. Chronic Stress Enhances Working Memory in Aging Male Rats via Corticosteroid Receptor-Dependent Adaptation of the Hypothalamic-Pituitary-Adrenal Axis

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Background: Working memory, which depends on the prefrontal cortex (PFC), declines with age. PFC neurons express glucocorticoid (GR) and mineralocorticoid (MR) receptors that bind stress hormones and regulate cortical and hypothalamic-pituitary-adrenal (HPA) axis activity when challenged during stressful experiences. While HPA axis dysregulation is evident with advancing age, no studies have systematically examined the effects of chronic stress on working memory over the full lifespan. This omission is significant because epidemiological data implies cumulative stress exposure can exacerbate risk for memory-related disorders in advanced aging. However, stress and stress hormone signaling may also impart short-term enhancement of working memory and PFC cellular activity, but the moderating effects of chronological age on PFC functions during chronic stress are not well-studied. To address this gap, we evaluated PFC-dependent working memory in normally aging male and female rats and, subsequently, investigated the role for GR or MR signaling and HPA axis adaptation on working memory during stress in aging.

Methods: Our studies used male or female F344 rats at 6-, 14-, or 24-months (mo.). We assessed PFC-dependent working memory using a delayed match-to-sample operant task. This task taxes working memory by requiring rats to remember the location of a pseudo-randomly selected lever for delays that range from 0 to 24 s. While working memory testing was ongoing, we assigned similar numbers of each sex and age to unstressed (UNS) or chronic variable stress (CVS) treatment. CVS entailed twice-daily exposure to stressors that included forced swims, physical

restraint, predator urine, or cage-flood, presented in an unpredictable order for 21 days; UNS rats underwent daily testing but without exposure to these experimental stressors. In a subset of 24-mo. males, we determined the role of GR and MR signaling by pre-treatment with mifepristone (MIF; GR antagonist), spironolactone (SPIRO; MR antagonist), or inert vehicle immediately after daily testing and 30 minutes prior to CVS; corticosterone (CORT) secretion stimulated by physical restraint was assessed at the start of CVS and every 7 days thereafter.

Results: We observed that CVS interacted with chronological age and biological sex to influence working memory accuracy. In males, CVS lowered accuracy of 6 mo. rats but, surprisingly, increased accuracy of 14- and 24-mo. rats, compared to age-matched UNS. Choice accuracy of females was significantly greater compared to males and not observed to decline with age or stress. These age- and sex-specific effects of CVS on working memory accuracy were selective as CVS otherwise reduced the number of daily trials completed, lowered body weight, and increased adrenal gland weight independent of age or sex. Reduced activity, weight loss, and adrenal hypertrophy are well-established behavioral and physiological sequelae of chronic stress and reveal that males and females retain sensitivity to chronic stress across the lifespan. Considering the positive effects of CVS on working memory accuracy in 24-mo. males, we followed up on the underlying role of the HPA axis on this phenomenon. Pre-treatment of aged males with either MIF or SPIRO prevented CVS-related memory enhancement compared to vehicle-treated rats. Furthermore, pre-treatment with either MIF or SPIRO attenuated suppression of CORT secretion during acute stress throughout the CVS period. Persistently elevated levels of CORT co-occurred with larger adrenal glands of rats receiving GR or MR antagonists compared to rats that were pre-treated only with vehicle.

Conclusions: Jointly, these findings reveal that the effects of chronic stress on working memory are highly contingent on biological sex and chronological age. Further, enhanced working memory during stress in aged males coincides with adaptation of HPA axis responses that depends on GR or MR signaling. More broadly, these discoveries may lead to new interventions to optimize HPA axis function and improve memory over the full lifespan.

Keywords: Acute and Chronic Stress, Corticosteroids, Working Memory, Prefrontal Cortex, Aging

Disclosure: Nothing to disclose.

P15. Transient Exercise-Induced Changes of Circulating Factors in the Mouse Muscle-Brain Axis

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Background: Exercise promotes healthy cognitive aging and could be an effective non-pharmacological alternative to prevent or delay pathology associated with neurodegenerative disorders. However, mechanisms underlying such positive effects are not fully understood. Irisin, a myokine that is secreted from skeletal muscle during exercise, is linked to mechanisms that become dysfunctional in as we age, such as glucose metabolism and hippocampal neurogenesis.

Nevertheless, there have been contradictory reports on changes in irisin levels following exercise. Such inconsistencies might be a result of variability in experimental conditions, such as duration of exercise protocols (e.g., acute or chronic) and/or timing of tissue collection (e.g., immediately after or hours later).

Methods: To determine the precise timespan of exercise-induced changes in circulating irisin, we designed exercise protocols that tested the effect of these experimental variables. We hypothesized that changes in circulating irisin concentration occur during or immediately after exercise and return to baseline shortly thereafter. To test this hypothesis, we subjected adult male and female mice to swimming or running exercise for 20 min and measured post-exercise serum irisin concentration (via ELISA) at: 0, 30, 60, or 120 min after.

Results: Compared to sedentary controls, we observed a ~20% increase in male mice immediately after either exercise protocol, which decreased to baseline levels within 60 min. No changes were detected in female mice. Next, we performed a chronic exercise protocol (swimming for 20min/ day for 21 days) with a separate group of male and female mice, and measured serum irisin 24 h after the last exercise session. As expected, no difference was measured between the exercise group and the sedentary controls.

Conclusions: Our results suggest that irisin is released during exercise and circulating levels return to baseline within an hour. Therefore, the timing of sample collection should be carefully considered to reliably detect exercise-induced changes in circulating irisin. In addition, we observed robust sex-dependent differences, including the potential relationship between Irisin release and stress.

Keywords: Physical Exercise, Acute Stress, Brain Aging, Neuroprotection, Sex-Specific Effects

Disclosure: Nothing to disclose.

P16. Alprazolam Impairs Fear Memory and Alters Dorsoventral CA1 Neuronal Ensembles in Female Mice

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Background: Benzodiazepines (BZDs) are commonly prescribed anxiolytic drugs that act on GABAa receptors and can result in anterograde amnesia or the inability to form new memories. Although BZDs have been in use for over 60 years, the brain regions and neuronal mechanisms responsible for this detrimental side effect are largely unknown. Additionally, continued use of BZDs could lead to memory impairment and cognitive decline later in life. Our lab has previously shown that anxiety is a strong predictor of dementia transition, specifically in women and female Alzheimer's disease (AD) mice. However, there are conflicting results on whether anxiolytics are beneficial for treating neuropsychiatric symptoms in AD. Women were also found to be twice as likely to use BZDs. Although aged populations account for 8.4% of BZD users, the relationship between BZD use and cognitive decline is highly controversial. Previous literature has indicated both support and opposition to the hypothesis of accelerated cognitive decline in both healthy and AD populations treated with BZDs. To better understand the long-term memory deficits initiated by benzodiazepines, we aimed to analyze neuronal ensemble activation following a benzodiazepine injection prior to learning and during consolidation.

Methods: To analyze the effects of BZDs on long term memory, ArcCreERT2 x eYFP mice were injected with Alprazolam 30 minutes prior to a 3-shock contextual fear conditioning (CFC) procedure. The ArcCreERT2 x eYFP mouse model allows us to tag active neuronal ensembles during an experience by using Arc as an immediate early gene (IEG) promotor. We then re-exposed mice to

the same context 5 days later and euthanized mice 1 hr after exposure to examine retrieval cell activation using a second IEG, cFOS. This method allows us to determine which brain regions undergo changes after BZD injection and how these cellular changes correlate to freezing behavior (a proxy for memory). Additionally, we address the question of whether BZDs induce state-dependent memory by altering the timelines of injection to include drug administration at both time points or a drug-vehicle administration to circumvent the sedative properties of BZDs. We also analyzed how BZDs affect the initial consolidation of a fear memory by injecting mice immediately after CFC training. 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 that 1) BZD-treated female mice exhibit a decrease in memory retention ($p < 0.0001$), 2) BZD-treated female and male mice show a decrease in memory retention with saline injection prior to re-exposure ($p < 0.05$), and 3) BZD injection immediately after CFC training enhances memory in male mice ($p < 0.05$). Cell counts revealed that BZD-treated female mice exhibit increased EYFP+ (encoding) and cFos+ (retrieval) activation in the dCA1 compared to controls ($p < 0.05$). However, the opposite pattern was observed in the vCA1, where BZD-treated females showed less EYFP+ and cFos+ activation ($p < 0.05$). These results suggest that ventral hippocampal activity is dampened with BZD injection, but the dorsal hippocampus is still actively encoding contextual information. We are further analyzing cell counts in other hippocampal and amygdala regions.

Conclusions: Completion of this project will help us to better understand the long-term memory deficits associated with BZDs. We also have the potential to discover novel brain regions involved in these side effects and therefore offer alternative treatment options. Our future studies will examine the impact of chronic use of BZDs on aging and Alzheimer's disease.

Keywords: Benzodiazepine, Memory, Engram, Ventral Hippocampus, Sex Differences

Disclosure: Nothing to disclose.

P17. Downstream Drainage to Cervical Lymph Nodes is Compromised in Cerebral Small Vessel Disease: Implications for Dysregulation of Brain Fluid Homeostasis

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Background: Alcohol use disorder (AUD) is associated with dysregulation of central nervous system (CNS) fluid homeostasis manifesting as enlarged ventricles which can be reversed with abstinence. Cerebral ventriculomegaly is also observed in neurodegenerative diseases including normal pressure hydrocephalus a common disorder in older adults and remains a poorly understood pathophysiological signature. Recent studies have demonstrated that the glymphatic system when coupled to meningeal lymphatics facilitates brain waste elimination and sustains CNS fluid homeostasis. The regulators of glymphatic and lymphatic systems cross-talk for solute waste and fluid drainage to the cervical lymph nodes remain poorly understood. A major issue resides in the technical challenge of quantifying glymphatic clearance from the brain to cervical lymph nodes without direct administration of a tracer into parenchyma, a procedure that by itself can negatively impact clearance. We have developed an imaging approach with CSF administration of a tracer which when combined with computational fluid dynamic (CFD) analysis allows for tracking of mass 'gain' and 'drain' at the voxel level. The CSD approach is based on an "unbalanced" regularized optimal mass transport (urOMT) model which incorporates a source/sink term thereby allowing for quantifying mass gain and mass loss (clearance) over time. Here we demonstrate that urOMT analysis uncovers surprising defects in cervical lymph node drainage in a rat model cerebral small vessel disease known to have glymphatic system impairment. Our study highlights the potential therapeutic importance of considering compromised downstream lymph node drainage function for correcting upstream dysregulation of cerebral fluid homeostasis and glymphatic transport function observed in neurodegenerative disease states including AUD.

Methods: We performed dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) with GadoSpin-P contrast injection into the CSF in 10-month-old female Wistar Kyoto (WKY) rats ($N = 7$) and spontaneously hypertensive stroke prone (SHRSP) rats ($N = 6$) which is rat model of cerebral small vessel disease. We used a custom-made transmit RF coil for signal excitation and a 2-cm surface radiofrequency loop coil, positioned over the neck of the rat was used as a receiver. 3D T1-weighted images were acquired at a voxel size of 0.008 mm3, before and 160min after GadoSpin-P infusion. The raw time series of 3D DCE-MRI data underwent intensity normalization and smoothing with the full-width, half-maximum Gaussian smoothing kernel of 0.1 mm, and the voxel-wise percent signal change from baseline was calculated. An anatomical mask of the base of the brain and cervical lymph nodes was used to extract the corresponding time series of DCE-MRI data which was fed into the urOMT model. Sample sizes were chosen on the basis of similar experiments. No randomization was performed. Two-group comparisons were made using a two-tailed unpaired independent t-test assuming unequal variances. For the urOMT time trajectories of mass gain and mass loss rates, a linear mixed model for repeated measures (LMM-RM) with independent variables including strain (WKY vs SHRSP), time and the time x strain interaction were fit to compare the mean differences of different outcomes between strains and between time points after tracer administration within each strain of rats. Group differences were calculated using a post-hoc pairwise Fisher's least significant difference (LSD) with adjustments for multiple comparisons using Bonferroni correction.

Results: There were no differences in MRI defined anatomical features or size of the deep cervical lymph nodes (dcLN) across WKY and SHRSP rats (volume, $p\text{-value} > 0.05$). Conventional kinetic analysis of the time signal curves extracted from the raw parametric DCE-MRI images at the level of the deep cervical lymph nodes (dcLN) did not reveal differences in across WKY and SHRSP rats: Time-to-peak ($p\text{-value} = 0.391$), full width at half maximum ($p\text{-value} = 0.578$) and peak magnitude ($p\text{-value} = 0.104$). We next analyzed the series of urOMT 'r-flux' maps, which was used to extract the time varying spatial patterns of mass 'gain' and drainage from the lymph nodes. In WKY rats the progression of mass gain in the lymph node dominated for the 60-min and then rapidly declined, whereas the time trajectory of drainage followed a sigmoid curve pattern with rapid increases at ~80-90 min which was sustained for the rest of the experimental time period. In contrast, in the SHRSP rats mass gain in the lymph node dominated over mass loss over the first 120-130min, and drainage was minimal. The LMM-RM analysis revealed statistically significant time*group differences in dcLN trajectories of mass gain ($p\text{-value} = 0.014$) and loss ($p\text{-value} = 0.002$) across groups.

Conclusions: We uncovered significant impairment of cervical lymph node function in a rat model of cerebral small vessel disease known to be associated with a decrease in glymphatic transport. The new information was uncovered by applying the urOMT analysis to DCE-MRI data which allows for tracking of mass gain and drainage in real time. Our study highlights the potential

therapeutic importance of considering compromised downstream lymph node drainage function for correcting upstream dysregulation of cerebral fluid homeostasis and glymphatic transport function observed in neurodegenerative disease states including cerebral small vessel disease.

Keywords: Fluid Homeostasis, Glymphatic System, Lymphatics, Neurodegenerative Disease, Waste Clearance

Disclosure: Nothing to disclose.

P18. Taste Processing in a Mouse Model of Frontotemporal Dementia

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Background: Frontotemporal dementia (FTD) is the second most prevalent form of presenile dementia. Patients with FTD show a pathological sweet tooth and decreased ability to identify flavors. Taste perception depends on neural processing in chemosensory regions such as the insular cortex - a brain region that also contains the primary taste cortex, gustatory cortex (GC). The chemosensory deficits in FTD may be related to GC damage as insular cortex is one of the primary targets in FTD disease progression. Little is known on how circuitry changes related to FTD lead to abnormal activation of GC and to deficits in taste processing in FTD.

Methods: The goal of this project is to test the hypothesis that the chemosensory deficits in a mouse model of FTD are related to abnormal patterns of neural activity in GC. TDP-43 inclusions are a significant pathological feature in 50% of FTD cases, thus we use a transgenic mouse model overexpressing human transactivating response region (TAR) DNA binding protein (TDP-43) with a Q331K mutation (Q331K mutants) To assess chemosensory deficits, we relied on a taste-based two alternative forced choice (2AFC) task probing the ability to discriminate sucrose/NaCl mixtures. Behavior performances at different concentrations were fitted to sigmoid functions to obtain the psychometric functions of Q331K mutants and control (Q331K mutants $n = 4$, control $n = 5$). The sigmoidal fits were compared using extra sum-of-squares F test. To monitor neural activity, we relied on electrophysiological recordings using chronically implanted tetrodes in alert Q331K mutants and control mice. Activity in GC was probed as mice licked multiple gustatory stimuli (sucrose 200mM, NaCl 50mM, quinine 0.5mM, citric acid 10mM) (Q331K mutants $n = 6$, control $n = 5$). Taste selectivity was assessed using two-way ANOVA (taste x time (0- 5 s after taste delivery)). Taste selectivity was defined as either the taste identity main effect or the interaction term is significant for a neuron. A 'best window' analysis was employed to probe the taste responsiveness, where firing rate from 0 to 5s after taste delivery was scanned using a 250ms sliding window moving in 25ms steps. Activity during the 250ms window greater than 2 standard deviations above or below the average baseline firing rate was considered as a significant response. A classification analysis using a population decoder based on max correlation coefficient as well as a principal component analysis were applied to neural activity 0 to 5s post taste delivery to understand the temporal dynamics of taste processing in the GC of Q331K mutants and control animals. Both sexes were included in these studies.

Results: Q331K mutants make more mistakes and show significant deficits in the mixture discrimination 2AFC task ($p = 0.014$). There is a larger proportion of taste responsive neurons while a smaller proportion of taste selective neurons in Q331K mutants compared to control mice. Classification analysis shows that taste decoding decays faster in Q331K mutant mice relative to control mice. Principal component analysis confirms that population activity dynamics evoked by the different gustatory stimuli are more similar in Q331K mutants than in control mice. No sex differences were found between female and male animals.

Conclusions: Overall, these results demonstrate taste deficits in a mouse model of FTD and provide evidence for altered taste processing in GC of Q331K mutants compared to control mice.

Keywords: Single-Unit Electrophysiology in Vivo, Frontotemporal Dementia, Behavioral Tasks

Disclosure: Nothing to disclose.

P19. Dopamine Release in Response to Cues Predicting Avoidable Aversive Stimuli Differentiates Behavioral Responses: Escape Versus Helplessness

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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. In some situations, negative outcomes are avoidable, while in others they are not. The inability to effectively discriminate between these situations and update information about these relationships is a hallmark of pathological disease states, such as PTSD and anxiety disorders. Here we utilized a negative reinforcement paradigm – where mice can perform an operant response to avoid a series of footshocks - to investigate dopamine release patterns throughout learning and determine how these neural signals can differentiate animals that adaptively learn to avoid versus those that do not.

Methods: The optical dopamine sensor dLight in conjunction with in-vivo fiber photometry was used to monitor real-time dopamine responses in the nucleus accumbens core (NAc). The negative reinforcement paradigm lasted 15 days, where mice underwent 30-minute training sessions. Each trial began with a 15-second tone and lever presentation, followed by a train of five 0.5 sec, 0.5mA shocks separated by 1 sec. Mice could respond at any time from cue onset to the last shock presentation (i.e. during the discriminative cue). After which, a house light went on (also presented with a lever press and cessation of future shocks). There was a variable ITI (30-90sec), then a new trial began. Mice then went through an FR1 positive reinforcement schedule with sucrose presentation, until the number of active lever presses accounted for 80% or greater of the total lever pressing. After completing, this instrumental learning phase, mice went back into the initial negative reinforcement paradigm for 10 days with white noise instead of a tone for the discriminative cue. Dopamine release was assessed around behaviorally relevant events in male and female mice. Sample sizes range from 3-10 mice per group.

Results: Mice were separated into groups based on task performance. Female learners displayed increased lever pressing to greater than 80% correct trials and a decrease in freezing time to the cue as they learned to avoid the shocks. Female non-learners displayed a comparable initial freezing time to the predictive cue that was maintained across days and minimal operant responses. Female learners displayed an increase in dopamine release to the cue, while non-learners displayed a decrease. The dopamine response increased to the first shock over time similarly in both groups compared to the following shocks; however, by the last day of the task, there was a significant increase in dopamine release to the first shock in learners and

non-learners compared to the first day. In males, a similar pattern emerged where non-learners displayed a decrease in dopamine release to the cue over time and an increase in dopamine release to the first shock. Following positive reinforcement acquisition, the initially classified non-learner females were still unable to acquire negative reinforcement despite changing the discriminative cue. Non-learner females displayed a decrease in dopamine release to the cue that did not change over time and an increase in dopamine release to the first shock that was larger than the release to subsequent shocks and increased over time.

Conclusions: Despite the escapable nature of these shocks, many mice developed a freezing response to the cue, rather than learning to avoid. The mice that did not learn to avoid had future deficits in negative reinforcement learning as well, despite successful instrumental learning, suggesting that this learned experience ultimately influenced behavior in other contexts. Interestingly, there were still changes in dopamine release throughout training in non-learners, such as changes in the neural responses to the shock itself. These data demonstrate behavioral changes dependent on learning ability and changes in dopamine signaling over time may underly how a subject navigates their environment and perceives the availability of behavioral outcomes (ability to escape shocks vs perceived inescapability).

Keywords: Negative Reinforcement, Dopamine, In Vivo Fiber Photometry

Disclosure: Nothing to disclose.

P20. Spatial Analysis of Transcriptional Changes and Connectivity After Threat Conditioning

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Background: Maladaptive transcriptional alterations within complex neurocircuitries are central to many stress- and trauma-related disorders. However, how gene expression changes concertedly across brain regions remains largely unknown.

Methods: We generated spatial transcriptomics RNAseq (stRNAseq) data in C57BL/6 mice exposed to auditory threat conditioning ($n = 8$ per group), capturing the early memory consolidation period. Bioinformatic analyses focused on between group differences and mapping transcriptional connectivity.

Results: Unsupervised clustering analysis of stRNAseq data revealed 33 subregions in concordance with known anatomical structures. Cluster specific expression markers were in alignment with in-situ hybridization data of the Allen Brain Atlas. Differential gene expression analysis between threat conditioned and control animals resulted in a total of 415 DEGs ($p.FDR < 0.05$, $n = 8$ pergroup) across subregions and RNAscope analyses confirmed expression differences in selected subregions. In addition, transcriptional connectivity network analyses showed a coordinated transcriptional response to threat conditioning across 15 brain regions (permutation $p < 0.05$) including the piriform area, caudoputamen, cortical layers, and the thalamic reticular nucleus.

Conclusions: Our results indicate a brain-wide, coordinated transcriptional response to threat conditioning with transcriptional network analyses suggesting additional brain regions as part of the threat neurocircuitry.

Keywords: Spatial Transcriptomics, Threat Conditioning, Brain Circuits

Disclosure: Nothing to disclose.

P21. Decoding Claustrum Neuropeptidergic Control of Stress-Induced Binge Eating

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Background: The experience of stress promotes the development and persistence of binge eating behaviors. However, how and where stress-induced binge-eating behaviors happen is still largely unknown. The dynorphin (dyn)/kappa opioid receptor (KOR) neuropeptide system is well known for playing a crucial role in mediating the dysphoric and anhedonic aspects of stress, which act to promote reward-seeking and drug abuse-related behaviors. Recent studies show a reduction in binge eating behavior while maintaining normal hunger states when treated with opioid receptor antagonists. Given these findings, we aimed to explore the neural circuit basis of dynorphinergic control of stress-induced binge-like eating behavior in a mouse model.

Methods: We first established a behavior model for stress-induced binge-like eating behaviors. Mice ($N > 10$) experienced either 15 mins of the forced swim or in an empty bucket before being given ad-lib access to both palatable foods (high fat, high sugar, HPD) and regular chow for 1 hr. Their brains were extracted later for histology analysis for cFos activation. For the knockout experiment, we inject AAV-CRE-GFP virus in either WT ($N = 9$) or Kor-cKO ($N = 4$) mice and perform behavior analysis. Pharmacology testing was done with an IP injection of 5mg/kg U50 ($N = 8$) or local norBNI infusion (20ul/ml) in the CLS. For single-photon in vivo recordings, AAV-DIO-GCaMP was injected in the CLS in Kor-Cre mice ($N = 3$), and their neural activities were recorded during 1 hr feeding in stress and non-stress conditions. Similarly, either AAV-CaMKII-GCaMP ($N = 10$) or AAV-DIO-klight ($N = 6$) was injected in the CLS, and neural activities were recorded using fiber photometry. To identify the dynorphin input to the CLS, Pdyn-cre mice ($N = 3$) were co-injected with AAV-CaMKII-mcheery + rAAV-DIO-GFP in the CLS, and their entire brain was examined for GFP+ cells. For the circuit manipulation experiment, AAV-CaMKII-GCaMP was injected in the CLS, and either AAV-DIO-Chrimson ($N = 5$) or AAV-DIO-mcherry ($N = 4$) was injected in the anterior insular cortex in Pdyn-Cre mice. We performed fiber photometry recordings in the CLS population while stimulating the terminals from the anterior insular cortex.

Results: We found mice that experienced 15 mins of the forced swim will significantly increase their food intake of familiar palatable food (high fat, high sugar, HPD) compared to non-stressed mice. We also found increased cFos-positive cells in the claustrum (CLS), a subcortical structure with highly abundant expression of KORs, after mice developed this stress-induced binge-eating behavior. When targeting CLS-KOR+ cells through viral-mediated knockout, we have found intact KOR signaling in CLS is necessary for elevated binge-like eating in stressed mice. In vivo cell recordings in CLS-CaMKII+ cells using fiber photometry revealed increased neural activity in initiating HPD feeding bouts compared to the non-stressed mice. And this effect is further enhanced when mice are given U50, which is a KOR agonist. When infusing norBNI (KOR-antagonist) locally in CLS, we were able to block stress-induced binge eating, which indicates that dynorphin release is critical for this behavior. We further characterize and dissect the dynorphinergic components of this behavior using dynorphin biosensors Klight in CLS-KOR+ cells and single-cell imaging while mice perform binge-eating behaviors. To identify the source of dynorphin releases, we performed retrograde tracing and found anterior insular cortex (AIC) sends dynorphinergic inputs to CLS. When we stimulated AIC-Pdyn+ to CLS

terminals, we observed increased activities in the CLS-CaMKII+ populations.

Conclusions: Forced swim stress induces binge-eating behaviors in mice, activating neurons in the Claustrum (CLS). This behavior relies on the dynorphin (dyn)/kappa opioid receptor (KOR) neuropeptide system in the Claustrum. We have established a functional dynorphinergic input from the anterior insular cortex (AIC) to the CLS. We hypothesize that AIC releases dynorphin in the CLS during stress-induced binge eating behaviors and forms a disinhibition circuit within CLS. By characterizing the neural circuit mechanisms and cell activity dynamics within the CLS, we aim to understand how neuropeptides regulate stress-induced binge eating.

Keywords: Dynorphin, Binge Eating Disorder, Claustrum, Insular Cortex

Disclosure: Nothing to disclose.

P22. Testing Optimal Glucocorticoid Mediated Stress Conditions for Modeling the Multi-Omic Signatures of Stress-Associated Psychiatric Disorders With iPSC Derived Neural Cell Types

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Background: Posttraumatic Stress Disorder (PTSD) is a common complex psychiatric disorder affecting 6.1% of Americans, and no efficacious cellular models exists for testing novel therapeutics. Our group recently published (Chatzinakos, Pernia et al. AJP 2023) the first single nucleus RNA-seq (snRNA-seq) study of human DLPFC brain samples across PTSD, MDD, and healthy controls to identify cell type specific transcriptional effects, and recapitulated PTSD unique effects in vitro with iPSC derived neurons exposed to 100nM Dexamethasone (DEX). What combination of in vitro cell types and DEX doses that best recapitulate individual stress related disorders (PTSD, MDD, etc) are still unknown. In this work, we attempt to identify in vitro conditions that best recapitulate PTSD and other stress-associated disorders' multi-omic signatures to assist future psychiatric disease modeling studies, and to better understand the role glucocorticoids play in molecular psychiatry.

Methods: Two healthy control iPSC lines were differentiated into neural stem cells (NSCs), then immature neurons (INs), and neuron cultures. At each stage, three samples were treated with a DEX dose curve (0nM, 5nM, 25nM, 100nM), and harvested for RNA and DNA methylation (DNAm). RNA and DNAm triplicates were pooled into 32 separate samples, then RiboZero sequenced at ~70M read depths or Illumina 850K EPIC arrayed respectively. RNA and DNAm differential analysis was performed by voom/limma, and gene networks were studied with weighted gene co-expression network analysis (WGCNA). We examined if previous published stress associated psychiatric disorders clinical datasets could be recapitulated by our DEX treated cells using spearman correlations, rank-rank hypergeometric overlap (RRHO), and gene set enrichment analysis (GSEA).

Results: After demonstrating each iPSC derived cell type expresses varying levels of the glucocorticoid receptors (GR), RNA-seq and EPIC arrays were performed. Less developed cell types (iPSC and NSCs) displayed fewer differentially expressed genes (DEGs) then the more neuronal cell types (INs and neuron) when exposed to DEX, while only the INs and neurons had consistent DEGs across the DEX dose trajectory. WCGNA of DEX DEGs in INs and neurons identified gene modules associated with protein regulation, vesicle transport, and the synapse. RRHO and normalized enrichment score (NES) analysis of DEX DEGs with stress associated psychiatric datasets showed distinct enrichments across various in vitro conditions with PTSD and MDD. iPSC derived neuronal cells' DEX DEGs were also enriched for PTSD and MDD GWAS genes. Most differentially methylated genes (DMGs) due to DEX across all cell types were associated with stress or psychiatry. GSEA of DMGs due to DEX in the INs and neurons were enriched for neuronal and synaptic processes, similar to the RNA analysis.

Conclusions: While many psychiatric disorders have been studied in recent years with innovative iPSC disease modeling paradigms, PTSD research has yet to fully embrace such approaches. We have previously shown iPSC derived neurons treated with DEX are an efficacious model for studying PTSD gene expression. In this study, we explore various iPSC derived cells types and DEX dosages for best modeling individual stress associated psychiatric disease based off published clinical datasets. Intriguingly, we observed GR activation was not consistent across cell types, and that the separate analytical approaches associated certain cell types to the various psychiatric datasets. Overall, our most developed neurons treated with 100nM DEX best recreated PTSD GWAS, post mortem bulk and cell type specific gene expression, and DNAm signatures. In the future, we will use additional stem cell based approaches for modeling the functional impact of glucocorticoid mediated stress in the context of PTSD and other stress associated psychiatric disorders, in the hopes of generating a high fidelity in vitro platform for focused drug screening.

Keywords: hIPSCs, Glucocorticoids, MDD, PTSD

Disclosure: Nothing to disclose.

P23. Group 2 Innate Lymphoid Cells at the Choroid Plexus Mediate Psychological Stress-Induced Anxiety-Like Phenotype

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Background: Prolonged or severe stress can cause structural and functional changes in the brain. Such changes in the brain contribute to various neuropsychiatric disorders like depression and anxiety. The dysregulated immune function is known to mediate stress-induced anxiety-like behavior. Innate lymphoid cells (ILCs) are tissue-resident innate immune cells, but their role in regulating anxiety-like behavior under stress conditions is poorly understood.

Methods: We used a well-validated mouse model of stress where animals were exposed to trauma and trigger via inescapable electric foot shocks to study anxiety-like phenotype. Flow cytometry analysis was performed to examine the ILC population. Realtime qPCR was performed for the mRNA expression analysis. The data were presented as mean ± SEM. Unpaired Student's t-test was used for two-group comparisons, while Analysis of Variance (ANOVA) was employed for multiple-group comparisons. Post hoc analyses were conducted using Tukey's test. A significance level of $p < 0.05$ was considered statistically significant.

Results: We found an increased anxiety-like behavior in male C57BL6/J mice ($N = 12$) exposed to stress in the open field test (OFT) and light/dark test (L/DT) compared to the no-stress group. The flow cytometry analysis showed that out of three (ILC1, ILC2, and ILC3) ILC subpopulations, stress induces an increase in the ILC2 population in the choroid plexus (CP) but not in the meninges. Further, depletion of ILC2s (Rag2-/-Il2rg-/- mice) significantly attenuates stress-induced anxiety-like behavior in

both OFT and L/DT. The stress-induced increase in mRNA expression of IL-5 and IL-13, cytokines known to be elevated following ILC2 activation were significantly attenuated in the CP of Rag2-/-Il2rg-/- mice. The cytokines IL-25 and IL-33 are known to activate and expand ILC2s. We observed that anti-IL-25 antibody, but not anti-IL-33 antibody administration attenuated stress-induced increases in the ILC2 population in the CP and anxiety-like behavior. Glucocorticoid receptor (GR) activity plays a critical role in CNS stress circuitry modulating anxiety-like behavior. Flow cytometry analysis showed no significant effect of mifepristone, a GR antagonist administration in attenuating the stress-induced increase in the ILC2 population in the CP. However, mifepristone treatment resulted in a significant decrease in stress-induced anxiety-like phenotype in OFT and L/DT.

Conclusions: Our findings show that ILC2s mediate stress-induced anxiety-like behavior in mice in a GR-independent manner. We provide the first evidence on the role of ILC2 in stress-induced anxiety-like behavior. These results may help in the development of potential biomarkers and also may lead to the development of novel therapeutic strategies for anxiety disorders.

Keywords: Group 2 Innate Lymphoid Cells, Choroid Plexus, IL-25, Anxiety-Like Phenotype, Stress

Disclosure: Nothing to disclose.

P24. Stepwise Implementation of Virtual Reality Mindfulness and Accelerated Transcranial Magnetic Stimulation Treatments for Dysphoria in Neuropsychiatric Disorders

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Background: Dysphoria is a transdiagnostic symptom of unease or dissatisfaction experienced across a range of diagnoses including depression, anxiety, PTSD, and chronic pain. Those who experience dysphoria as a result of these conditions often have limited treatment options that, even if effective, can take weeks or months to improve symptoms. The goal of this project was to pilot a stepwise approach for quickly addressing symptoms using Virtual Reality (VR) Mindfulness followed by 2 different accelerated Transcranial Magnetic Stimulation (accel-TMS) protocols as needed. The sequenced approach allows for demonstration of improvement in those that did not previously respond. We hypothesized that the sequence of VR Guided Mindfulness and accelerated TMS protocols would provide a progressive, significant improvement in dysphoria symptoms.

Methods: Participants were recruited from the community. Inclusion criteria for dysphoria included minimum depressive (Patient Health Questionnaire-9 (PHQ-9) ≥10), anxiety (General Anxiety Disorder-7 (GAD-7) ≥10), PTSD (Posttraumatic Stress Disorder Checklist (PCL-5) ≥45), or chronic pain (Average Pain Intensity ≥4/10 for >3 months) symptoms. Participants were excluded for medical contraindications to TMS and severe psychiatric diagnoses such as psychotic, active substance use, and cognitive disorders. After meeting study criteria, participants were enrolled in Phase 1: VR Guided Mindfulness. Guided mindfulness treatments were 2x/day with a 50-minute break between treatments for 2 weeks (10 total visits). At Visits 1 and 10, clinician- and self-rated scales were obtained to assess for treatment benefit (clinician-rated were MADRS, HAM-A, CAPS-5, and PROMIS Pain Interference). Those without significant treatment benefit (at least a 30% reduction on the primary participant-rated scale); or those who did not tolerate the VR treatments well and wanted to stop prior to Visit 10 and who still met inclusion criteria were screened and offered to go to Phase 2A: left dorsolateral PFC accelerated TMS (iTBS 1,800 pulses/treatment, 110% MT, 5x/day for 5 days with 50-minute breaks between treatments). At Treatment A1 (first day of treatment) and Follow-Up A1 (one-week post treatment), clinician- and self-rated scales were obtained. Participants with a treatment benefit continued for 4 more weekly sessions over 4 weeks and were reassessed on the last day of follow-up (5 Follow-Ups total). Those without significant treatment benefit from Phase 2A on Follow-Up A1 or who still met inclusion criteria after completing all Phase 2A Follow-Ups were then offered Phase 2B: dorsomedial PFC accelerated TMS (iTBS 600 pulses/treatment and 10Hz for 1,200 pulses/treatment, 110% MT, 5x/day for 5 days with 50-minute breaks between treatments), plus 5 weekly sessions over 5 weeks. Clinician- and self-rated scales were obtained at Treatment B1, Follow-Up B1, and Follow-Up B5 to assess for treatment benefit. Paired t-tests assessed (significance $p \leq 0.05$) change in rating scales from baseline to the last day of treatment for VR or first follow up for TMS.

Results: There were 27 participants enrolled in the study, 24 of which were included in the analysis. For Phase 1 (VR mindfulness), 12 participants completed the full 10 days, 11 participants discontinued before 10 days, and 1 elected to not receive VR. There were 5 participants that did not continue to Phase 2A (3 responder with improvement in dysphoria and 2 chose to not continue). There were 19 participants that were enrolled in Phase 2A, 8 of which (42%) achieved remission, and 5 of those 8 completed the study. One participant elected not to move on to Phase 2B. Thirteen participants went on to Phase 2B with 8 of 13 (62%) achieving remission. All participants completed full acute courses of Phase 2A and 2B without any major adverse events.

For those participants meeting criteria for depression, across the sample of participants, the average MADRS for Phase 1 decreased from 29 to 27 ($p = 0.098$, $n = 20$); for Phase 2A from 29 to 21 ($p = 0.011$, $n = 19$); and for Phase 2B from 22 to 15 ($p = 0.010$, $n = 12$).

For those participants meeting criteria for anxiety, across the sample of participants, the average HAM-A decreased for Phase 1 from 23 to 2 ($p = 0.113$, $n = 20$); for Phase 2A from 23 to 16 ($p = 0.005$, $n = 19$); and for Phase 2B from 17 to 10 ($p = 0.025$, $n = 12$).

For those participants meeting criteria for PTSD, across the sample of participants, the average CAPS-5 decreased for Phase 1 from 30 to 26 ($p = 0.029$, $n = 20$); for Phase 2A from 27 to 19 ($p = 0.011$, $n = 19$); and for Phase 2B from 18 to 12 ($p = 0.063$, $n = 12$).

For those participants meeting criteria for chronic pain, across the sample of participants, the average PROMIS Pain Interference decreased for Phase 1 from 13 to 12 ($p = 0.604$, $n = 20$); for Phase 2A stayed at 12 ($p = 1.000$, $n = 19$); and for Phase 2B stayed at 12 ($p = 0.931$, $n = 12$).

Conclusions: This sequenced neuromodulation approach demonstrates progressive improvement in rapidly reducing symptoms of depression, anxiety, and PTSD, but not chronic pain. Virtual reality treatments were well tolerated but did not provide much response possibly due to degree of samples treatment resistance. For both Phase 2A accelerated TMS and subsequent Phase 2B accelerated TMS, there was a modest degree of response, despite only one week of treatment. As this a non-randomized pilot study with a rather small sample size without long-term follow-up, future assessments of the feasibility and efficacy of this approach should be examined in randomized, larger sample sizes with long-term follow-up.

Keywords: Transcranial Magnetic Stimulation, Virtual Reality, Dysphoria, Depression, PTSD

Disclosure: Neuronetics: Grant (Self). NIRx: Grant (Self).

P25. Combining Ketamine with Trauma-Focused Psychotherapy for Chronic PTSD - A Pilot Clinical Trial

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Background: Posttraumatic stress disorder (PTSD) is a leading cause of disease burden worldwide. There is a critical need for the development of novel treatment interventions for PTSD, as currently available treatments have limited efficacy. While trauma-focused psychotherapies have the highest evidence base and are considered the gold standard intervention, they are associated with significant rates of nonresponse or limited response, especially in individuals with severe chronic PTSD.

Glutamate is crucial to learning and memory formation via strengthening of synaptic connections. Key mechanisms underlying chronic PTSD include "synaptic disconnection" and impaired fear extinction learning, which might underlie limited response to trauma-focused psychotherapies in many individuals. Ketamine, a noncompetitive glutamate NMDA receptor antagonist, has been found to rapidly enhance brain plasticity in the short term, and to enhance fear extinction learning. While a course of six ketamine infusions yielded rapid and robust PTSD symptom improvement in a randomized controlled trial in civilians with chronic PTSD, most ketamine responders lost this response over the following weeks (without maintenance infusions). Median time to loss of response was four weeks following the course of six infusions.

Through opening a window of increased neuroplasticity, however, a course of ketamine infusions has the potential to enhance the efficacy of trauma-focused psychotherapy via a potential synergistic effect, while also reducing the need for maintenance infusions. The current preliminary clinical trial aimed to examine the effect of combining a course of six ketamine infusions with a brief, evidence-based and highly scalable trauma-focused therapy, written exposure therapy (WET), in patients with high-moderate to severe chronic PTSD.

Methods: Participants were eligible if they were between ages 18 and 70 and had a current primary diagnosis of chronic PTSD, defined as meeting DSM-5 criteria on the Structured Clinical Interview for DSM-5 (SCID-5), of at least three months' duration, and a score ≥30 on the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5). Key exclusion criteria included psychotic or bipolar disorder, serious unstable medical illness, substance or alcohol use disorder within the prior three months, current opioid medication or long-acting benzodiazepines, and currently receiving evidence-based psychotherapy for PTSD. Concomitant psychotropic medications at stable doses for three months prior to study start were permitted. Eligible participants were assigned to receive a course of six ketamine infusions (0.5 mg/kg administered over 40 minutes, three times a week for two consecutive weeks) and WET (five total sessions). WET sessions, delivered via video telehealth, began after the first four infusions –the first two WET sessions interleaved with the last two infusions –each on a different day– and the last three WET sessions delivered the following week. Total combined treatment duration was three weeks. WET begins with therapist-delivered psychoeducation, its core consisting of 30 minutes of writing by the patient per session, following instructions read by the therapist. Over the course of WET, writing instructions begin with a focus on the details of the primary trauma and then shift to the meaning of the traumatic event. Change in PTSD symptom severity was assessed weekly with the CAPS-5 through week 12. Treatment response was defined as ≥30% improvement in CAPS-5 score from baseline. The primary outcome was change in total CAPS-5 score from baseline to 12 weeks following the start of WET. Analyses are descriptive and hypothesis generating.

Results: Fourteen eligible participants began study treatment. Thirteen participants [100% female, mean (SD) age = 38.3 (7.5) years, mean (SD) CAPS-5 score = 41.6 (6.2) at baseline], who completed the full course of infusions and all five WET sessions, were included in data analyses. Eight (62%) participants presented with sexual assault or molestation as their primary trauma; the remaining participants had experienced physical assault or abuse, survived a serious motor vehicle accident, survived the 9/11 terrorist attacks on the World Trade Center, or witnessed a shooting. Six (46%) patients were on stable doses of psychotropic medications throughout the study. One participant (male, age = 64 years) was exited early (after the first three infusions) due to inappropriate behavior with study staff.

The combined treatment was associated with large-magnitude improvement in PTSD symptoms from baseline [CAPS-5 score = 41.6 (6.2)] to 12 weeks [CAPS-5 score = 20.7 (14.8)], Cohen's $d = 1.6$ (95% CI = 1.0 – 2.2, $p < 0.001$). One week after completion of the combined treatment, 10 (77%) participants were treatment responders. At 12 weeks, 9 (69%) participants maintained treatment response, and 6 (46%) no longer met DSM-5 diagnostic criteria for PTSD.

Conclusions: This preliminary study provides the first evidence of large-magnitude PTSD symptom improvement following a three-week novel combined treatment in patients with high-moderate to severe chronic PTSD, with the majority of patients remaining clinically improved three months following treatment start. A hypothesized synergistic effect of ketamine infusions combined with WET, a highly scalable, evidence-based, brief psychotherapy, in patients with more severe forms of the disorder warrants further study with a randomized controlled trial.

Keywords: PTSD, Clinical Trial, IV- Ketamine, Exposure therapy

Disclosure: I am named co-inventor on a patent application in the US, and several issued patents outside the US filed by the Icahn School of Medicine at Mount Sinai related to the use of ketamine for the treatment of PTSD. This intellectual property has not been licensed: Patent (Self).

P26. Methylone for the Treatment for PTSD: Initial Results From an Open-Label Study (IMPACT-1)

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Background: Post-traumatic stress disorder (PTSD) is a debilitating psychiatric illness affecting 12 million adults in the United States in a given year. Current treatment options for PTSD, psychotherapy or SSRIs, have limited effectiveness. Non-hallucinogenic compounds with rapid and sustained therapeutic benefits may be clinically useful as they may be more accessible to patients, compared to classical psychedelics. Methylone is a non-hallucinogenic neuroplastogen and a beta-ketone analog of MDMA. Although methylone targets a similar set of receptors as MDMA (e.g., SERT, NET, and DAT), the difference in affinity preference and its lack of off-target effects relative to MDMA produces a distinctive pharmacological and subjective effect. Studies on healthy volunteers have demonstrated that methylone produces similar, yet notably less intense, subjective effects compared to MDMA. Furthermore, a retrospective case series of

patients with PTSD and depression treated with methylone has shown positive clinical effects. Methylone has also been found to have fast-acting, robust, and long-lasting anxiolytic and antidepressant-like activity in preclinical studies. As such, methylone is currently being developed as a potential treatment for PTSD.

Methods: The IMPACT-1 study is a multi-center, two-part clinical trial: Part A entails an open-label evaluation involving approximately 15 participants with PTSD, while Part B involves a randomized, placebo-controlled evaluation with approximately 40 to 64 participants. Eligible participants are aged 18-65 with a confirmed diagnosis of severe PTSD using DSM-5 criteria, and must have failed at least 1 prior treatment (pharmacotherapy and/or psychotherapy) for PTSD.

In Part A, participants are treated with 4 doses of methylone, administered once a week for 4 weeks. Throughout each dosing session, participants are provided non-directive psychological support by one trained mental health practitioner. Following the 4-week treatment period, participants were followed for an additional 6 weeks to evaluate the durability of the therapeutic effect. Efficacy is evaluated on the CAPS-5, as well as other outcome measures. Safety is assessed by monitoring adverse events, vital signs, and C-SSRS.

Results: Enrollment into Part A is nearly complete, and the study's results will be presented at the meeting. Preliminary efficacy results are highly encouraging, current participants have reported a rapid improvement in PTSD symptoms on the CAPS-5, including several participants achieving remission. To date, treatment with methylone has been well-tolerated, with an overall adverse event profile typical of a psychostimulant with no increased risk of suicidal ideation or behavior.

Conclusions: Methylone is currently being investigated as a therapeutic intervention for PTSD, with promising initial results from the IMPACT-1 study.

Keywords: Methylone, PTSD, Transcend Therapeutics, Human Clinical Trial

Disclosure: Transcend Therapeutics: Employee, Stock / Equity (Self).

P27. Synergistic Transcranial Direct Current Stimulation and Virtual Reality for PTSD Modulates Ventromedial Prefrontal Cortex-Basolateral Amygdala Functional Connectivity

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Background: Posttraumatic Stress Disorder (PTSD) is a highly prevalent and debilitating disorder, disproportionally affecting returning Veterans. Despite its severity, many individuals with PTSD fail to adequately respond to current first-line treatments, including exposure-based cognitive behavioral therapy and pharmacotherapy. In recent years, non-invasive brain stimulation approaches have emerged as potential treatments that can target relevant neural structures thought to underlie psychiatric symptoms. Of these, transcranial direct current stimulation (tDCS) provides a low level of electrical stimulation that biases neurons towards or against depolarization allowing modulation of PTSD-relevant cognitive processes when combined with other therapeutic interventions. A convergent body of human and non-human studies suggests that the ventromedial prefrontal cortex (vmPFC) down-regulates amygdala-dependent threat responding. Disruption in this down-regulation is a core component of PTSD neurobiology, supported by functional neuroimaging studies of PTSD patients, who commonly exhibit hypoactivity in vmPFC and amygdala hyperactivity. Developing treatments that target these regions to correct the disrupted connectivity is the next step in reducing symptoms and improving quality of life in Veterans with PTSD. To do so, we conducted a double-blind, randomized, sham-controlled clinical trial of tDCS combined with virtual reality exposure for PTSD (NCT03372460).

Methods: 33 Veterans with PTSD (Mage = 45.24; SDage = 10.50; Nfemale = 3) received either 2 mA vmPFC-targeted tDCS with the anode placed over EEG coordinate Fp1 and cathode over P08 (n = 14) or sham tDCS (n = 19) during six, 25-minute sessions of warzone virtual reality exposure, delivered over 2-3 weeks using the Bravemind Virtually Better platform. PTSD symptom severity was assessed using the PTSD Checklist for DSM-5 (PCL-5) and Clinician-Administered PTSD Scale for DSM-5 (CAPS-5) at pre-treatment baseline, mid-treatment, end-of-treatment, and at one- and three-months follow-up. Twelve minutes of resting state functional connectivity (rsFC) was acquired at baseline and at the end of treatment. rsFC data were preprocessed using the fMRIprep (docker v.4.20.1) standard preprocessing pipeline. Major steps include: 1) realignment, 2) slice time correction, 3) registration to MNI-152 volumetric and FreeSurfer spaces, and 4) spatial smoothing with a 6 mm full-width half-max (FWHM) Gaussian kernel using SPM12. rsFC data were analyzed using CONN v22.a. ROI-to-ROI analyses were performed with the vmPFC and basolateral amygdala as a priori seeds.

Results: Following treatment, there was a significant relationship between changes in vmPFC to left basolateral amygdala functional connectivity and PTSD symptom improvement on both the PCL-5 ($r = -0.47$, $p = 0.006$) and CAPS-5 ($r = -0.34$, $p = 0.05$) across groups. Furthermore, greater connectivity between vmPFC and left BLA at baseline predicted better treatment response following treatment (PCL-5: $r = 0.47$, $p = 0.005$; CAPS-5: $r = 0.44$, $p = 0.011$) and also at the 1-month follow-up (PCL-5: $r = 0.52$, $p = 0.003$; CAPS-5: $r = 0.39$, $p = 0.029$, $n = 31$).

Conclusions: These results are consistent with prior studies demonstrating altered vmPFC-basolateral amygdala connectivity following non-invasive brain stimulation for chronic PTSD, underscoring that this relationship can be modulated through treatment, and that it is associated with clinical improvement. Interestingly, the current results identify increased baseline connectivity between vmPFC and left basolateral amygdala as a potential biomarker for PTSD treatment response. These data continue to indicate promise of non-invasive brain stimulation as a novel precision treatment for PTSD with outcomes that are generally consistent with known neurobiological models of PTSD, and thus can provide critical new information on how to develop innovative future treatments.

Keywords: Posttraumatic Stress Disorder, Virtual Reality, Transcranial Direct Current Stimulation (tDCS), Resting State Functional Connectivity, Combat PTSD

Disclosure: Nothing to disclose.

P28. Subjective Units of Distress Scale (SUDS) is a Psychometrically Valid and Reliable Outcome Measure for the Assessment of Anxiety in Social Anxiety Disorder (SAD) Clinical Trials

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Background: Subjective Units of Distress Scale (SUDS) is a self-reported Visual Analog Scale (VAS) that measures the subjective intensity of disturbance or distress currently experienced by an

individual and is a standard instrument for rating social, anticipatory, and performance anxiety in patients with SAD during role-playing situations. The individual self-assesses where they are on the scale, between 0 to 100, with higher numbers indicating more anxiety or greater discomfort (Wolpe, 1969).

SUDS (or similar VAS scales) has been used to assess the severity of anticipatory and performance-related anxiety in patients in industry-sponsored and academic studies using a public speaking challenge to evaluate the potential treatment effect of both psychotherapy and pharmacotherapy interventions. Specifically, SUDS was used as the primary endpoint in the acute dosing fasedienol Phase 2 clinical trial (Liebowitz et al., 2014) and two recent Phase 3 clinical trials (NCT04754802 and NCT05011396) in SAD patients. Other examples of the use of SUDS in a public speaking paradigm in SAD patients include evaluation of cognitive behavior therapy (CBT) and phenelzine (Heimberg et al., 1998), diazepam (Helmus et al., 2005), cannabidiol (Bergamaschi et al., 2011), D-cycloserine to facilitate CBT (Rodebaugh et al., 2013), and CBT (Morrison et al., 2016).

VAS, in general, have been used extensively in drug development and registrational trials. They are used when patient input about subjective symptoms and changes in the severity of symptoms are required, and examples are found in indications such as hereditary angioedema and acute and chronic pain, whether due to ankylosing spondylitis, neuropathic, post-surgical, or dry eye disease, etc. (CDER COA Compendium; U.S. FDA, 2021).

Methods: Data were collected during a Phase 2, randomized, double-blind, placebo-controlled, 3-arm, parallel-group study comparing the effects of single acute doses of BNC210 and placebo in participants with SAD (PREVAIL; NCT05193409). SUDS, State-Trait Anxiety Inventory (STAI), and Self-Statements During Public Speaking (SSPS) data were collected pre-dose (baseline) and post-dose, during a public speaking challenge (capturing anticipatory and performance anxiety) and post-challenge. In this study, BNC210 demonstrated a reduction of anxiety compared to placebo in a public speaking challenge using SUDS. The reduction in anxiety with BNC210 was observed during the speech preparation phase (anticipation phase) and the speech task itself (performance phase). Converging findings favoring BNC210 were observed in the STAI, a self-reported questionnaire with 20 anxiety-related questions.

Using data generated from the PREVAIL study, the psychometric properties of the SUDS were evaluated to assess the SUDS's fit for measuring the reduction in self-reported anxiety severity provoked by a public speaking challenge in patients with SAD. For the assessment of the psychometric properties of the SUDS, the following parameters were evaluated:

- Reliability: Pearson correlations, concordance correlation coefficient, intraclass correlation coefficient, and Bland-Altman limits of agreement using the SUDS at 20- and 30-minutes post-challenge (public speaking task)
- Validity: Pearson correlations between the SUDS at baseline and the following scales at baseline: STAI-State and SSPS-N (negative subscale), and the results of a Kruskal-Wallis test that the means of the SUDS are the same between groups defined by tertiles of the STAI-State and the SSPS-N.

Results: No floor or ceiling effects were observed in the use of the SUDS. At all measured time points, the full range of the scale was used.

- The reliability of the SUDS at 20- and 30-minutes post-challenge was very high, with a Pearson's correlation coefficient of 0.91 in both the placebo and full populations, respectively. The concordance and intraclass correlation coefficients were also high, with values of 0.84 and 0.86 in the placebo and full populations, respectively. The Bland-Altman plots also demonstrated strong agreement between the two-time points.
- Baseline SUDS scores demonstrated good convergent validity when compared with STAI-State scores; all Pearson correlation coefficients were statistically significant, and the strongest correlations with the SUDS were the following: STAI-State total score $r = 0.63$; Q12 (I feel nervous) $r = 0.55$; Q5 (I feel at ease) $r = -0.54$; Q13 (I am jittery) $r = 0.49$; Q9 (I feel frightened) $r = 0.49$; Q15 (I am relaxed) $r = -0.49$; and Q6 (I feel upset) $r = 0.47$. The STAI also demonstrated strong known-groups validity with the mean value of the SUDS within each tertile of the STAI-State total score tracking with the SUDS (mean SUDS [standard deviation] of 24.1 [15.1], 34.5 [15.8], and 52.9 [20.8] in the lower, middle, and upper tertiles).

Conclusions: This analysis concluded that SUDS is a psychometrically valid, sensitive, and reliable tool for the evaluation of social anxiety across multiple time points. Results demonstrated strong convergent validity of baseline SUDS and STAI-State, and the absence of appreciable floor or ceiling effects indicates that the SUDS is highly sensitive to change over the entirety of its 100-point range. Additional data on the psychometric characteristics of the SUDS will be collected during Phase 3 studies with BNC210 in SAD.

Keywords: Social Anxiety Disorder, Clinical Trials Methodology, Novel Assessment Tools for Clinical Trials

Disclosure: Nothing to disclose.

P29. MDMA's Impact on Fear Extinction and Subjective and Observer Rated Effects in Healthy Adults

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Background: Psychiatry is in the midst of a "psychedelic renaissance" (Sessa, 2012), in which emerging evidence indicates the promise of psychedelic-assisted therapy for a range of conditions. 3,4-methylenedioxymethamphetamine (MDMA)-assisted therapy for posttraumatic stress disorder (PTSD) has demonstrated strong promise (Mitchell et al., 2023) and may receive FDA approval soon. It is important to investigate MDMA's effects to inform therapeutic mechanisms optimization of MDMA treatment models. Fear conditioning and extinction are validated translational models of fear-related symptoms, and rodent models support that MDMA enhances fear extinction retention (Young et al., 2017).

Methods: In a randomized placebo-controlled, two-group, trial in a sample of healthy adults (NCT0318176), we investigated the impact of MDMA on fear extinction retention, subjective effects (i.e., self-reported subjective effects and Mystical Experiences Questionnaire; MacLean et al., 2012), and observer rated drug effects (Rush et al., 1998). Participants ($N = 34$) completed an experimental paradigm including fear acquisition day 1, fear extinction day 2, 2 hours following MDMA or placebo administration, and extinction recall 48 hours later. Participants ($N = 34$; 70.6% male) were randomly assigned in 1:1 manner to 100 mg MDMA or placebo.

Results: Repeated (measures analysis of variance (RM-ANOVA) results found extinction learning in the combined sample NA; $F(3, 96) = 21.50$, $p < 0.001$) with no significant differences across group, and a return of fear at extinction recall in the combined sample ($F(1, 32) = 26.87$, $p < 0.001$) with no differences across groups. Participants were then classifying as extinction retainers or

non-retainers (i.e., increase in fear-potentiated startle of < 0 (i.e. no increase), and results indicate that there were significantly more retainers in the MDMA group (6/17) as compared to the placebo group (0/17; $\chi 2 = 7.29$, $p = 0.007$). The MDMA group reported significantly greater mystical experiences, including positive mood, transcendence of time and space, and ineffability, greater subjective drug effects, and greater observer rated drug effects, with effects primarily driven by the observer estimating the strength of drug effect, and the participant appearing stimulated.

Conclusions: These finding suggest that MDMA may enhance retention of extinction retention in healthy adults; research in PTSD samples is needed. This is notable as extinction is the foundation of Prolonged Exposure, a gold standard PTSD treatment, indicating the potential promise of MDMA in combination with exposure-based therapy for PTSD, and subjective and observer related effects indicates potential drug effects relevant to therapeutic processes.

Keywords: PTSD, Extinction Recall, MDMA

Disclosure: COMPASS Pathways: Consultant (Self).

P30. Effective Connectivity Using a Naturalistic in-Scanner Worry Induction Task in Late-Life

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Background: Severe worry is a core component of multiple anxiety disorders. It is highly prevalent in depressive disorders and is associated with significant morbidity and mortality. Thus, understanding the neural basis of worry is critical in late life. Previously, we have reported on the neural activation of worry induction and reappraisal. In this study, we investigate task-based effective connectivity using a naturalistic worry induction functional magnetic resonance imaging (fMRI) task.

Methods: We collected demographic, clinical, and fMRI data from 112 participants aged 50 and older with varying worry severity and clinical comorbidity (depression and anxiety disorders). Participants completed a worry induction and reappraisal fMRI task with personalized scripts and rated in-scanner worry severity. We calculated context-dependent connectivity in neutral, worry, and reappraisal conditions with generalized psychophysiological interactions (gPPI). We computed voxel-wise connectivity of the subgenual anterior cingulate cortex (ACC), dorsal ACC, and left and right amygdalae, and compared across conditions with paired t-tests. We identified significant clusters with SnPM (10,000 permutations, cluster-forming threshold of $p = 0.001$, and cluster-wise inference to control family-wise error rate at 0.05) and assessed for association with in-scanner worry severity with linear regression, controlling for age, sex, race, education, and cumulative illness burden.

Results: All four seeds showed robust context-dependent connectivity. In worry compared to neutral: subgenual ACC connectivity to the default mode network (DMN) was greater; dorsal ACC connectivity to bilateral insula, motor, and occipital cortices was lower, while connectivity to left dorsolateral prefrontal cortex (dlPFC) and angular/supramarginal gyri was greater; and left amygdala to posterior cingulate cortex (PCC) connectivity was greater. In reappraisal compared to neutral: subgenual ACC connectivity to key regions of the DMN and subcortical regions including the thalamus and hippocampus was greater; and left amygdala connectivity to the bilateral dlPFC and left inferior parietal lobe was lower. In worry compared to reappraisal: subgenual ACC connectivity to the right insula and left executive regions was greater; and left amygdala connectivity to posterior DMN and prefrontal regions was greater. The context-dependent connectivity of the left amygdala was positively correlated with in-scanner worry ratings.

Conclusions: Worry induction and reappraisal alter the connectivity in key regions of the default mode, executive control, and salience networks. In general, DMN connectivity is robustly increased during worry, while connectivity to key regions of the executive control network is increased during both worry and reappraisal, indicating a complex interplay of implicit and explicit emotional regulation during the worry process. These findings could inform targets for neuromodulatory treatment of severe worry in older adults.

Keywords: Worry, Anxiety, Effective Connectivity, fMRI, Network Neuroscience

Disclosure: Nothing to disclose.

P31. Trauma Exposure and Attentional Bias: Unraveling Reward and Contextual Cue Processing in a Virtual Reality Paradigm

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Background: Trauma-related psychopathology often leads to diminished pursuit and anticipation of rewards. However, the impact of trauma exposure on attention allocation to conditioned reward cues remains poorly understood. We utilized eye-tracking in a virtual reality (VR) reward conditioning task to investigate how trauma exposure affects attention to rewarding versus contextual cues. This research offers valuable insights into the cognitive processes involved in reward processing in trauma-affected individuals.

Methods: We recruited trauma-exposed (TE; $n = 10$) and non-trauma-exposed (HC; $n = 6$) participants for the study. Both groups completed an eye-tracking task both before and after engaging in the VR reward conditioning task. In the eye-tracking task, participants were presented with matrices of pictures representing the conditioned reward stimuli (MN) and the surrounding environment (CX) from the VR task. We quantified attentional bias by using a 2x2 ANOVA (group; block) comparing the total dwell time and number of first fixations on each cue type. Both sexes were used in the study and use as covariates in the analysis.

Results: Although not reaching statistical significance, our preliminary findings indicate that non-trauma-exposed participants (HCs) demonstrated a slight increase in the percentage of first fixations on CX images from pre-task to post-task. In contrast, trauma-exposed participants (TEs) exhibited a slight decrease in this measure during the same period. Additionally, we observed that HCs maintained a higher percentage of total dwell time on CX images compared to TEs at both pre-task and post-task stages, but there was no significant increase in total dwell time on CX images in either group from pre-task to post-task.

Conclusions: In light of our preliminary findings, it appears that trauma exposure might influence attentional bias, directing focus towards valenced cues while diverting attention away from contextual cues during initial environmental scanning. This distinctive attentional pattern towards valenced cues could potentially serve as a recognizable marker of trauma exposure, presenting an opportunity for targeted treatment interventions. Further research and validation are warranted to harness these insights for the development of more effective and personalized approaches in addressing trauma-related psychopathology.

Keywords: Trauma Exposure, Virtual Reality, Attentional Bias, Reward Processing

Disclosure: Nothing to disclose.

P32. General Functional Connectivity and Everyday Negative Affect Instability in Internalizing Psychopathology

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Background: Internalizing psychopathology may be characterized by negative affect instability, yet there is substantial heterogeneity in these disorders. The salience network (SN) is involved in detecting and responding to emotional information. On the other hand, the central executive network (CEN) is associated with goal-driven, cognitive control of emotional processing. Individual differences in the intrinsic connectivity of the SN and CEN might explain why affective instability is a more prominent feature for some individuals with internalizing psychopathology than others.

Methods: Participants with internalizing symptoms (N = 54; 38 female) completed symptom measures, as well as resting-state and passive picture viewing tasks performed in an fMRI scanner (32 minutes of fMRI data in total). Following their lab visits, participants completed 10 days of ecological momentary assessment on their smartphones, to assess real-time negative affect instability. Principal components analysis was used to quantify symptom load across measures. General functional connectivity (GFC) provided a reliable alternative to traditional intrinsic functional connectivity. GFC of the CEN and SN was examined as a moderator of associations between internalizing symptoms and negative affective instability in everyday life.

Results: Participants with higher levels of internalizing symptoms were characterized by greater negative affect instability, ($\beta = .324$, $p = .015$), only when SN connectivity was strong (internalizing symptoms X SN; $\beta = .303$, $p = .023$). In addition, higher levels of internalizing psychopathology were associated with higher negative affect instability ($\beta = .340$, $p = .010$), only when CEN connectivity was weak (internalizing symptoms X CEN; $\beta = -.380$, $p = .004$).

Conclusions: Stronger connectivity in the SN is associated with enhanced emotional reactivity, which may amplify fluctuations in negative affect in internalizing psychopathology. Weaker connectivity in the CEN is associated with deficits in cognitive control over emotion, which may cause difficulty in regulating mood among individuals with higher internalizing psychopathology. Differences in SN and CEN connectivity might explain heterogeneity in negative affect instability in internalizing psychopathology.

Keywords: Internalizing Disorders, Ecological Momentary Assessment, Affective Instability, fMRI Functional Connectivity, Moderators

Disclosure: Nothing to disclose.

P33. Intergenerational Transmission of the Risk of Post-Traumatic Stress Disorder: Preliminary Data on Observational Fear Learning in Children of Trauma-Exposed Mothers

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Background: Children of mothers diagnosed with PTSD have a higher risk of developing this disorder. By virtue of their traumatic symptoms, adults with PTSD have dysregulated fear levels and maladaptive fear regulation strategies. Moreover, parents with PTSD report that their children tend to reproduce some of their PTSD-related behaviors, notably hypervigilance. It has been proposed that these learned behaviors promote the intergenerational transmission of PTSD. This study examined observational fear learning and fear regulation patterns in healthy children as a function of their mother's trauma exposure history.

Methods: To date, 106 non-trauma-exposed healthy children aged 8-12 years old (43 boys) and their mothers with varying levels of trauma exposure (60 children for whom the mother had no history of trauma) have been recruited. On Day 1, the mother is filmed while being exposed to two different stimuli, where one stimulus is reinforced with electrical stimulation (conditioned stimulus; CS+) and the other is not paired with a shock (CS-). This procedure is then shown to the child. Thereafter, the child is directly exposed to the stimuli and told that he/she may receive a shock, although no shock is delivered (test of acquisition). As the presentation of the stimuli continues without electrical stimulation, a process of fear extinction occurs. The next day (Day 2), the child is presented once again with the same stimuli. For both days, skin conductance responses (SCRs) are recorded for each stimulus presentation for the child and mother.

Results: A Stimulus X Group analysis revealed that trauma exposure did not modulate SCRs in the mothers during conditioning, all ps > .05. In children, a Stimulus X Group X Sex interaction was found during the test of acquisition, $p = .019$, $\eta p2 = .053$. Post-hoc analyses were conducted for each sex to decompose this interaction. In boys, the Stimulus X Group interaction did not reach significance, $F(1,41) = 1.027$, $p = .317$, $\eta p2 = .024$. In girls, a Stimulus X Group interaction was found, $F(1,61) = 6.628$, $p = .012$, $\eta p2 = .098$. Independent sample t-tests revealed that girls of trauma-exposed mothers tended to have higher SCRs to the CS+ relative to girls of non-trauma-exposed mothers, $t(61) = 1.782$, $p = .08$. No difference was found between the two groups for the CS-, $t(61) = 0.641$, $p = .524$. At the end of extinction, a Stimulus X Group X Sex ANOVA was conducted. The analysis did not reveal any significant main effects nor interactions, all ps > .05. When investigating fear recovery at Day 2, a main effect of Group was found, $p = .065$, $\eta p2 = .034$, with children in trauma-exposed dyads showing a stronger increase in fear levels relative to their counterparts (irrespective of CS type).

Conclusions: Healthy young girls of trauma-exposed mothers may be more prone to acquire stronger fear responses for threat-related stimuli when learning from their mothers. Moreover, children of trauma-exposed mothers exhibit higher fear recovery, which may highlight a certain physiological vulnerability to fear-related disorders.

Keywords: Observational Fear Learning, PTSD, Mother-Child Dyads, Skin Conductance Responses, Sex Differences

Disclosure: Nothing to disclose.

P34. Hot and Cold: Neural Function Underlying Emotional and Non-Emotional Inhibitory Control and Associated Anxiety and Impulsivity Symptoms

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Background: Clinical symptoms of anxiety and impulsivity differentially interact with various inhibitory control processes (e.g., emotional interference and motor inhibition). Regions of the

prefrontal cortex such as the anterior cingulate cortex (ACC) and dorsolateral prefrontal cortex (dlPFC) are central to these processes. Few studies have specifically interrogated the overlap in patterns of activation related to both emotional and non-emotional inhibitory control. In this presentation, we summarize results from a study examining PFC function associated with emotional interference and non-emotional motor inhibition, and how these processes relate to symptoms of anxiety and impulsivity.

Methods: Participants (n = 2264; M age = 10.02; SD age = 0.62) were early adolescents from the Adolescent Brain Cognitive Development (ABCD) dataset. fMRI scanning was conducted during the Emotional N-Back (EN-Back) Task and Stop Signal Task (SST). Participants also completed measures of anxiety (i.e., CBCL) and impulsivity (i.e., UPPS-P, BIS/BAS). Patterns of PFC activation were identified for the emotion contrast on the Emotional N-Back (EN-Back) Task and the failed inhibition contrast on the Stop Signal Task (SST). We used structural equation models examine relationships between the unique patterns of PFC activation on each task and clinical symptoms.

Results: A conjunction analysis revealed activation specific to the EN-Back Task in the middle frontal gyrus (MFG) and specific to the SST in the ACC and dlPFC, as defined by the Desikan-Killiany-Tourville Atlas. We found positive relationships between anxiety and both impulsivity (b = 0.06, p = 0.01) and MFG activation during the EN-Back Task (b = 0.07, p = 0.001). There were no significant relationships between impulsivity and neural function on either task.

Conclusions: These results reveal commonalities and distinctions in PFC function associated with emotional and non-emotional inhibitory control. We also observed differences in how anxiety versus impulsivity related to neural function during inhibitory control. This work suggests that nuances in the measurement of impulsive constructs may play a role in the mixed findings surrounding these relationships.

Keywords: Anxiety, Impulsivity, ABCD Study, Inhibitory Control

Disclosure: Nothing to disclose.

P35. Differences in the Principal Gradient of Hippocampus Functional Connectivity Track Individual Differences in Sensory-Perceptual Properties of Trauma-Related Intrusive Memories

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Background: Trauma-related intrusive memories (TR-IMs) are prevalent among individuals exposed to trauma and are leading predictors of transdiagnostic post-traumatic sequelae. Conceptual models of TR-IMs highlight their sensory vividness and perceptual fragmentation as phenomenological properties distinguishing them from other forms of episodic memory. However, the extent to which TR-IMs are truly unique in these sensory-perceptual properties remains inconclusive, suggesting significant heterogeneity in the manifestation of TR-IMs.

This heterogeneity alludes to individual differences in the neural correlates of TR-IMs. Biological models of TR-IMs heavily implicate the hippocampus (HPC) given its critical role in episodic memory. The HPC demonstrates a functional specialization along its long (anterior-posterior) axis to support the different phenomenological properties of memory, with anterior regions contributing to general reconstruction and posterior regions supporting detailed sensory elaboration. Recent evidence suggests this specialization emerges along a gradient rather than hard, segregated boundaries. This is mirrored in the organization of HPC functional connectivity, which is characterized by a smooth gradient along the long axis between two maximally divergent patterns of connectivity at the anterior and posterior HPC poles. This principal gradient reflects the organizational structure of HPC connectivity that supports the flexible recruitment of different hippocampal-cortical circuits to give rise to the diverse phenomenology of memory. Therefore, it is well-positioned to provide mechanistic insights into the properties of TR-IMs.

In this study, we combined ecological assessments of TR-IMs with functional imaging of the HPC in trauma-exposed adults to test the hypothesis that differences in TR-IM properties are linked to differences in the organization of HPC functional connectivity.

Methods: Ninety-two (92; 76 female) trauma-exposed adults were assessed using the Clinician-Administered PTSD Scale and completed two weeks of ecological momentary assessments (EMA) of TR-IM properties followed by resting-state functional magnetic resonance imaging (rs-fMRI). K-means clustering of EMA data was performed to identify groups of participants demonstrating distinct patterns of TR-IM properties. Individual voxel-wise hippocampal-cortical connectivity matrices were generated from rs-fMRI data and submitted to diffusion map embedding, a dimensionality reduction technique, to compute individual HPC gradients. These gradients reflect a one-dimensional pattern that explains the maximal amount of variance in hippocampal-cortical connectivity patterns across HPC voxels. These gradients were projected back into voxel space to generate individual gradient maps. These maps were submitted to group contrasts in SPM to compare differences between TR-IM groups identified in the K-means clustering. Additionally, individual TR-IM properties were regressed onto the maps to situate them along the gradient.

Results: K-means clustering of TR-IM properties identified two groups (Group 1/2 n's = 46/46) of participants that were maximally separated by differences in fragmentation (Group 1/2 means = 1.24/2.59; t = -3.91, p < 0.001) and visual features (Group 1/2 means = 3.12/1.80; t = 2.80, p = 0.006). The groups did not differ on other clinical or demographic variables.

Diffusion map embedding confirmed a principal gradient of HPC connectivity that followed the long axis, with the two gradient poles residing in the anterior (peak gradient value = -0.11, MNI coordinates = 28, -6, -26) and posterior HPC (peak gradient value = 0.12, MNI = 24, -36, 4), and the transition point (gradient value = 0) positioned in the middle (MNI = 34, -24, -13).

Group differences in these gradients emerged within the poles of the HPC. Group 2 (high fragmentation, low visual features) demonstrated lower absolute gradient values in the anterior (k = 64, peak MNI coordinates = 20, -14, -16; t = -3.23, p = 0.001) and posterior HPC (k = 37, peak = 34, -36, -8; t = -2.54, p = 0.006), suggesting a weaker distinction between anterior and posterior connectivity patterns relative to Group 1. Regression analyses confirmed these group differences were driven by fragmentation and visual features – fragmentation was associated with lower absolute values within the anterior/posterior poles (k = 118/52, peak = 24, -6, -28/26, -34, 4; r = -0.38/-0.35, p's < 0.001) while visual features were associated with higher absolute values (k = 97/81, peak = 34, -10, -22/28, -32, -2; r = 0.39/0.34, p's < 0.001).

Conclusions: K-means clustering revealed distinct groups of trauma-exposed individuals that differed in the sensory-perceptual properties of their TR-IMs, confirming heterogeneity in the phenomenology of trauma intrusions. Examination of the principal gradients of HPC functional connectivity revealed differences between these groups at both ends of the long axis, particularly as a function of the fragmentation and visual features of TR-IMs. These data suggest the organization of functional connectivity along the long axis of the HPC plays a critical role in

the sensory-perceptual integrity of TR-IMs and offers novel mechanistic insights into the heterogeneity of TR-IMs. This positions the functional organization of HPC connectivity as a target for personalized neurotherapeutics in the treatment of trauma-related intrusions.

Keywords: Posttraumatic Stress Disorder, Ecological Momentary Assessment, Memory, Hippocampus, Connectivity Gradients

Disclosure: Nothing to disclose.

P36. Both cTBS and iTBS Increase Anxiety When Delivered to the Right dlPFC in Healthy Volunteers

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Background: The dorsolateral prefrontal cortex (dlPFC) is thought to be a key region involved in the expression and regulation of emotion. Indeed, noninvasive neuromodulation aimed at this site has been used to treat several mental health disorders, most notably depression and anxiety. In common practice, the left dlPFC is targeted for depression, while the right dlPFC is targeted for anxiety. My work focuses on this link between the right dlPFC and anxiety. In previously published work, we showed that continuous theta burst stimulation (cTBS) increases anxiety potentiated startle (APS) in healthy subjects. In the current project, we aimed to contrast these findings by administering intermittent TBS (iTBS) to the same site in a novel cohort.

Methods: We used a double-blinded cross-over design where 28 healthy men and women completed an unpredictable threat task 24 hours after 4 sessions of either active or sham iTBS. Each standard iTBS session included 600 pulses grouped into 10, 2-second trains (5 Hz train of 50 Hz triplicate bursts) separated by an 8-second gap. We used our previously published fMRI and e-field modelling approach to define the right dlPFC target. During each testing session, we administered the neutral, predictable, and unpredictable (NPU) threat task and measured the acoustic startle reflex throughout. Anxiety potentiated startle (U startle > N startle; APS) was our primary outcome measure.

Results: Consistent with our previously published cTBS results, subjects showed greater APS following active compared to sham iTBS ($f(1,27) = 5.1$; $p = 0.032$; eta-squared = 0.16), suggesting that the iTBS increased their anxiety expression. In contrast, subjects rated their anxiety similarly following active compared to sham, suggesting that the startle effect was not due to insufficient blinding.

Conclusions: Together with the previously published cTBS findings, the current results suggest that modulation of right dlPFC circuits leads to increased physiological anxiety in healthy subjects, possibly due to impaired anxiety regulation. These findings are surprising because the right dlPFC is the most common stimulation site for anxiety, suggesting that the right dlPFC circuits targeted for treatment may function differently in anxiety patients.

Keywords: TMS, Theta-Burst Stimulation, Anxiety, Working Memory, Fear-Potentiated Startle

Disclosure: Nothing to disclose.

P37. Resting-State Functional Connectivity Between Right Dorsolateral Prefrontal Cortex and Right Anterior Hippocampus in Relation to Trauma Memory Frequency and Intrusiveness in Posttraumatic Stress Disorder

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Background: Recurrent trauma-related intrusive memories (TR-IMs) are hallmark symptoms of posttraumatic stress disorder (PTSD) and have been associated with social and daily life functioning impairment. Although TR-IMs may represent critical treatment targets, their neural correlates remain partly unknown. Task-based functional magnetic resonance imaging (MRI) studies in healthy and non-PTSD trauma-exposed individuals have demonstrated that voluntary suppression of memory retrieval (i.e., suppression-induced forgetting) recruits top-down inhibitory modulation (i.e., increased anticorrelation) of the right dorsolateral prefrontal cortex (dlPFC) on the right anterior hippocampus (aHPC). This suppression-induced forgetting gradually disrupts memories and makes them less likely to be involuntarily retrieved. This neural mechanism is impaired in PTSD, and a negative association between suppression-induced forgetting deficit and PTSD symptom severity has been reported. Therefore, the deficit in the brain inhibitory control of IMs in PTSD may partly contribute to more frequent and intrusive TR-IMs. However, no study has investigated the relationship of TR-IM properties such as their frequency and intrusiveness with resting-state functional connectivity between the right dlPFC and right aHPC in PTSD. We hypothesized that higher TR-IM frequency and intrusiveness would be associated with weaker resting-state functional connectivity anticorrelation between these regions.

Methods: We enrolled trauma-exposed adults ($N = 92$; mean age: 32.03 (10.23); 83% female; 77% full diagnostic of PTSD; 23% subthreshold PTSD) who experienced a Criterion A trauma event and at least two TR-IMs in the past week. Participants were asked to complete three ecological momentary assessments (EMAs) per day for fourteen consecutive days to assess TR-IM properties. TR-IM frequency was defined as the total number of TR-IMs over the EMA period. TR-IM intrusiveness was rated using a 5-point Likert scale (from not at all out of the blue to completely out of the blue), and we computed the average intrusiveness score over the EMA period. Participants also underwent a 3T MRI scan. We defined two regions of interest (ROIs) based on the Brainnetome atlas, namely the right rostral HPC (i.e., aHPC) and the right dlPFC resulting from the combination of Brodmann area 46 (BA46) and ventral Brodmann area 9/46. We first performed a seed-based functional connectivity analysis using the right aHPC template as seed to identify the voxel within the right dlPFC demonstrating the maximum anticorrelation with the right aHPC for each participant. We then placed a 6-mm radius sphere at the voxel coordinates and conducted an ROI-to-ROI analysis between the right aHPC and right dlPFC. We applied a Poisson regression model to assess the relationship between TR-IM frequency (outcome) and right aHPC-right dlPFC resting-state functional connectivity (predictors), controlling for age, sex, and number of surveys completed. We also used a linear model to investigate the relationship of average TR-IM intrusiveness (outcome) with right aHPC-right dlPFC resting-state functional connectivity (predictors), controlling for age, sex, and number of surveys completed.

Results: The Poisson model showed that TR-IM frequency was not significantly negatively associated with right aHPC-right dlPFC resting-state functional connectivity (incidence rate ratio = 0.99; $p < .589$; 95% confidence interval (CI) = [0.94; 1.03]). Results from multiple linear regression showed a significant positive association of TR-IM intrusiveness with right aHPC-right dlPFC resting-state functional connectivity (weaker anticorrelation; beta = 0.20; $p = .028$; 95% CI = [0.02; 0.38]).

Conclusions: Our findings indicate that resting-state functional connectivity between the right dlPFC and right aHPC may represent a biomarker of greater intrusiveness of TR-IMs in PTSD. As hypothesized, higher average TR-IM intrusiveness was significantly associated with a weaker anticorrelation between the right aHPC and right dlPFC. Contrary to our hypothesis, higher TR-IM frequency was not significantly associated with resting-state functional connectivity between the right aHPC and right dlPFC. These novel findings shed light on the neural correlates of TR-IM intrusiveness and suggest that the strength of the negative coupling between the right aHPC and right dlPFC involved in suppression-induced forgetting may contribute to the perceived intrusiveness of TR-IMs in PTSD patients.

Keywords: Posttraumatic Stress Disorder, Traumatic Memories, Resting-State fMRI, Ecological Momentary Assessment, Hippocampal-Prefrontal

Disclosure: Nothing to disclose.

P38. Psychological Distress and Stress Hormones During the Pandemic: Do Mother and Child Influence Each Other?

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Background: During the COVID-19 pandemic, some children reported increased psychological distress (Panchal et al., 2021). It has been suggested that parents may have played an important role in modulating their own children's psychological distress during the pandemic. Indeed, several studies have shown that the parent's distress was related to their child's distress (Hanetz-Gamliel et al., 2021; Romero et al., 2020; Spinelli et al., 2020; Zhang et al., 2022). However, most of these studies are cross-sectional and therefore do not allow to study the potential temporal dynamics between a parent's distress and his/her child's distress levels. Moreover, studies have found that pandemic-related stressors (e.g., social distancing) were not only associated with psychological distress, but also with increased levels of cortisol, a major stress hormone, in children (Hasting et al., 2021; Perry et al., 2022; Taylor et al., 2022). Parents can also influence their children's cortisol levels (Ouellette et al., 2015; Lupien et al., 2000). Within the context of the pandemic, a study found an association between the mother's and her child's cortisol levels and showed that higher maternal cortisol levels predicted greater child's internalizing symptoms (Perry et al., 2022). However, the impact of pandemic-related maternal distress on children's cortisol levels remains unknown. Furthermore, for both psychological distress and stress hormones, the influence of the child on the mother has rarely been studied. The present study pursued three principal objectives: 1) explore the role of maternal distress on her child's distress levels longitudinally during the COVID-19 pandemic; 2) investigate the influence of maternal distress on her child's cortisol levels and 3) examine the moderating role of maternal distress on the association between mother-child cortisol levels. Moreover, for the three objectives, we aimed to investigate the reverse order, i.e., the influence of the child's distress on the mother's distress and cortisol levels, as well as the interaction with the child's sex.

Methods: Fifty-one healthy mother-child dyads (children aged 9 to 14 years old, 59% girls) participated in the study and 44 provided a 3-cm hair sample in June 2020. As hair grows one centimeter per month (Stalder et al., 2017), we assessed retroactive hair cortisol concentrations (HCC) during the first three months of the pandemic in Quebec, Canada (March-May 2020). The mothers' post-traumatic stress (PTS), depressive, anxious, and stress symptoms were assessed every three months between June 2020 (T1) and March 2021 (T4) via the Impact of Events Scale – Revised and the Depression, Anxiety and Stress Scale. The children's PTS, anxiety, and depressive symptoms were measured at the same timepoints using the Children's Revised Impact of Events Scale, the State-Trait Anxiety Inventory for Children (State Form), and the Children's Depression Inventory.

Results: Multilevel analyses revealed that the mother's symptoms did not influence the child's symptoms, and the child's symptoms did not influence the mother's symptoms. When examining the impact of the mother's symptoms on her child's distress at the subsequent time (and vice-versa), interesting results emerged. Maternal anxiety symptoms negatively predicted children's PTS symptoms at the next timepoint ($B = -0.41$, $t(72) = -2.00$, $p = .050$), while controlling for other maternal symptoms. The child's anxiety symptoms predicted the mother's depressive symptoms at the next timepoint ($B = 0.40$, $t(74) = 2.45$, $p = 0.017$), while controlling for the child's other distress symptoms. Hierarchical regressions showed that maternal stress symptoms at T1 (June 2020) were positively associated with children's HCC ($B = 0.51$, $p = .003$), while controlling for the child's distress and other maternal distress symptoms ($F(8, 28) = 2.71$, $p = .024$, adjusted $R2 = 0.27$). The child's distress at T1 did not influence the mother's HCC. A multiple regression indicated that maternal stress symptoms at T1 moderated the association between the mother's and the child's HCC ($F(7, 26) = 3.96$, $p = .047$). Specifically, this association was only present when maternal stress was high (+1 SD) ($B = 0.37$, $p = .018$). However, when the mother's other symptoms were considered, this effect fell out of significance. The child's distress had no impact on the mother-child HCC association.

Conclusions: Our findings serve as preliminary evidence for the influence of maternal physiological and psychological stress on her child's physiology during the pandemic. Furthermore, our results suggest that mother and child can influence each other's distress during a lasting stressful event (e.g., pandemic). Importantly, this result points to the importance of considering distress longitudinally as dynamics between the mother's and the child's distress could evolve over time. Finally, our study highlights the importance of considering the mother-child dyad in future strategies to promote well-being during future pandemics.

Keywords: COVID-19 Pandemic, Mother-Child Dyads, Psychological Distress, Cortisol

Disclosure: Nothing to disclose.

P39. Brain State Characteristics During Movie-Watching are Related to Generalized Anxiety Symptoms in Children

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Background: Anxiety is associated with lack of neural habituation to controlled negative and ambiguous stimuli. Less is known about how anxiety alters complex, naturalistic emotion processing. Recent work has demonstrated that brain states during movie-watching represent a combination of exogenous (i.e., movie content) and endogenous (i.e., feeling states) factors. Characterizing how brain state characteristics differ in high anxiety children could therefore lend insight to their day-to-day experiences, linking laboratory observations to real-world emotional functioning. The aim of this study is to examine: 1) how brain states shift during emotionally evocative videos; 2) how individual differences in anxiety are related to brain state maintenance and shifting; and 3) if there are developmental

differences in these associations across childhood and early adolescence.

Methods: We characterized brain states in a large sample of 740 5-to-15-year-old children while they watched two emotionally-evocative videos. Data were from the Healthy Brain Network Biobank. Data were split into discovery and replication datasets for both model fitting and follow-up univariate analyses. We trained a Hidden Markov Model on the temporally concatenated and network-averaged discovery dataset for 2-15 states. Model fit was assessed on the replication dataset, and scree plots of fit indices were inspected to determine the optimal number of states. The final model was next applied to each individual's dataset for state classification. Time spent in each state and moment-to-moment probability of being in each state was extracted for further analysis. Videos were annotated for emotion-specific and emotion non-specific information using the EmoCodes system. Group level state probabilities were compared against the emotion-specific content using correlations. Self-reported anxiety symptoms were assessed using the screen for anxiety related disorders (SCARED). Percent time spent in each state across the video, during peaks in negative content, and right before and after negative content peaks was correlated with the generalized anxiety and social anxiety subscales, covarying age, sex, and mean motion.

Results: We identified 3 brain states across the sample which were each characterized by unique cognitive network activation patterns. Brain state 1 had increased ventral attention (VAN), visual (VIS), auditory (AUD), and default mode (DMN) activation and decreased dorsal attention (DAN), cingulo-opercular (CON) and frontopa xrietal (FPN) activation. State 2 was characterized by increased AUD and CON activation and decreased DAN and VAN activation. State 3 was characterized by increased DAN and decreased AUD and DMN. Across the sample, mean percent likelihood of being state 1 was associated with increased negative content in the videos ($r(739) = 0.42$, $p < 0.001$). Self-reported generalized anxiety scores were positively associated with the percent of time that each individual spent in state 1 ($r(619) = 0.14$, $p = 0.002$) and negatively with percent of time spent in state 2 ($r(619) = -0.14$, $p = 0.004$) when negative emotions were on the screen. No associations were found for social anxiety symptoms.

Conclusions: Our findings replicate and extend previous work that found sustained activation to negative stimuli in individuals with anxiety symptoms. Specifically, we found that increased generalized anxiety was associated with spending more time in a brain state with increased sensory, DMN, and VAN activation, which are associated with self-thinking and stimulus-driven attention respectively. Based on the activation profiles of state 1 versus state 2, it is possible that children high in generalized anxiety are more immersed in negative emotions when they are presented, making it more difficult to disengage or self-regulate in the moment. Further work is needed to link concurrent affect to emotion processing in children with anxiety.

Keywords: Emotion Circuitry, Generalized Anxiety Disorder, Computational Neuroscience

Disclosure: Nothing to disclose.

P40. Connectome Predictive Modelling: A Robust and Reproducible Marker of PTSD Symptom Severity

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Background: Posttraumatic stress disorder (PTSD) is a debilitating disorder with just two Food and Drug Administration-approved treatments, in part due to innumerable failed psychiatric drug trials. The first step of drug development in the context of excessive Phase II psychiatric drug trials is developing robust biomarkers for PTSD. There is increasing acknowledgement that coordinated patterns of altered brain activity may be implicated in PTSD, making it essential to study such multivariate patterns. To do so, we used data-driven approach that combines network restricted strength and connectome based predictive modelling (CPM) in which functional connections of the entire brain are mapped to provide a unique fingerprint of PTSD with network-informed results.

Methods: We examined a cohort of 54 United States Veterans who completed functional magnetic resonance imaging (fMRI) (64% male, mean age = 44, SD = 14). All participants had a clinical diagnosis of PTSD as measured by the Clinician Administered Interview for DSM-5 (CAPS-5) (i.e., moderate to severe). The PTSD Checklist for DSM-5 (PCL-5) was used as a continuous measure of PTSD symptom severity to derive the predictive models. We generated a network restricted strength predictive model (NRS-PM) by taking a pairwise average connectivity of all modules as AA-50 (hierarchical connectivity at 50 modules) and AA-24 (24 modules). 1000 iterations of tenfold cross-validation were conducted to ensure statistical significance. After identifying the PCL connectome network, we extracted the network (average connectivity in each participant) to generate AA-50 and AA-24 for each participant. AA-424 using all 424 modules was generated for each participant to test models with no network restrictions.

Results: We identified a connectome fingerprint signature of PTSD using CPM. Using NRS-PM at AA-50 (i.e., features extraction using the atlas hierarchy at 50 modules) as the primary analysis for identifying a connectome fingerprint, we found a brain network that significantly predicted PTSD symptom severity as measured by the PCL (NRS: $r = 0.28$, $p = 0.006$). Higher PCL scores were associated with lower functional connectivity between default mode network (DMN) and visual nodes, DMN and dorsal salience (DS), and visual and somatosensory nodes. PCL was severity was also associated with higher functional connectivity between central executive and visual nodes, DMN with ventral salience (VS) and subcortical nodes, and higher within network connectivity in visual nodes. Secondary analysis showed significant predictive models of PCL at AA-24 (NRS $r = 30$, $p = 0.003$) and without network restrictions ($r = 0.31$, $p = 0.002$). These effects were not moderated by age or sex.

Conclusions: Our findings identify a robust and cross-validated connectome predictive model of PTSD symptoms as reflected by PCL scores. Overall, our findings were partly consistent with the triple network model, which suggests hyperconnectivity of the salience network and hypoconnectivity of DMN and CEN underlies PTSD pathophysiology. Our finding that PCL scores are associated with lower DMN connectivity with the dorsal salience network may reflect reduced ability of top-down attention to task-focused salient stimuli (a DS function) to disengage self-referential mental processes mediated by the DMN, such as rumination, dissociation, and reexperiencing. Lower connectivity between the CEN and DMN associated with greater PTSD symptom severity may impede the CEN inhibitory capacities over the DMN, resulting in reduced ability to switch between the CEN 'external mind' and DMN 'internal mind' and exacerbating self-referential mentation. Given the role of ventral salience networks in reorienting in response to involuntary salient internal and external stimuli, higher DMN and VS connectivity associations with more severe symptoms may suggest greater reorienting to involuntary internal/external salient stimuli such as intrusive thoughts and trauma reminders. Future work should 1) examine whether heterogeneous PTSD presentations are associated with differing connectome fingerprints, and 2) identify and test drugs that target alterations in the observed networks.

Keywords: Anxiety and PTSD, Human Neuroimaging, Brain Connectome

Disclosure: Nothing to disclose.

P41. Infant Nonhuman Primate White Matter Microstructure is Associated With Components of Trait-Like Anxiety

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Background: Childhood behavioral inhibition, when extreme, is a risk factor for the development of stress-related psychopathology, particularly anxiety disorders (ADs). However, its underlying neural correlates and potential neurodevelopmental origins remain unclear. We developed a rhesus monkey model that has allowed for in-depth studies of the factors that underlie the development of behavioral inhibition or threat-induced freezing, as well as the trait-like anxious temperament (AT), which incorporates measures of threat-induced freezing with decreases in vocalizations and increases in cortisol. In relation to individual differences in the expression of anxiety, our studies suggest that white matter (WM) microarchitecture within the anxiety neural circuit is a promising area of study as it likely functions to modulate threat-related neuronal signaling. Our previous work in preadolescent children and nonhuman primates (NHPs) demonstrated region-specific and distributed anxiety-related WM microstructural alterations, generally highlighting negative associations with subjective anxiety, ADs, and freezing behavior. Most previous work in this domain has utilized diffusion tensor imaging (DTI) measures as proxies for properties of WM microstructure. Though highly sensitive to myelination, DTI metrics are non-specific, whereas quantitative relaxometry (QR) is an alternative method of assessing WM microarchitecture that may be more specific to myelination. To understand the earliest origins of WM alterations associated with the development of pathological anxiety, we extend our work using DTI and QR metrics in our NHP model to longitudinally examine their respective associations with individual differences in anxiety-related behaviors across the first year of life.

Methods: 34 rhesus monkeys (23 F, 11 M) underwent 30 minutes of the No-Eye-Contact (NEC) session of the Human Intruder Paradigm (HIP) at approximately 2, 6, 12, 24, and 52 weeks old. During the NEC, a human experimenter stands 6 feet away from the testing cage and presents their profile to the monkey, representing an indirect, potential threat. Behaviors key to AT, including freezing duration, were scored during each 30-minute NEC session. At approximately the same timepoints, all animals also underwent imaging with both MPnRAGE and single-shell DTI sequences. Images were co-registered into a common template space. A previously published WM atlas was used to delineate 5 major WM regions of interest (ROIs) across the brain that prior studies have implicated in the pathophysiology of ADs: the uncinate fasciculus, cingulum, internal capsule, corpus callosum, and superior longitudinal fasciculus. QR metrics (i.e., longitudinal relaxation rate [qR_1], generated from MPnRAGE images) and DTI metrics (i.e., fractional anisotropy [FA]) were calculated per subject at each study timepoint. Freezing durations were log-transformed and converted to z-scores. Three sets of ROI-specific linear mixed-effects (LME) models were built in R to assess within-subject associations among: 1) FA and freezing; 2) qR_1 and freezing; and 3) both FA and qR_1 in relation to freezing to determine the unique contribution of each metric to NEC-related freezing behavior. All analyses controlled for gestational age at scan.

Results: FA-focused analyses demonstrated that, on a within-subject level, FA in all five ROIs negatively predicted NEC-related freezing duration across the first year of life (mean $R^2 = 0.16$, $p < 0.001$). qR_1-focused analyses revealed similar findings, however with slightly larger effects sizes, such that qR_1 measures in all five ROIs exhibited stronger negative associations with freezing duration (compared to FA) on a within-subject level during the first year of life (mean $R^2 = 0.25$, $p < 0.001$). Finally, LME models that simultaneously estimated the effects of FA and qR_1 on NEC-related freezing showed that, in all five ROIs, the qR_1 metric was a more robust predictor of freezing relative to FA. In other words, when assessed alongside qR_1, the FA metric was a nonsignificant predictor of freezing, while qR_1 remained highly significant and negatively predicted freezing in all ROIs (qR_1: mean partial-$R^2 = 0.12$, $p < 0.001$).

Conclusions: Our results serve as a cross-species replication of our recent findings in preadolescent children and NHPs with pathological anxiety, indicating a dynamic, within-subject relation between WM microarchitectural integrity and anxiety-related behavior. Importantly, the current work extends this association to the earliest postnatal developmental period, suggesting the importance of WM in the neurodevelopmental underpinnings of maladaptive anxiety-related responses. More specifically, simultaneous analysis of distinct WM metrics derived from multimodal imaging reveal robust qR_1-specific relationships with AT-related behavioral constructs, particularly freezing. These data support the notion that QR and DTI metrics exhibit differential and/or enhanced sensitivity to unique biophysical properties of WM microstructure and, moreover, that QR may be sensitive to components of WM microstructure that are particularly relevant to AT-related behaviors. In turn, to the extent that QR is a more specific measure of myelination compared to DTI, our results point to the possibility that, on an individual level, myelination processes are critical to the development of AT-related behaviors in the earliest phases of life.

Keywords: White Matter Development, Longitudinal Multimodal Imaging, Nonhuman Primate Models, Early Life Anxiety, Anxiety-Like Behavior

Disclosure: Nothing to disclose.

P42. Non-Rapid Eye Movement Sleep Oscillations in Anxious Youth

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Background: Anxiety disorders typically manifest in childhood and adolescence, at a time of rapid changes in cortical maturation, and are commonly accompanied by sleep disturbances. Despite the well-known association between sleep and anxiety, there is a dearth of studies examining the biological basis of the precipitating role of abnormal sleep in anxiety. Non-Rapid Eye Movement (NREM) sleep oscillations are critical for cognition and emotional regulation: these include sleep spindles, 12-15 Hz waxing and waning thalamocortical oscillations, and slow oscillations (SO), highly synchronous 0.5-4 Hz oscillations of prefrontal cortical origin. Emerging work in rodent models suggest that SO and spindles also play a causal role in brain maturation. Here, across two studies that utilize sleep EEG to characterize sleep macro and microstructure, we examine the relations between NREM oscillations, anxiety symptoms, development and affect.

Methods: Study 1 involved 40 non-help seeking young adults (18-21 yrs) with low vs moderate-to-high levels of self-reported anxiety symptoms, who were monitored during a 2 hr mid-day nap opportunity. Study 2 consisted of 35 children (9-13 yrs) either typically developing or with an anxiety disorder (generalized

anxiety, separation anxiety or social phobia), who were monitored for nocturnal sleep and completed 7T MRI scans (T1, resting-state fMRI and diffusion-weighted imaging) one week later. In both studies sleep was monitored with a 32 channel EEG device and NREM sleep oscillations were identified using validated automated detectors.

Results: While we observed no differences between high vs low anxiety groups in any of the macro features of sleep (sleep duration, latency, efficiency or sleep stage distributions; all p's >.3there was a significant effect of anxiety on slow oscillations (SO). Across all frontal electrodes, normalized averaged peak-to-peak amplitude of NREM SO was significantly reduced in young adults with heightened anxiety symptoms (10 electrodes, $F(1,40) = 5.7$, $p = .02$). Although there were no group differences in the density or morphology of sleep spindles, in the entire sample of young adults, spindle density was associated with more negative affect ($r = .38$, $p = .02$), higher depressive symptoms ($r = .31$, $p = .04$) and higher state anxiety $r = .33$, $p = .03$).

Conclusions: In a sample of youth enriched for the presence of self-reported anxiety, we found a significant reduction in NREM SO amplitude and significant relations between sleep spindles and mood symptoms, which was observed in the context of no group differences in macro features of sleep. This reflects that alterations of NREM microcircuitry are evident even for midday naps and in non-clinical and non-help seeking individuals with heightened symptoms. Data analysis for Study 2 is ongoing and will allow for replication of NREM microcircuitry abnormalities in adolescents with established anxiety disorders, compare age-related changes in NREM oscillations between groups and their relations with structural and functional MRI estimates of cortical maturation.

Keywords: Anxiety, Sleep Spindles, Slow Oscillation, Adolescence, NREM Sleep

Disclosure: Nothing to disclose.

P43. Cognitive Control Task Performance Predicts PTSD Symptoms in the First-Year Post-Trauma

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Background: Cognitive control is the ability to sustain attention and inhibit behavioral responses to distracting stimuli, and deficits in cognitive control are commonly considered a risk factor for the development of posttraumatic stress disorder (PTSD) based on prior work. The proposed neural mechanism is dysregulated prefrontal cortical function that inhibits elevated neural activation in threat-related regions such as the amygdala. However, most prior studies examining cognitive control deficits in PTSD used retrospective datasets in which the current impact of PTSD on cognitive control cannot be distinguished from pre-trauma deficits. Prospective datasets in which participants complete cognitive control tasks acutely post-trauma before the development of PTSD can test the hypothesis that cognitive control deficits will predict future PTSD symptoms during the first-year post-trauma. Findings from prior work with small samples supported this hypothesis, but testing this hypothesis using larger samples with participants from diverse cultural backgrounds will increase reliability and representativeness of these findings.

Methods: In the present study, data from 2,943 ambulatory patients (mean age = 35.90 years; 1,815 women; mean education level = 14.9 years; race/ethnicity: 11.67% Hispanic, 34.80% non-Hispanic white, 49.74% non-Hispanic Black, 3.79% Asian, Pacific Islander, American Indian) from the multi-site AURORA (Advancing Understanding of RecOvery afteR traumA) study were analyzed. All patients had presented to the emergency department (ED) after acute trauma exposure, and patients completed the PTSD Checklist for DSM-5 (PCL-5) and a neurocognitive task battery that included a not-X gradual onset continuous performance test (gradCPT; designed to measure sustained attention/response inhibition). Participants repeated the PCL-5 during the first-year post-trauma (2 weeks, 8 weeks, 3 months, 6 months, 12 months) and repeated the gradCPT in rotating groups across time [weeks 2-4 ($n = 2170$), weeks 4-8 ($n = 2046$), months 3-6 ($n = 568$), months 6-9 ($n = 875$), months 9-12 ($n = 381$)]. A series of linear mixed-effects regressions (LMEs) were used to examine the trajectory of PCL-5 scores, gradCPT d prime scores (discrimination ability between trials) and gradCPT reaction times (correct responses on Go trials). LMEs were then used to test (1) if gradCPT task performance measures predict PTSD symptoms at the following time point across the first-year trauma and (2) if PTSD symptoms predict gradCPT task performance at subsequent times across the first-year trauma. LMEs use maximum likelihood estimation, which can provide estimations despite patients not completing the gradCPT across time. Covariates for LMEs were age, sex, education level, and race/ethnicity.

Results: Over the first-year post-trauma, the trajectory of PCL-5 scores showed a significant increase at 2 weeks post-trauma ($p < .001$; $d = .131$) followed by significant reductions in PCL-5 scores at all subsequent time points, including 12 months post-trauma ($p < .001$; $d = .321$). The trajectory of gradCPT d prime scores was nonlinear with an initial increase 2 weeks post-trauma ($p < .001$; $d = .113$), followed by a decrease at 8 weeks ($p < .001$; d's = -.217) and 3 months ($p < .001$; $d = -.123$), and then returned to baseline levels at 6 and 12 months (p's > .182; d's < .033). GradCPT reaction times became significantly faster over time consistently through 12 months post-trauma ($p < .001$; $d = -.605$). Due to non-linearity, gradCPT d prime scores were excluded from prediction analyses. LMEs showed gradCPT reaction times at earlier time points had a significantly positive relationship to PCL-5 scores at later time points ($p = .009$; $d = .183$), such that those with slower reaction times had higher PCL-5 scores over time. PCL-5 scores at earlier time points did not significantly predict reaction times at later time points ($p = .973$; $d = .024$).

Conclusions: Findings showed that cognitive control task performance predicts PTSD symptom development and contributes to symptom maintenance throughout the first-year post-trauma. Future work can examine the predictive value of other tasks collected in the AURORA study. Modifiable factors that influence cognitive control could also be identified to inform novel early interventions that may bolster resilience to PTSD development. Interventions such as transcranial magnetic stimulation could stimulate prefrontal cortical areas contributing to cognitive control.

Keywords: Cognitive Neuroscience, Continuous Performance Task, PTSD, Prediction, Causal Modeling, Trajectory Modeling, Cortisol, Trauma Exposure, Posttraumatic Stress Disorder

Disclosure: Nothing to disclose.

P44. Heart Rate Variability: Threat Conditioning, Appraisal, and Approach-Avoidance Behavior

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Background: Dysregulated threat processing may play an important role in anxiety. Threat conditioning studies often measure sympathetic arousal (i.e., skin conductance response),

but fewer studies have measured parasympathetic function using high frequency Heart Rate Variability (HF-HRV). While previous research suggests that individuals with lower resting HF-HRV may exhibit more exaggerated responses to threat, task-related HF-HRV may be more closely associated with behavior (Whitney et al., under review). However, few studies have examined individual differences in HF-HRV during threat learning or investigated the impacts of task-related HF-HRV on subsequent appraisals and approach-avoidance behavior to threats.

Methods: In an MRI session, thirty-one adults (17 females), ages 18-30 ($M = 20.32$, $SD = 2.55$) underwent a threat conditioning task. Two women with neutral facial expression served as the conditioned stimuli, CS+ and CS-. A fearful facial expression of the CS+ co-terminating with a loud, aversive scream served as the unconditioned stimulus (US). The CS+ was paired with the US using a 50% reinforcement rate, whereas the CS- was never paired with the US. Subjective ratings of the CS+ and CS- were obtained to assess threat conditioning. Following threat conditioning, individuals viewed morphed images of the CS+ and CS- in the context of two tasks. In response to each morphed image during a threat appraisal task, individuals rated the explicit probability of the US, current fear of the CS, and likelihood of avoiding the CS in the future. All morphed images were assessed in each appraisal block. In an implicit approach-avoidance task (AAT), individuals viewed morphed images on a colored background (e.g., blue or green). Participants identified the background color via button press until the image disappeared. With each button press, the image either increased or decreased size, mimicking approach and avoidance of the image, respectively. Heart rate, skin conductance (SCR), and respiration were collected continuously during each task: rest, threat conditioning, threat appraisal, and implicit AAT. For each task, HF-HRV was extracted using the spectral power within the 0.15-0.40 Hz frequency band and log transformed. In SPSS, statistical significance of main effects and group differences were determined using alpha = 0.05.

Results: In the whole group, evidence of threat conditioning was detected in subjective report of US awareness (CS + >CS-, $t(28) = 6.69$, $p < .001$) and skin conductance differences (US>CS + >CS-, $F(1, 29) = 14.38$, $p < 0.001$). Analysis of self-reported anxiety ratings revealed two patterns: a group that failed to exhibit threat conditioning ($n = 11$; CS+Anx = CS-Anx) and a group that exhibited successful threat conditioning ($n = 18$; CS+Anx > CS-Anx). Although group differences in HF-HRV were only at trend-level significance ($p < 0.09$), we investigated group differences in HF-HRV during each task, controlling for resting state HF-HRV. In the group that failed to exhibit threat conditioning, HF-HRV reactivity increased throughout the threat conditioning task, such that HF-HRV was significantly greater in the late acquisition phase ($M = 2.76$, $SD = 0.45$) compared to pre-acquisition ($M = 2.52$, $SD = 0.59$), $t(10) = -1.93$, $p = 0.04$. Within this group, HF-HRV was significantly lower in the threat appraisal condition ($M = 3.19$, $SD = 0.28$) compared to the explicit memory condition ($M = 3.27$, $SD = 0.29$, $t(12) = 2.620$, $p < 0.02$). In the group that demonstrated successful threat conditioning, no changes in HF-HRV reactivity were observed in conditioning ($p = 0.16$) and no differences in HF-HRV across appraisal types were observed ($p < 0.73$). Group differences in HF-HRV were not observed in the AAT task between the non-conditioning and threat conditioning groups ($p = 0.66$).

Conclusions: Individuals that self-reported unsuccessful or successful threat conditioning exhibited divergent patterns of HF-HRV during threat conditioning and threat appraisal. Lower HF-HRV during pre-conditioning compared to later acquisition of conditioning among individuals that failed to demonstrate successful threat conditioning may indicate greater demands on emotion regulation during early threat conditioning. Therefore, lower HF-HRV when individuals are learning and appraising threat may have clinical implications. In addition, these data highlight differences in HF-HRV between explicit and implicit threat processing, which may suggest that explicit and implicit threat processing differentially captures active demands on emotion regulation. Regardless, future threat processing studies should expand the investigation of autonomic function to include HRV as a measure to understanding parasympathetic mechanisms underlying anxiety disorders.

Keywords: Threat Conditioning, Heart Rate Variability, Approach/Avoidance

Disclosure: Nothing to disclose.

P45. Rostral Anterior Cingulate Response to Emotional Conflict is Modulated by Trauma Exposure Burden in Resilient World Trade Center Responders

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Background: People vary widely in ability to successfully adapt after potentially-traumatic events. For example, a subset of 9/11 World Trade Center (WTC) responders have remained consistently psychologically well despite having endured considerable WTC-related exposures. Emotion regulation is one psychological factor that may support resilience: top-down cortical regulation of subcortical fear processing structures supports adaptive responding to environmental demands. Most neuroimaging studies have focused on effortful (explicit) emotion regulation. However, recent studies suggest that automatic (implicit) emotion regulation abnormalities constitute a core dysfunction in PTSD and could be key to resilience as well: Offringa and colleagues (2013) found greater rostral anterior cingulate (rACC) activation during implicit emotion regulation in trauma-exposed controls vs. symptomatic individuals. However, few neuroimaging studies investigated implicit emotion regulation in trauma-exposed adults across both dimensions of exposure severity and presence or absence of psychopathology.

In the present study, we sought to examine rACC functioning (as a region subserving automatic emotion regulation) using an established picture-word emotional Stroop task. We hypothesized that (1) highly resilient WTC responders with high WTC-trauma exposure burden [Resilient-High group] would demonstrate the greatest degree of rACC BOLD activation in response to emotional conflict, while the lower-WTC-exposed responders [Resilient-Low group] would show lesser but still positive rACC activation, and (3) responders with PTSD would not exhibit significantly different rACC activation on incongruent (vs. congruent) trials.

Methods: Rescue and recovery workers ($N = 97$) involved in WTC recovery efforts following the 9/11/01 attacks in New York City were enrolled in a neuroimaging study. All participants were administered the Structured Clinical Interview for DSM-5 and the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5). We categorized WTC trauma-exposed responders with no current or lifetime psychopathology (i.e., resilient) into two groups (Resilient-High and Resilient-Low) based on number of specific 9/11-related exposures (e.g., participated in search and rescue, exposed to human remains, experienced death of a colleague or loved one). The PTSD group met DSM-5 criteria for WTC-related PTSD, based on the CAPS-5. Groups were matched on age and sex, with approximately 82% males in each group.

Participants attended a fMRI session during which they completed a task involving photos of fearful or happy faces, overlaid with a word ("AFRAID" or "HAPPY") that was either

congruent or incongruent with the face. They were instructed to indicate the emotion in the photo by pressing a button as quickly as possible, while ignoring the overlaid word.

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 smoothed with a 6-mm FWHM Gaussian kernel before single-subject and group-level analysis via SPM12.

Ninety-six participants completed the FaceStroop task, of which 85 were included in the final FaceStroop task analyses (Resilient-High n = 31, Resilient-Low n = 26, PTSD n = 28) after excluding data from 7 participants for excessive motion in the scanner and from 4 participants for task performance below conventional thresholds for this task (>25% missed trials and/or <75% accuracy).

Results: There were no significant group differences in FaceStroop task performance (mean reaction time and accuracy), matching results from Offringa et. al (2013).

Incongruent > Congruent fMRI single-subject contrast images were aggregated across the sample to assess for whole-brain effects of task and group. We found typical task-related activation in regions reflecting conflict processing (e.g., dorsal ACC, bilateral anterior insula) and cognitive control (inferior frontal gyrus, dorsolateral prefrontal and parietal cortices), among others (FWE-corrected $p = .05$, $k = 10$).

To test our a priori region of interest hypotheses, we created an 8-mm sphere around the rACC peak MNI coordinates reported by Offringa et al. (2013) [x = 16, y = 36, z = 34] and extracted participants' Incongruent > Congruent BOLD activation estimates within the mask. As predicted, there was an omnibus effect of group, $F(2,82) = 3.20$, $p = .046$, such that the Resilient-High group showed the greatest rACC response to emotional conflict, followed by the Resilient-Low group, with minimal to no rACC activation in the PTSD group. Linear contrast results supported our specific directional hypotheses: rACC in Resilient-High > PTSD ($t(82) = 2.48$, $p = .015$), Resilient-High > Resilient-Low ($t(82) = 0.72$, $p = .48$), and Resilient-Low > PTSD ($t(82) = 1.67$, $p = .098$). We obtained similar results using an anatomically-defined rACC mask.

Conclusions: We replicated prior findings that rACC engagement during automatic regulation of emotional conflict is modulated by presence/absence of psychopathology, and further expanded these results by identifying differences in trauma exposure severity as another modulator in resilient individuals. These findings contribute to understanding the neural instantiation of psychological factors that enable some individuals to be highly resilient despite high trauma exposure burden.

Keywords: Stress Resilience, World Trade Center Rescue and Recovery Work, PTSD, fMRI, Trauma Exposure

Disclosure: Nothing to disclose.

P46. Fear Generalization and Extinction are Related to Peripheral 2-AG Levels and Distinct Neural Connectivity Patterns in Humans

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Background: The endocannabinoid system is believed to play a critical role in fear learning. In particular, the ability to distinguish between fear-related and safe stimuli is a process critical to well-being but believed to be impaired in some individuals following trauma exposure. As several cannabinoid-targeted compounds are moving forward in clinical trials related to trauma exposure (i.e., PTSD), we aimed to explore the relationship between fear learning, neural connectivity, and eCB function in a clinically heterogenous sample of adults with or without prospectively assessed histories of childhood trauma exposure or substance use disorders.

Methods: In this study, adult male and female humans (N = 102 total) with or without histories of prospectively assessed childhood trauma exposure and/or substance use disorders (N = 24-26/group) completed a single-day laboratory model of fear conditioning and extinction and provided blood samples for analysis of peripheral endocannabinoid levels (AEA, 2-AG) using mass spectrometry. A resting state functional neuroimaging scan was collected on a separate occasion. Given the role of the amygdala as a mediator of fear behavior, the left/right amygdala were used as seeds in a seed-based connectivity analysis with target regions of the dorsolateral prefrontal cortex (dlPFC) and anterior cingulate cortex (ACC) and were assessed for covariance with peripheral measures of endocannabinoids and behavioral measures of fear conditioning. Results were further validated by extracting beta coefficients from the significant clusters for each participant to use in a regression analysis.

Results: Overall, those with trauma exposure histories had lower 2-AG levels ($F(3, 60) = 2.81$, $P = 0.047$). When the sample was assessed as a whole, lower levels of 2-AG were associated with generalization of fear ($p = 0.025$, 95% CI 0.007, 0.132), i.e., an impaired ability to distinguish between a fear-associated cue and a cue not associated with a fear stimulus. Higher levels of 2-AG were also associated with facilitated within-session fear extinction ($p = 0.005$, 95% CI 0.020, 0.130). Fear generalization was associated with reduced amygdala-dorsolateral prefrontal cortex activity ($p < 0.001$, 95% CI 0.022, 0.051), while fear extinction was associated with greater connectivity between the amygdala and anterior cingulate cortex ($p < 0.001$, 95% CI 0.037, 0.085).

Conclusions: Together, this data highlights potential novel therapeutic targets for individuals with histories of trauma exposure. New therapeutic interventions could include pharmacological interventions, such as increasing 2-AG via inhibition of the degradative enzyme monoacylglycerol lipase, or non-pharmacological interventions, such as neurostimulation of the dlPFC, which would constitute innovative treatment opportunities for individuals histories of trauma exposure

Keywords: Endocannabinoids, Childhood Trauma, Fear Conditioning

Disclosure: Nothing to disclose.

P47. Posttraumatic Stress Disorder (PTSD) is Associated With Abnormal Amygdala and Striatal Signaling of Temporal Prediction Errors During a Passive Reward Learning Task

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Background: Posttraumatic stress disorder (PTSD) is characterized by prominent symptoms of diminished positive affect (DPA), e.g., inability to experience pleasure, diminished interest in valued activities, and difficulty experiencing interpersonal closeness. In PTSD, these symptoms are subsumed by the symptom domain of "emotional numbing", where they are associated with greater distress and chronicity, greater functional impairment, poorer treatment outcomes, and increased suicidality. Despite these relationships to important clinical outcomes, the biological bases of PTSD DPA symptoms are not well characterized.

Reward processing reliably recruits circuitry implicated in positive affect and is therefore a useful experimental model for the study of PTSD DPA symptoms. This includes canonical nodes of the reward circuit, i.e., ventral striatum, ventromedial prefrontal cortex/ventral cingulate, and midbrain nuclei including the ventral segmental area (VTA), as well as subcortical and limbic structures such as the amygdala and insula. A limited number of prior studies have investigated PTSD reward-processing abnormalities through use of operant conditioning tasks, which typically involve choosing behaviors to earn money/points and avoid losses and learning contingencies between behaviors and outcomes. Abnormal neural prediction error (PE) signaling has been observed in PTSD in such tasks, which is a neural representation of the difference between outcomes expected vs. those received. PEs are used to guide learning and update knowledge of choice-outcome contingencies. However, such paradigms only probe PE signaling of reward magnitude/quantity following voluntary choice (operant conditioning), which is only one aspect of real-life reward learning and reinforcement. Another critical aspect involves associative learning (classical conditioning), wherein a neutral cue is associated by experience with a rewarding outcome independent of voluntary behavior. For example, lights dimming in a movie theater is a reliable predictor of a rewarding outcome (movie starting). Moreover, temporal relationships between predictor and reward can also be learned/acquired and, if violated, leads to temporal PEs (TPEs), which signal the difference between expected vs. actual timing of a reward following a predictive cue. For example, lights dimming in a movie theater typically signals an imminent movie start, but a substantial delay between the two will induce a TPE to signal violation of the expected temporal relationship.

Here, we probed PTSD reward processing abnormalities with functional magnetic resonance imaging (fMRI) to examine brain responses to TPEs during a passive reward learning task. Deprived of liquids for 2-3 hours, participants completed a task in which neutral visual cues were reliably paired with either delayed delivery of a juice bolus (primary reinforcer) or a neutral outcome (another visual cue) 4-6 sec later. A subset were "catch" trials, in which the temporal relationship between cue and outcome was extended to 10-12 sec. Through examining distinct temporal windows on catch vs. normal trials, it is possible to map neural signaling of positive TPEs (temporally unexpected outcome received vs. temporally expected outcome received) and negative TPEs (temporally unexpected outcome absence vs. temporally expected outcome absence). Consistent with prior work for abnormal reward PE signaling in PTSD, we expected PTSD vs. trauma-exposed healthy controls (TEHCs) to display abnormal neural signaling of TPEs.

Methods: Participants with PTSD and TEHC participants ($N = 45$ each) underwent fMRI in a liquid-deprived state (2-3 hrs) and completed two runs of a passive reward learning task. One of two visual cues reliably preceded delivery of a juice bolus (1ml of participant's preferred juice), while the other reliably predicted another neutral visual cue 4-6 sec later. Catch trials, when temporal delay between cue and outcome was extended to 10-12 sec, were instituted on the second run and always followed by reinstatement of the learned temporal dependency (4-6 sec) on the next presentation. Regressors corresponding to onset of each predictive cue, juice bolus, or visual cue outcome were modeled on the first-level in AFNI. Within-subject contrasts were computed to map positive and negative TPEs for reward (juice) vs. non-reward (visual cue) cue-outcome relationships. Voxelwise independent t-tests comparing PTSD vs. TEHC were conducted and constrained by an anatomical mask of the striatum, midbrain nuclei, amygdala, insula, and ventral/subgenual cingulate. T-maps were then subjected to probabilistic threshold-free cluster enhancement (pTFCE) at a corrected $p < 0.05$.

Results: Relative to TEHCs, PTSD displayed attenuated signaling of positive TPEs (failure to properly activate) in the left amygdala, left midbrain (VTA and parabrachial nucleus), and left ventral putamen. For negative TPEs, PTSD displayed exaggerated signaling (failure to properly deactivate) in the left amygdala and left ventral putamen (all pTFCE $p < 0.05$) relative to TEHCs.

Conclusions: Findings implicate abnormal amygdala and striatal TPE signaling in PTSD, which extends prior findings for abnormal reward PE signaling in operant decision-making reinforcement paradigms. These findings expand knowledge of PTSD circuitry abnormalities and identify potential novel treatment targets for remediating PTSD symptoms of DPA, which are not directly addressed with existing treatments.

Keywords: PTSD, Reward, fMRI, Prediction Error, Anhedonia

Disclosures: Alto Neuroscience: Stock / Equity (Self). Synapse Bio AI: Consultant (Self).

P48. Striatal Engagement During Encoding Differentially Relates to Memory Bias for Social and Monetary Feedback

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Background: Reward processing and recall are fundamental for learning. Yet biases often distort the accuracy of recall. While often linked to appetitive experiences, recent work shows that striatal engagement facilitates learning about motivationally significant events, regardless of valence. However, the striatum's role in recall bias has rarely been assessed. Moreover, research on reward processing has often probed striatal response to feedback in the monetary domain, leaving potential differences in response to social feedback poorly understood. Likewise, research on memory bias often focuses on neural response to lists of memoranda or non-verifiable autobiographical events, leaving degree of bias elicited in response to verifiable social feedback untested. To bridge these gaps, we developed the Recall After Feedback Task (RAFT), which assess memory and prediction biases about being liked or disliked. Our prior work shows a positivity bias for both true and false memories for social feedback. Moreover, memory bias was associated with social anxiety in a developmentally sensitive way. Among adults, more severe symptoms of social anxiety were associated with a negativity bias. During the transition from adolescence to adulthood, age strengthened the positivity bias in those with less severe symptoms and strengthened the negativity bias in those with more severe symptoms. Biases did not emerge for predictions about social feedback. The present work replicates behavioral findings in the social domain using a new version of the RAFT that tests generalizability to the monetary domain. We also describe the relationship between striatal engagement during the encoding of social and monetary feedback and subsequent memory bias.

Methods: After submitting a photo purportedly sent to peers, participants ($N = 62$; 20.86 ± 1.71years; 77% female) underwent fMRI while completing well-matched social and monetary encoding tasks. During the social task participants saw pairs of purported peers and selected the peer they thought would like them before receiving positive (like) or negative (dislike) feedback. During monetary task participants saw pairs of doors photos and selected the door they thought contained a $1.50 win or a $0.25 loss before receiving positive (win) or negative (lose) feedback. During a surprise cued response task (post fMRI) participants were asked which peers and doors they had selected, then recalled the valence of the feedback they received. For peers and doors participants did not recall selecting, they then predicted if each

would have given them positive (like/win) or negative (dislike/lose) feedback.

The first question during the cued response task probed memory for feedback. Responses identified trials for which subsequent bias reflected true and false memories and predictions. The second question was then used to quantify bias. For each of the four response types, a bias score was calculated that reflected the number of peers or doors a participant recalled as giving them (or predicted would give them) positive feedback, divided by the total number of peers or doors the participant recalled as giving them (or predicted would give them) either positive or negative feedback. Thus, scores nearing 1 or 0 respectively reflected a strong positivity or negativity bias. One-sample and paired samples t-tests were used to test magnitude of memory and recall bias within, and across domains, respectively. fMRI analyses focused on striatum as a region of interest (ROI) and used linear regression to test the relation between bias and functional engagement during the encoding of social and monetary feedback.

Results: Replicating prior work, there was a robust positivity bias for perceived memories for social feedback, regardless of whether memories were true (0.63 ± 0.21; $t(49) = 4.48$, $p < .001$) or false (0.59 ± 0.21; $t(49) = 3.06$, $p = .00356$), yet no bias for true (0.48 ± 0.17; $t(49) = -0.68$, $p = 0.50$) or false (0.49 ± 0.18; $t(49) = -0.38863$, $p = 0.70$) predictions. A somewhat different pattern emerged in the monetary domain. There was a positivity bias for true memories (0.59 ± 0.19; $t(51) = 3.38$, $p = 0.0014$) and predictions (0.43 ± 0.18; $t(51) = -2.84$, $p = 0.006$), but not false memories (0.54 ± 0.21; $t(51) = 1.38$, $p = 0.17$) or predictions (0.45 ± 0.19; $t(51) = -1.966$, $p = 0.05469$). Despite generalized results for true memories, positivity bias was greater in the social vs. monetary domain, $t(47) = 2.17$, $p = 0.03$. Moreover, there was a positive relation between memory bias and caudate engagement during the encoding of social feedback ($\beta = 135$, $t(39) = 2.685$, $p = 0.01$) that failed to emerge during the encoding of monetary feedback ($\beta = -46.6$, $t(40) = -0.97$, $p = 0.34$).

Conclusions: There was a robust positivity bias for true autobiographical memories of social and monetary feedback. Compared with monetary domain, bias was stronger in the social domain, where associations with striatal engagement during encoding also emerged. Moreover, in the social domain there was bias for perceived experiences (i.e., true and false memories), whereas in the monetary domain there was bias for actual experiences (i.e., true memories and predictions). Results provide intriguing initial evidence suggesting distinct processes, potentially related to striatal engagement, may promote memory and prediction bias across social and monetary domains. Further work is needed to determine if these effects vary in the presence of internalizing or externalizing symptoms or across development.

Keywords: Memory Bias, Social Reward, Monetary Reward, Functional MRI (fMRI), Striatum

Disclosure: Nothing to disclose.

P49. The Role of Glutamatergic Astrocyte-Neuron Interaction in Adult Anxiety Susceptibility Induced by Adolescent Repeated Alcohol Exposure

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Background: Adolescence is a critical period for brain development and maturation. Thus, individuals exposed to alcohol early in life during this period are at a greater risk for developing multiple psychiatric and medical conditions in adulthood. Indeed, epidemiological studies show that early initiation of alcohol use increases the future risk of developing generalized anxiety disorder, which is accompanied by alcohol use disorder (AUD) in adults. Due to the character of anxiety as a state of arousal and hypervigilance to external environmental stimuli, the thalamic circuits controlling arousal have been identified as a link between the adolescent experience and psychiatric behavioral hallmarks in adults. Specifically, the paraventricular nucleus of thalamus (PVT), a part of epithalamus that projects to emotion-related brain regions including the amygdala, nucleus accumbens, and medial prefrontal cortex, has been considered a hub brain area controlling brain anxiety network. Indeed, recent anatomical and functional studies suggest that the PVT presents a variety of neural signals according to the early-life experience and the activities are significantly correlated with anxiety-like behavior. However, gaps remain to be filled with respect to how adolescent repeated alcohol exposure (AAE) impacts maladaptive activities in the PVT and consequent behavioral outcomes in adulthood.

Methods: To investigate this, we compared the cellular activities in the PVT and behavioral consequences of mice withdrawn from adolescent repeated alcohol exposure (AAE) and ethanol naïve counterparts (CON), using electrophysiological, biochemical, chemogenetic, transgenic, and behavioral approaches. Briefly, mice were exposed to air or vaporized ethanol in a vapor inhalation chamber for three weeks from P28 to P49. Each daily cycle consisted of ethanol vapor for 16 h followed by 8 h of abstinence in their home cage. This was repeated each day for 4 consecutive days, followed by 3 days of abstinence. The cellular activities and anxiety-like behaviors were evaluated after 3 to 4 weeks withdrawal from the AAE paradigm.

Results: We observed the increase in anxiety-like behaviors in the AAE mice compared to those in the ethanol-naïve CON mice. In parallel, the firing rates and the expression levels of ΔFosB immediate early gene in the PVT neurons were increased in the AAE group. Interestingly, chemogenetic inhibition of the PVT neurons alleviated the anxiety-like behavior in the AAE mice. The neuronal excitability in the PVT of AAE mice was increased, at least partly, via reduction of the GLT1 (an astrocyte dominant glutamate transporter, as known as EAAT2, slc1a2). Our noninvasive magnetic resonance spectroscopy (MRS) measures also showed the increase in glutamate levels in the thalamic area including the PVT of the GLT1 conditional knock-out mice. Those GLT1 conditional knock-out mice showed the increased anxiety-like behavior, whereas the selective upregulation of GLT1 in the PVT astrocytes via the expression of GFAP-promoter driven Cre recombinase in the PVT of GLT1 Ai9 mice alleviated the anxiety-like behavior induced by the AAE.

Conclusions: These findings highlight the significant role of PVT astrocytic GLT1 in the anxiogenic phenotype in adulthood induced by long-term withdrawal from adolescent repeated ethanol exposure, reinforcing the importance of the glutamatergic interaction between astrocytes and neurons in the AAE-induced adult anxiety susceptibility. It also suggests that GLT1 in the PVT could serve as a therapeutic target for alcohol use disorder and comorbid emotional disorders.

Keywords: Adolescent Alcohol, Anxiety, Glutamate Transport, Neuron-Astrocyte Interactions

Disclosure: Nothing to disclose.

P50. Sex-Specific Contribution of the Endocannabinoid and Immune Systems to Posttraumatic Stress Disorder

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Background: Posttraumatic stress disorder (PTSD) – a severe and debilitating trauma-related psychiatric disorder – exhibits greater prevalence and severity in women compared to men. The root causes of such sex-disparities are complex and appear to be linked to sex-differences in brain systems and immunological processes implicated in stress and fear response. In particular, animal and human studies converge in indicating that PTSD is characterized by profound dysregulations of the endocannabinoid (eCB) system, an endogenous lipid signaling system critically implicated in modulating fear and anxiety. The eCB system is also an important regulator of inflammatory processes, with deficits in eCB signaling favoring the emergence of an hyperinflammatory state, as observed in PTSD. Importantly, both eCB signaling and inflammatory response considerably differ between sexes, with females showing decreased eCB tone and heightened proinflammatory activity compared to males, both at baseline and in response to stressors. This evidence suggests the compelling hypothesis that sex-differences in trauma-induced eCB dysregulation and eCB-mediated inflammation may contribute to sex-biases in PTSD risk and severity. However, to date no study has examined the interplay between endocannabinoids and pro-inflammatory molecules in individuals with PTSD, and sex-differences therein. To begin addressing these questions, we investigated the relationship between levels of circulating eCBs and peripheral pro-inflammatory markers in a cohort of men and women with PTSD and non-psychiatric controls. Furthermore, we assessed whether such relationship varied by sex, with the goal to uncover sex-specific mechanisms contributing to PTSD vulnerability.

Methods: We used a large biorepository database (Mass General Brigham Biobank) to identify individuals aged 18- 45, with a diagnosis of PTSD based on ICD-10 criteria (n = 88), and sex- and age-matched non-psychiatric controls (n = 88). Subjects were included based on available electronic health records, serum samples and genetic data [Fatty Acid Amide Hydrolase (FAAH) 385C->A substitution]. Exclusion criteria included history of autoimmune, endocrine and/or chronic inflammatory disorders; and use of medications known to alter inflammatory response and/or eCB levels at the time the biospecimens were collected. Serum samples were processed to assess circulating levels of eCB (Anandamide [AEA], 2-Arachidonyl glycerol [2AG], Oleoylethanolamide [OEA], Arachidonic acid [AA]) and levels of several pro-inflammatory molecules (IL-1b, IL-6, IL-8, IL-18, TNF-α and CRP). We performed logistic regression to assess the effect of each molecule of interest on the probability of PTSD diagnosis while controlling for age, sex, race, FAAH genotype, smoking status, and presence of psychiatric comorbidities (alcohol and cannabis use disorder, any anxiety disorder, and major depressive disorder). Analyses were performed across the whole sample (n = 176) and stratified by sex (females = 90; males = 86). Molecules significantly associated with PTSD were selected based on False Discovery Rate (FDR) Benjamini-Hochberg Procedure (FDR < 0.02). Finally, we used a data-driven approach to construct a network across eCBs and pro-inflammatory molecules and reveal their interconnectivity.

Results: Whole-sample logistic regression analysis indicated that AEA, 2-AG, CRP, IL-6, IL-8, and TNF-α were significant predictors of PTSD diagnosis. Exploring the network of eCBs and pro-inflammatory molecules, we observed that 2-AG and IL-6 both independently influenced CRP levels. Linking the network to logistic regression and controlling for the effect of confounding molecules, we found that the effect of IL-8 on the probability of PTSD diagnosis was confounded by TNF-α and not significant after adjusting for the effect of the confounder.

Sex-disaggregated analyses revealed that the relationships between eCBs and pro-inflammatory molecules and PTSD significantly differed between sexes. Specifically, we found that IL-6 and TNF-α were positively associated with PTSD diagnosis in women (FDR < 0.1), suggesting that an increase in their levels exacerbates PTSD risk in female individuals. Conversely, AEA were negatively associated with PTSD in men (FDR < 0.05), indicating that an increase in its concentration reduces the vulnerability to PTSD in male individuals.

Conclusions: This is to our knowledge the first study showing that eCBs and pro-inflammatory molecules differentially contribute to PTSD risk in men vs. women. Our findings contribute to a better understanding of sex-specific biological mechanisms underlying PTSD and may have important implications for personalized medicine.

Keywords: Endocannabinoids, Inflammation, PTSD, Sex-Differences

Disclosure: Nothing to disclose.

P51. An Amygdala to Bed Nucleus of the Stria Terminalis Circuit Reduces Learned and Innate Defensive Responses

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Background: The bed nucleus of the stria terminalis (BNST) has been implicated in the regulation of anxiety, fear, and reward-related behaviors. Lesion studies of the BNST demonstrate reductions in anxiety-like behaviors and fear generalization (Duvarci et al., 2009). Subsequent studies suggest that the BNST mediates sustained fear and apprehension (Davis et al., 2010), which are part of the hypervigilance symptoms present in PTSD. It has been proposed that the adBNST reduces anxiety via outputs to lateral hypothalamus (LH) (Kim et al., 2013). The basomedial nucleus of the amygdala (BMA) has been implicated in top-down negative regulation of anxiety and fear-related behaviors and projects heavily to the anterodorsal subdivision of the BNST (adBNST) (Adhikari et al., 2015).

Methods: Male and female mice were injected with AAVs encoding ChR2 under control of the CaMKII promoter in the BMA and optic fibers were implanted above adBNST. Following 6 weeks of recovery and time for viral expression, mice were exposed to elevated plus maze, fear conditioning, open field, and predator odor assays while blue light was delivered to BMA-BNST terminals through the optic fiber at 10 Hz with 10 ms pulse width and and 10 mW of power.

For the slice electrophysiology experiments, mice were injected with AAVs encoding ChR2 in BMA and retrograde AAV expressing TdTomato in LH. Brains were harvested for slice physiology after 5 weeks following surgeries.

Results: (Adhikari et al., 2015). Using optogenetic stimulation, we show that activating the BMA-BNST pathway reduces conditioned fear and generalization, as well as innate avoidance and fear. Using slice electrophysiology, we show that LH projecting neurons in the BNST receive direct inputs from BMA. We further show that mu opioid receptor activation reduces excitatory drive in this circuit.

Conclusions: Altogether, our results show that BMA can exert control over LH activity through the BNST and its impact over LH function could be moderated by endogenous or exogenous opioids. Our results are in line with the idea that BMA-BNST-LH may an important circuit component linking cortical to subcortical control of PTSD-relevant behaviors and which its dysregulation could increase the risk of co-morbid opioid use disorder.

Keywords: Amygdala, Bed Nucleus of Stria Terminalis, Circuit Optogenetics, Slice Electrophysiology, Fear

Disclosure: Nothing to disclose.

P52. Trauma-Related Intrusive Memory Frequency is Associated With Altered Macrostructure of the Inferior Longitudinal Fasciculus and Lower Microstructure of Cingulum Tracts

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Background: Trauma-related intrusive memories (TR-IMs) are a common phenomenon following trauma exposure and are characterized by reexperiencing the sensory-perceptual details of the trauma without contextual binding, resulting in a 'here-and-now' experience. Evidence suggests that TR-IMs arise due to concurrent hyperactivity of affective brain regions such as the amygdala and altered activity of regions providing autobiographical context including the hippocampus and its affiliated cortical regions. Communication between the temporal lobe and cortex occurs within two major brain systems: the anterior-temporal (AT) network, connecting prefrontal cortex and temporal lobe via the uncinate fasciculus, and the posterior-temporal (PT) network, linking the occipital, retrosplenial, parietal cortex, and temporal lobe through the cingulum and inferior longitudinal fasciculus. The AT network predominantly is involved in affective and item memory whereas the PT network is involved in contextual and spatial memory. Previous research has implicated alterations in the macrostructure (global connectivity) of whole-brain structural networks and lower microstructure (bundle-averaged fractional anisotropy; FA) of white matter in individuals with posttraumatic stress disorder. However, it is currently unknown if altered macrostructural connectivity of AT and PT networks or microstructure of specific tracts within white matter bundles are associated with TR-IM frequency. In this investigation, we examined whether the macrostructure of AT and PT white matter networks or microstructure of specific tracts were associated with higher TR-IM frequency, assessed using ecological momentary assessment (EMA), in a sample of trauma-exposed adults. Specifically, we investigated whether (1) global measures of AT and PT white matter networks were associated with TR-IM frequency and whether (2) long-range connections between network communities (highly interconnected structures within networks) were associated with TR-IM frequency. Additionally, recognizing that bundle-average FA might not be sensitive to changes within small portions of white matter, we used correlational tractography to investigate if (3) the microstructure of specific tracts within bundles correlated with TR-IM frequency.

Methods: Participants were ninety-seven individuals (76 female) exposed to a DSM-5 Criterion A trauma who endorsed at least two TR-IMs per week over the past month. EMA involved 3 daily surveys assessing TR-IMs over a two-week period, with participants completing at least 70% of EMA surveys. TR-IM frequency was quantified as the number of TR-IMs that each participant reported over the EMA period divided by the EMA period duration. Following the two-week EMA period, subjects underwent 3T magnetic resonance imaging using Human Connectome Project protocols. Employing DSI Studio, we applied a restricted network topology approach to quantify global network measures reflecting white matter macrostructural properties, including graph density, global efficiency, and weighted clustering coefficient of AT (90 regions) and PT (70 regions) networks, separately. Networks were derived using the number of tracts (edges) connecting each node (region) pair. We defined the AT and PT networks using the Brainnetome atlas, as it offered a whole-brain parcellation of 246 regions that provided detailed connectivity information for precise selection of regions belonging to each network. Community detection algorithms identified nodes that belonged to distinct communities within each network, and large inter-community tracts were defined using a threshold of at least 50 tracts on average between node pairs belonging to different communities. FDR was used to correct for multiple comparisons in testing of resulting edge-lists. Correlational tractography was conducted in DSI Studio, with varying t-thresholds (2, 2.5, 3), to determine the sensitivity and specificity of results (tracts with significantly lower FA). FDR was set to 0.05 for each iteration. Covariates for network analyses and correlational tractography included age, sex, motion, and number of EMA surveys completed. Spearman partial correlations tested relationships of TR-IM frequency with network outcomes.

Results: TR-IM frequency was not significantly associated with global network measures of AT and PT structural connectivity networks. Greater TR-IM frequency was associated with lower tract count between network edges reflecting the right temporal pole and lateral occipital cortex ($rho = -0.37$, $p = 0.0002$, $pFDR = 0.014$). Correlational tractography revealed that TR-IM frequency was associated with lower FA within portions of the cingulum bundle, namely tracts of the right paraolfactory and right frontal parietal cingulum ($T \geq 3.0$, $FDR = 0.0036$).

Conclusions: We provide evidence that TR-IM frequency relates to altered macrostructure of white matter tracts reflecting the inferior longitudinal fasciculus and lower microstructure of tracts located within the cingulum bundle. These findings suggest that TR-IMs are associated with alterations in white matter circuits involved in affective memory, top-down inhibitory control, and spatial memory. Future work should consider sub-components of the hippocampus within the AT and PT networks, given evidence of different cortical and functional connectivity patterns in relation to TR-IMs.

Keywords: Post Traumatic Stress Disorder, Intrusions, White Matter, Temporal Lobe, Diffusion Weighted Imaging

Disclosure: Nothing to disclose.

P53. Pilot Study of Neurofeedback Enhanced Cognitive Reappraisal Training

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Background: Growing evidence shows that ineffective emotion regulation may contribute to the development and persistence of anxiety, transdiagnostically. Despite the existence of evidenced-based treatments, remission rates are low. Cognitive reappraisal, a core therapeutic skill that involves changing the meaning of a situation (e.g., threatening) to alter its emotional content (e.g., lower anxiety), relies on a set of frontoparietal brain regions, particularly the dorsomedial prefrontal cortex (dmPFC) and the left ventrolateral prefrontal cortex (vlPFC), which are underrecruited in anxiety patients compared to healthy participants.

To date, translations from advancements in the neurobiology of emotion and cognitive processes have been slow to inform novel efficacious treatments. However, real-time functional magnetic resonance image neurofeedback (rt-fMRI-nf) has emerged as a tool to teach volitional control over brain activity, thereby training a skill available to apply in everyday life and offering the potential

to translate neuroimaging research into clinical practice. Previous rt-fMRI-nf has shown promise in training participants to down-regulate emotion production regions, however, less work has focused on upregulation of emotion regulatory regions. We report here work from an ongoing pilot study to 1) develop a cognitive reappraisal task that activates the dmPFC and left vlPFC at the single subject level and 2) establish feasibility to calculate and deliver rt-fMRI-nf targeted at the vlPFC and dmPFC. This pilot study will provide the foundation for a larger sham-controlled design in patients with anxiety.

Methods: Healthy participants, aged 18-55, complete a diagnostic interview, questionnaires, and a 30-minute practice session where they are taught to use cognitive reappraisal. Within 1-5, days, they undergo an fMRI scan while engaging in an Emotion Regulation Task (ERT), adapted from Keller et al. (2021). To optimize the task for rt-fMRI-nf, participants are recruited in phases: During phase 1, participants completed the ERT without neurofeedback to establish recruitment of the dmPFC and vlPFC during cognitive reappraisal. In phase 2, they complete the task with neurofeedback. In phase 2, healthy adults complete the task with neurofeedback designed to increase recruitment of dmPFC and vlPFC. Finally, in phase 3, adults with anxiety complete the task with neurofeedback instructions.

The ERT consists of 5 runs of 18 trials, during which participants view negatively valanced images while instructed to either 'look' at the image without altering their emotional response or to 'reappraise' the image by reinterpreting the situation to reduce negative affect. After each run, participants rate their anxiety on a visual analog scale. A sliding window of 8 TRs is used for the NF signal, beginning 8s after end of the reappraise image presentation of each trial to allow for hemodynamic delay. Participants receive real-time feedback about dmPFC or vlPFC activity from the most recent reappraise>look contrast based on an established reappraisal mask (Langner et al., 2018). The feedback display shows a thermometer, reflecting perfect signal change. Offline standard preprocessing of the data is conducted with SPM12, and first level models for each participant during each run are generated for the reappraise>look contrast.

Results: Results from phase 1 are presented. To date, 5 healthy adults (M age = 37.2 years old; 1 females, 4 males) have been recruited for phase 1. Significant left vlPFC and dmFPC activation was seen at the whole brain level ($pFWE < 0.05$) in 4 out of 5 participants, when instructed to reappraise compared to look at negative images. Only one participant, who self-reportedly had difficulty following task instruction, did not show expected activation. For each individual, activation between the left vlPFC and dmPFC was highly correlated across runs (rs ranged from 0.85-0.99). Participants reported a variety of reappraisal strategies including: imagining that suffering was for the greater good; picturing that the situation was different than initially imagined; thinking the situation would improve in the future; creating a silly story; and imagining the image is a scene from a movie. Subjects with greater activation in both regions reported less stress, anxiety and depression in the past week on the Depression, Anxiety and Stress Scale (rs = -0.22 to -0.38). During the scan, however, within-person increases in dmPFC were associated with decreased anxiety (B = - 10.8), while within-person increases in the vlPFC were associated with increased anxiety (B = 11.4).

Conclusions: Consistent with meta-analyses of emotion regulation, the left vlPFC and dmPFC showed significant activation during cognitive reappraisal compared to passive viewing. Such reappraisal-related brain activity was demonstrated at the single subject level, a necessary prerequisite for rt-fMRI-nf. Participants reported successfully implementing cognitive reappraisal strategies to reduce negative affect, however activation patterns showed different relationships with anxiety during the scan compared to anxiety reported in daily life. Future work will examine if vlPFC and dmPFC activity can be modulated over time with rt-fMRI-nf in a sham-controlled design in patients with anxiety and test the relationship between such neural modulation and changes in self-reported negative affect.

Keywords: Real-Time fMRI Neurofeedback, Cognitive Reappraisal, Prefrontal Cortex

Disclosure: Nothing to disclose.

P54. The Enduring Impact of Maternal Childhood Maltreatment on Brain Structure and Epigenetic Age Acceleration: A Multigenerational Study

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Background: Early life adversity can have long-lasting, detrimental impacts on brain development and behavior. For example, previous work has shown that early life adversity in the form of childhood maltreatment may alter the structure of brain regions involved in threat circuitry, as well as regions involved in sensory processing. These changes have been hypothesized to result from an accelerated pace of biological aging, which can be measured using current approaches such as structural neuroimaging and DNA methylation. Such alterations are believed to be indicative of increased allostatic load, a measure of cumulative chronic stress and life events on biological processes. Additionally, evidence suggests that the effects of childhood maltreatment during an individual's lifetime can be intergenerationally transmitted to subsequent offspring. However, investigations into the intergenerational consequences of childhood maltreatment on epigenetic aging have yielded inconsistent findings, necessitating a more comprehensive understanding of the factors influencing the relationship between maltreatment, age acceleration, and brain structure. Here, we examined this relationship in a deeply-phenotyped cohort of mothers and infants at 4 months and 15 months of age to better understand the long-term impact of early life adversity on brain development and behavior.

Methods: Data was collected from (93 mothers) with previous history of childhood maltreatment and their infant offspring. Childhood trauma history was assessed through the administration of self-report questionnaires that included the Maltreatment and Abuse Chronology of Exposure scale and the Adverse Childhood Experiences (ACE) questionnaire. DNA was extracted from saliva samples and analyzed for DNA methylation levels across a collection of sites shown to be strongly associated with aging. Additionally, structural T1-weighted brain images were acquired to evaluate the integrity of brain regions previously linked to childhood maltreatment.

Results: Results showed that witnessing multiple episodes of interparental and sibling violence was related to lower cortical thickness in both the inferior occipital cortex ($t(34.45) = -4.09$, $p < .001$) and fusiform cortex ($t(24.03) = -2.48$, $p < .05$), in contrast to the control group. Moreover, there was a significant association between the epigenetic age acceleration in mothers and infants at 15 months of age ($R = 0.37$, $p < .05$). Notably, we also observed significant relationships between epigenetic age acceleration and post-natal depression ($t = -0.25$, $p < .05$), exposure to abuse ($t = -3.16$, $p < .01$), and overall PTSD severity ($t = -2.82$; $p < .01$).

Conclusions: These findings demonstrate the enduring consequences of childhood maltreatment on brain development and biological aging. The observed reduced cortical thickness associated with the maltreatment measures highlights the profound impact of early-life adversity on brain structure. Additionally, these

structural alterations, potentially resulting from accelerated biological aging, signify the burden of chronic stress and life events experienced by individuals exposed to childhood maltreatment. Furthermore, the significant association between epigenetic age acceleration in mothers and their infants at 15 months of age suggests a potential intergenerational transmission of epigenetic marks related to early life adversity. This adds further evidence to previous work describing the long-term consequences of childhood maltreatment that potentially affect subsequent generations. Together, these results stand as examples of both the macroscale brain effects and microscale epigenetic effects of early life adversity and contribute to the growing understanding of the profound and lasting impact of early life adversity on brain development and behavior.

Keywords: Childhood Maltreatment, Epigenetic Age Acceleration, Intergenerational Transmission of Trauma, Cortical Thickness, DNA Methylation

Disclosure: Nothing to disclose.

P55. Attentional Focus Modulates Associations Between Pediatric Anxiety Severity and Brain Function During Emotion Processing

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Background: Across the clinical to non-clinical range, anxiety in youth associates with vigilance to negative emotional stimuli and difficulty disengaging from such stimuli. Facilitated initial attention to negative emotions is likely subserved by hyperactivity in bottom-up, automatic threat detection regions like the amygdala. By contrast, impaired top-down attentional control may underlie difficulties disengaging from threat, which is mediated by prefrontal, executive control networks. These networks undergo functional development through childhood and adolescence, complicating inferences drawn from research in anxious youth. More research is needed to identify the shared versus distinct neural mechanisms of anxiety-related attentional patterns in relation to the full spectrum of severity, and to determine how these relationships may vary with age.

Methods: While undergoing functional magnetic resonance imaging, 160 youth (7-18 years) ranging the nonclinical-clinical spectrum of anxiety matched emotional faces or matched shapes in the context of emotional face distractors, probing bottom-up and top-down attention, respectively. Anxiety severity and age were tested as independent and interactive predictors of whole-brain activation, controlling for sex.

Results: During face-matching, anxiety severity was associated with greater recruitment of the left ventrolateral prefrontal cortex, but not slower response times. During shape-matching, anxiety severity predicted greater activation in the left posterior superior temporal sulcus and temporoparietal junction, and slower response times. Across both attention states, anxiety severity was associated with greater right inferior parietal lobule activation. Anxiety also interacted with age to predict activation in the right thalamus and bilateral posterior cingulate cortex during shape-matching, such that anxiety was associated with greater activation in the right thalamus and bilateral posterior cingulate cortex at younger, but not older ages.

Conclusions: Changing attentional focus modulated associations between anxiety severity and brain function. Explicitly attending to emotional stimuli led to the recruitment of emotion regulatory networks in more anxious youth, possibly supporting normal performance during face matching. By contrast, during implicit emotion processing, anxiety severity associated with greater activation in posterior temporal regions and slower responding, suggesting that excessive engagement of early attention networks may be maladaptive in anxious youth. Age also moderated associations between anxiety severity and brain activation during shape-matching, suggesting that the neural substrates of anxiety may change across development. These findings support the potential utility of attention modulation for anxiety and raise the possibility that treatments targeted to neural substrates of attention may need to be tailored to youths' developmental stage.

Keywords: Anxiety, Children and Adolescents, Facial Emotion Processing, Attentional Bias, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P56. The Decision Neuroscience of Craving: Computational Insights from the Lab, the Field, and the Brain

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Background: Although craving is ubiquitous and robustly predicts drug use and eating, we lack an explanatory model that connects the psychological experience of craving to its observed consequences: pursuit of craved options over other valuable alternatives. To address this gap, we developed an experimental framework to measure people's in-the-moment decisions for drugs and food while experiencing craving and tested this framework across several lab-based, EMA, and neuroimaging studies.

Methods: Participants were adults with opioid use disorders and community controls (N=118 lab-based, N=113 EMA, N=45 neuroimaging; ~45% women). The lab-based studies induced specific cravings (e.g., for chocolate or opioids) and tracked changes in subjective value (inferred from economic decisions) for the object of craving and other options as craving evolved, varying both the identity and amount of each option. The EMA study used similar smartphone-adapted tasks across 28 days as participants engaged with their natural environments. The ongoing fMRI study used the lab-based task following craving induction in a single or five repeated scanning sessions.

Results: Craving induction and natural fluctuation in real-world craving selectively increased value in an outcome-specific way: specific cravings mapped to changes in specific values, with limited impact on dissimilar alternatives. For both drug and food craving, these increases were best captured by a multiplicative scaling mechanism. Activity in canonical decision circuits, in particular ventromedial prefrontal cortex, tracked changes in outcome-specific value encoding during craving.

Conclusions: Craving narrows and focuses motivation through a selective, multiplicative gain-like, modulation of value computations, explaining how craving systematically biases decisions toward the object of craving.

Keywords: Subjective States, Computational Psychiatry, Addiction, Valuation

Disclosure: Nothing to disclose.

P57. Virtual Reality Biofeedback to Enhance Emotion Regulation in At-Risk Youth

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Background: Childhood exposure to trauma and adversity is common among youth, and nearly universal among youth in the juvenile justice system. Unfortunately, psychiatric problems are endemic in this population, particularly those featuring impairments in self-regulation. Deficits in self-regulation - the adaptive modulation of physio-affective experience – are a well-identified sequela of adverse childhood experiences known to mediate psychiatric risk. Though treatments targeting self-regulation in exposed youth may be helpful, improvements are modest and often in-accessible to marginalized youth at greatest risk. Innovative approaches are needed to reach this population.

This presentation details findings from a pilot investigation into the efficacy and tolerability of DEEP, a virtual reality video game that integrates respiratory biofeedback to condition adaptive physiological regulation through diaphragmatic breathing. Video games are an ideal treatment vehicle for adolescents (Granic et al., 2014) – nearly 97% of whom play video games on a regular basis – as well as an established vehicle for training cognitive processes, such as effortful autonomic regulation (Grupe et al., 2020). Respiration-based biofeedback is an efficacious technique for augmenting effortful autonomic regulation broadly (Zafar et al., 2020), and in individuals with trauma-related psychopathology specifically (Fonkoue et al., 2020). Drawing on prior work with an affected youth population (Weerdmeester et al., 2020), we anticipated regular game play would enhance self-regulation and reduce trauma-related symptom severity. Extant theory describing the neurobiological substrates of self-regulation further suggested that improvements would be linked to progressive change in bio-signals reflecting activation of the sympathetic and parasympathetic nervous systems.

Methods: DEEP is a virtual-reality (VR) video game providing an immersive underwater adventure that integrates respiratory biofeedback into player controls thereby conditioning the use of diaphragmatic breathing as a means of self-regulation. Respiration is translated in-game to a 'breathing' circle overlaying game play, as well as responsive changes in the color, form, and movement of flora and fauna within the game environment. Youth completed a once-a-day game playthrough (15 min) up to six times during pre-adjudicative detention, with ECG and skin conductance level (SCL) recorded during each session. Prior to game play, youth acclimated to VR over 5 minutes in a 'demo' environment, then provided a 5-minute resting baseline in a calm underwater scene. During gameplay, event markers demarcated incoming biosignal data into six sequential 'zones' of the game environment. Respiratory sinus arrythmia (RSA) and SCL were calculated over the six zones to measure within-session change in parasympathetic and sympathetic levels respectively. At baseline and following game play, youth completed assessments of emotional and behavior problems (CBCL-YSR), Difficulties with Emotion Regulation Scale (DERS), and post-traumatic stress (Child PTSD Symptom Scale – 5). Male and female youth detained in a local juvenile detention center were recruited to participate over a six-month period. Informed consent/assent was obtained from youth and their parent/guardian, a process carefully designed to minimize risk of coercion in this highly vulnerable population.

Results: 17 youth (83% male, 76% Black) completed up to six sessions with DEEP (mean 2.7). As anticipated, 2/3 of youth exhibited clinically significant levels of traumatic stress and over half of participants reported a history of serious abuse or neglect, while depression and anxiety were indicated in 28% and 14% of youth, respectively. Youth broadly found DEEP engaging and enjoyable ($M = 6.3$;$SD = [1.3]$), highly immersive (5.6;[1.7]), and were able to use the biofeedback-integrated navigation (6.14;[1.3]). Conversely, youth reported few difficulties with the game/equipment (1.7;[1.6]) and limited symptoms of 'VR sickness' (e.g., nausea; 2.0;[1.5]). Mixed effects models mapped change in RSA and SCL across game zones. Within-session changes in RSA and SCL activation across zones were moderated by symptom severity. Here, decreasing levels of parasympathetic activation and increasing levels of sympathetic activation were observed among youth with high (+2SD) levels of internalizing problems ($p < .01$; $p < .001$, respectively), greater severity of PTSD symptoms generally ($p < .05$; $p < .001$), and intrusive symptoms specifically (both $p < .001$), while the inverse pattern (increasing parasympathetic, de-creasing sympathetic) was observed among youth with low levels of psychiatric problems. Youth further demonstrated a marked between-sessions decline in emotion regulation impairment ($p < .01$, DERS total score).

Conclusions: Broadly, youth found the VR paradigm tolerable and enjoyable. The trial itself was highly feasible, with no adverse events during these initial pilot trials. Findings suggest that detained youth may improve emotion regulation and autonomic regulation, albeit with moderation by symptom severity. Based on these pilot data, future studies will aim to employ a randomized control trial design to formally test the efficacy of DEEP VR-biofeedback. These initial findings suggest a novel intervention design that is highly engaging and scalable for at-risk youth.

Keywords: Virtual Reality, Adolescent PTSD, Emotional Regulation, Biofeedback, Autonomic Nervous System

Disclosure: Jazz Pharmaceuticals: Consultant (Self).

P58. Psychiatric Disorders and Clinical Diagnoses of Long COVID in US Veterans

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Background: Psychiatric disorders may be a risk factor for long COVID, broadly defined as post-COVID-19 conditions continuing three months post-acute infection. In a sample of US Veterans with a high burden of psychiatric conditions, we examined associations between psychiatric disorders and clinical diagnosis of long COVID.

Methods: We conducted a retrospective cohort study using health records from 660,217 VA patients (12% female) with a positive SARS-CoV-2 test from February 2020 to February 2023. Generalized linear models were used to estimate associations between any psychiatric disorder and the likelihood of subsequent diagnosis with long COVID (i.e., two or more long COVID clinical codes). Models were adjusted for socio-demographic, medical, and behavioral factors. Secondary models examined individual psychiatric disorders and age-stratified associations.

Results: Among 660,217 VA patients with positive SARS-CoV-2 tests (mean [SD] age, 60 [16.4] years; 79,229 [12.0%] female), 56.3% had at least one psychiatric disorder diagnosis and 1.4% were diagnosed with long COVID. Individuals with any psychiatric disorder had higher risk for long COVID diagnosis, both in models adjusted for socio-demographic factors (adjusted Relative Risk, $aRR = 1.32$, 95%CI 1.24-1.41), and additionally adjusted for vaccination status, medical comorbidities, and smoking ($aRR = 1.28$, 95%CI 1.21-1.35). Most specific psychiatric disorder diagnoses were associated with higher long COVID diagnosis risk, including mood, anxiety, and stress-related disorders. Associations between psychiatric diagnoses and long COVID diagnosis were strongest in magnitude among younger adults (age < 40).

Conclusions: Psychiatric disorder diagnoses were associated with increased long COVID diagnosis risk in VA patients, with the strongest associations observed in younger individuals. Improved surveillance, treatment, and prevention for COVID-19 and its long-term sequelae should be considered for individuals with psychiatric conditions.

Keywords: Veterans, Mood Disorders, Anxiety and PTSD, COVID-19 Pandemic, Post-Infectious Sequelae

Disclosure: Nothing to disclose.

P59. Molecular Insights Into FKBP51-Mediated Chronic Posttraumatic Pain Development Using the Single Prolonged Stress Model in Rats and Mice

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Background: Chronic pain is a common and morbid sequela of traumatic stress exposure, and there is an urgent need for novel interventions to prevent chronic posttraumatic pain (CPTP). FKBP51 is a promising therapeutic target for the prevention of CPTP. We recently demonstrated that the administration of the FKBP51 antagonist SAFit2 substantially reduces CPTP-like behavior in rats exposed to the single prolonged stress (SPS) model. These effects depended on the time of administration of SAFit2, with early post-SPS administration resulting in the longest duration of effect. We sought to validate this finding across laboratories and animal species and evaluate differences in circulating corticosterone, immune modulators, and multi-tissue gene expression profiles over time.

Methods: C57BL/6 mice were exposed to SPS +/- SAFit2, a potent and specific FKBP51 antagonist, administered immediately post-SPS. Mechanical thresholds were measured using Von Frey filaments. Blood samples and eight tissues (hypothalamus, left and right hippocampus, amygdala, dorsal root ganglia [L4-L6], DRG, spine, heart, and muscle) were collected from mice and Sprague Dawley rats unexposed to SPS and 3-min, 2-hour, 24-hour, and 72-hours following SPS ($n > 6$ animals per group). Serum corticosterone levels were measured via ELISA, immune modulators via multiplex assays (Meso-Scale Discovery V-PLEX panel-2 including nine biomarkers), and tissue RNA via sequencing. Differential mRNA expression was assessed using DESeq2 and pathway analysis was conducted in IPA (Qiagen).

Results: In mice, similarly to published data in rats, exposure to SPS resulted in prolonged hypersensitivity that was reversed with early administration of SAFit2. In rats, plasma corticosterone levels increased 648x from baseline levels at the 3 min post-SPS timepoint ($t(16) = 44.91$, $p < 0.001$), decreased 0.47x relative to baseline levels at the 2 hour timepoint ($t(24) = 2.17$, $p = 0.035$) and returned to baseline levels at the 24 and 72 hour timepoints. Six immune biomarkers were reliably detected (IL-6, IL-10, IL-13, KC/GRO, IFN-γ, TNF-α) and most of these biomarkers showed SPS-induced changes over time. In each tissue at the 2-hour timepoint, Fkbp5 was amongst the most SPS-induced transcript (in top 1% of all transcripts), with the greatest magnitude of SPS-induction in muscle (7.7x-increase, $t(40) = 13.52$, $p < 0.001$). Though Fkbp5 expression returned to SPS-unexposed levels by 24-hours, dynamic expression changes in other transcripts remained through the 72-hour timepoint, including substantial circadian rhythm pathway gene expression differences in peripheral tissues at the 24 hour timepoint ($p < 0.001$). SPS-induced molecular changes described above were also assessed in mice and/or in the presence of SAFit2; these results will be provided at the meeting.

Conclusions: FKBP51 inhibition in the immediate aftermath of traumatic stress exposure prevented pain behaviors across species. Prevention of these pain behaviors was associated with time-dependent effects on Fkbp5 and downstream gene expression across many tissues, and with changes in post-stress levels of circulating corticosterone and immune modulators. Future studies are needed to understand the exact molecular mechanisms by which FKBP51 inhibition prevents chronic pain behaviors after traumatic stress exposure, and to evaluate behavioral and molecular effects across e.g., sex, age, animal strain, and stress model.

Keywords: Chronic Pain, Non-Opioid, Non-Addictive Therapeutics, Acute Traumatic Stress

Disclosure: Nothing to disclose.

P60. Mapping DNA Methylation Across Major Depression and PTSD in Human Subcortical Regions

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Background: Post-traumatic stress disorder is a debilitating psychiatric disorder with an approximately 7% prevalence in the general population. The differential risk determining those who do versus those who do not develop PTSD is multi-factorial: part genetic and part personal history. Therefore, PTSD is among the most likely of psychiatric disorders to be understood from the perspective of environmental influence interacting with genetic vulnerability because a diagnosis requires a specific highly traumatizing experience. In so far as MDD is one of the most common comorbid psychiatric diagnoses in the PTSD patient population (58.4%) it is the best possible psychiatric control group, and its inclusion allows us to disentangle the unique and divergent epigenetic processes of both disorders.

Methods: We generated DNA methylation (DNAm) data from six postmortem brain regions from 171 individual donors using the targeted next-generation sequencing of bisulfite converted DNA (targeted methyl-seq). Rigorous quality control provided genomic coverage across 1,065,750 CpG sites representing 22,544 genes across two unique diagnostic groups (PTSD and major depressive disorder) compared to neurotypical controls in brain regions (subregions of the amygdala and hippocampus) not extensively examined in previous studies.

Results: We find differential DNAm signatures across all six regions including many non-overlapping, sex-specific differences. Many DNAm signals are present near genes regulating GABAergic transmission such as ELFN1 which has previously been implicated in PTSD interneuron dysfunction and glucocorticoid signaling including CRH1. We also find that DNAm changes aggregate at genes previously implicated in genetic risk for PTSD and its associated clinical phenotypes including KANSL1, MAD1L1, and CRHR1. Remarkably, we also identified significant changes in methylation of the ELFN1, MAD1L1, and WNT5A genes in response to the rapid-acting antidepressant ketamine in a cohort of PTSD patients.

Conclusions: The goal of this effort was to comprehensively measure epigenetic responses (i.e., DNA methylation differences) across discrete brain regions with known involvement in stress pathophysiology and assess these effects on gene expression. Further, we identified convergent and divergent epigenetic regulatory mechanisms between PTSD and MDD highlighting

common pathways and genetic risk links. More generally, we illustrate the value of integrating epigenetic data across multiple brain regions to study complex psychiatric disorders. Our results implicate DNAm as an epigenetic mechanism underlying the molecular changes associated with MDD and PTSD.

Keywords: DNA Methylation, PTSD, Depression, Postmortem Brain Tissue

Disclosure: Nothing to disclose.

P61. Multi-Ancestry Polygenic Risk Scores for PTSD Capture Racialized Structural Inequities and Fall Short of Predictive Utility in Black and Hispanic Individuals

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Background: Polygenic risk scores of PTSD (PTSD-PRS), which aim to quantify an individual's genetic risk for PTSD, can be predictive of PTSD status but often account for a low amount of variance. Advancements in PRS construction methods have included the development of a new multi-ancestry PRS methods (including PRS-CSx) that can improve the PRS' predictive utility in non-European populations; however, the influence of gene-by-environment (GxE) interactions with these scores remain unclear. We examined whether environmental factors, including residential greenspace and neighborhood socioeconomic disadvantage, moderated the association between multi-ancestry PRS (META-PRS) and PTSD symptoms in recent trauma survivors. These factors reflect structural inequities which disproportionately affect Black and Hispanic individuals. Therefore, we also investigated whether the racialization of environmental factors was captured in META-PRS and may affect the predictive utility of these scores in Black and Hispanic participants.

Methods: As part of the AURORA study, trauma survivors (N = 2,139; 52% non-Hispanic Black, 37% non-Hispanic White; 11% Hispanic; 63% female) reported their PTSD symptoms at 4 timepoints post-injury (2-weeks, 8-weeks, 3-months, 6-months). At the first visit, participants provided a blood specimen used to extract DNA. META PRS for PTSD were derived for all participants using PRS-CSx which combined PRS scores that relied on European and African ancestry genome-wide association study summary statistics. Normalized Difference Vegetation Index (NDVI), a measure of greenspace, was derived within a 100m buffer around each participant's home address using high-resolution satellite imagery. Area Deprivation Index (ADI) was calculated using participants' home addresses. Multilevel models evaluated whether interactions between META-PRS and NDVI or ADI predicted PTSD symptoms across time after adjusting for sex, age, lifetime trauma, education, and the first two ancestral principal components. Follow-up analyses examined the relationship between META-PRS and PTSD by ethnoracial group.

Results: Multilevel models revealed significant GxE interactions. In individuals with lower genetic risk, greater NDVI was associated with significantly lower PTSD symptoms across time whereas greater ADI was associated with significantly higher symptoms (pcorrected < .05). There was no significant effect of NDVI or ADI in individuals with higher genetic risk. However, META-PRS was strongly correlated with both greenspace ($r(2137) = -0.15$, $p < .001$) and neighborhood disadvantage ($r(2137) = 0.41$, $p < .001$). In addition, ethnoracial differences in META-PRS mirrored disparities in greenspace and neighborhood disadvantage such that Black participants had significantly higher META-PRS compared to White and Hispanic participants (pcorrected < .001). Finally, META-PRS alone was not related to PTSD development in Black or Hispanic participants but did predict PTSD symptoms in White participants (pcorrected < .05).

Conclusions: Our findings suggest that for individuals with a lower genetic risk for PTSD, malleable environmental factors are related to symptoms across time. However, these GxE interactions may be contaminated by the significant overlap between META-PRS and structural inequities. Participants with the highest META-PRS were more likely to be Black, reside in more disadvantaged neighborhoods, and have less exposure to greenspace. Ultimately, these results suggest that META-PRS may perpetuate socially constructed ethnoracial biases. In addition, we found that for over half of the AURORA sample, the META-PRS did not significantly predict PTSD development. This work emphasizes the need to address diversity and equity in PRS development.

Keywords: PTSD, Polygenic Risk Scores, Race Disparities

Disclosure: Nothing to disclose.

P62. Systems Biology Dissection and Modeling of Traumatic Stress Response Across Brain Regions and Cell Types

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Background: Posttraumatic Stress Disorder (PTSD) and Major Depressive Disorder (MDD) are common and debilitating stress-related disorders. Initial studies focused on profiling molecular alterations within blood samples, while more recent studies have started to explore alterations within relevant brain regions using postmortem brain tissue. However, the underlying molecular mechanisms of stress-related mental disorders still remain unclear. Here, we applied a multi-omic systems biology approach to identify causal gene variants that induce disease-like changes in transcriptomic, epigenomic and proteomic profiles of stress-exposed individuals.

Methods: In this study, we established the largest brain multi-omic, multi-region database to date (n = 300), focusing on PTSD and MDD. Our aim was to identify both shared and distinct molecular alterations across three key brain regions: the central nucleus of the amygdala (CeA), medial prefrontal cortex (mPFC), and hippocampal dentate gyrus (DG). We included bulk brain tissue explorations of all three brain regions, and single-nucleus (sn) RNA-sequencing (RNA-seq data from the dorsolateral PFC (dlPFC; n = 120) to determine cell-type-specific gene expression.

Results: The mPFC exhibited the most significant molecular differences across various omics datasets. Differentially expressed genes (DEGs) and exons were found to be the primary contributors to disease signals, while peptide expression played a crucial role in explaining a significant portion of phenotypic variance. We observed altered methylation patterns primarily in the DG of individuals with PTSD, and unique gene networks were identified in the CeA. Pathway analyses further elucidated the disease-associated expression profiles, linking them to stress hormones, immune, and extracellular-matrix-organization dysregulation. Notably, EPHA2, FKBP5, ICAM1, STAT3, and TNFRSF members were identified as top genes. Additionally, upstream regulators and transcription factors such as TNF, IL1B, NR3C1 (glucocorticoid receptor (GR) gene), and STAT3 were prominently implicated. Through sn-RNA-seq in the dlPFC, we discovered differentially expressed genes mostly in neurons and dysregulated pathways, and upstream regulators in both neuronal and non-neuronal cell types. By conducting multi-omic fine mapping of

PTSD and MDD GWAS data, we identified risk genes that overlapped with the top genes and transcription factors. GR-signaling in iPSC-derived cortical neurons demonstrated greater relevance for PTSD pathology and the opposite direction of regulation compared to MDD, especially in Ex-neurons with the maturity of the neuronal culture influencing the level of concordance.

Conclusions: Overall, our comprehensive dataset suggests a shared molecular pathology underlying PTSD and MDD but also identified differences even in well-understood stress biology pathways (e.g., glucocorticoid signaling). We identified genetic and cell-type-specific mechanisms contributing to these disorders. highlighting potential novel therapeutic targets and brain-informed biomarkers.

Keywords: Prefrontal Cortex, Hippocampus, Amygdala, Multiomics, Single Nucleus RNA Sequencing

Disclosures: Biovie, Circular Genomics, Feel: Advisory Board (Self). Biogen: Employee (Spouse/Partner).

P63. Employing an Improved Serotonin Biosensor to Track Serotonin Release in Cortical and Subcortical Circuitry During Acute Vs. Chronic SSRI Administration

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Background: Serotonin (5-HT) has been linked to a large array of behavioral and affective states, yet technological limitations have historically constrained interrogation of this circuitry. Originating from the raphe nuclei, the 5-HT system exhibits distinct projections to both cortical and subcortical networks, engaging in widespread communication with downstream targets. Notably, the orbitofrontal cortex (OFC) assumes a pivotal role in top-down modulation of learning, while a separate pathway showcases prominent 5-HT projections to the bed nucleus of the stria terminalis (BNST), influencing anxiety circuitry.

In this study, we employed our lab's improved iSeroSnFR1.4 serotonin biosensor, coupled with in vivo fiber photometry, to characterize endogenous 5-HT release in both OFC and BNST during Pavlovian aversive learning. Additionally, we examined the effects of acute (1 day) and chronic (28 days) oral administration of the selective serotonin reuptake inhibitor (SSRI) fluoxetine on 5-HT release in these regions, alongside behavioral measures of aversive learning.

Methods: Experiment 1: All recording was done via fiber photometry using either iSeroSnFR1.0, iSeroSnFR1.4, or iSeroSnFR2.0 expressed in BNST or OFC. Mice then underwent a 3-day fear conditioning paradigm. During the first day of testing mice were exposed to 10 tone trials. On the second day of testing mice were exposed to 20 tone/shock trials. On the third day of testing mice were exposed to 10 tone/shock trials. ($n = 3$-6)

Experiment 2: All recording was done via fiber photometry using iSeroSnFR1.4 expressed in BNST or OFC. Mice were either chronically treated (28 days) or acutely treated (1 day) with 10mg/kg fluoxetine, or were placed into a no drug control group. Mice then underwent the same fear conditioning paradigm as described in Experiment 1. ($n = 2$-5)

Raw photometry data was down sampled to 30 samples per second. The raw data 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∗(raw 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. The opensource tracking software ezTrack was used to calculate freezing behavior.

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 ($p < 0.01$), iSeroSnFR1.4 BNST ($p < 0.01$), and iSeroSnFR1.4 OFC ($p < 0.01$). However, using iSeroSnFR2.0 there was no tone response recorded in BNST while a robust one was recorded in OFC ($p < 0.01$). Histology revealed that iSeroSnFR2.0 did not express in BNST while iSeroSnFR1.0 and iSeroSnFR2.0 both showed regular expression in BNST. All iSeroSnFR constructs showed regular expression in OFC. In the beginning tone/shock trials during, 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 in OFC and iSeroSnFR1.4 in BNST. Freezing behavior increased across all day/trials for all experimental groups.

Experiment 2: AUC analysis shows significantly higher tone response in mice treated with acute fluoxetine in both OFC ($p < 0.05$) and BNST ($p < 0.05$) compared to no drug controls. Mice treated with acute fluoxetine also showed more freezing on day 1 of testing (tone only) compared to no drug controls ($p < 0.05$). We found no significant differences in mice treated with chronic fluoxetine although this may be due to the low sample size ($n = 2$) and we hypothesize we may find differences between chronic fluoxetine treated mice and acute fluoxetine and/or no drug treated mice as sample size increases.

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 specificity. Here my work demonstrates that both iSerSnFR1.4 and iSeroSnFR2.0 are 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 the improved iSeroSNFRs allows for the collection of a completer and more accurate picture of serotonin release dynamics than is possible with iSeroSNFR1.0.

Result 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. We also show that the administration of fluoxetine alters serotonin dynamics at the synapse during distinct behavioral events. As we continue to gather data we expect to gain a deeper understanding about how acute versus chronic SSRI administration impacts serotonin availability at the synapse and how that correlates to fear behavior.

Keywords: Functional and Structural Neuroimaging, Serotonin, Selective Serotonin Reuptake Inhibitors (SSRIs), Auditory Fear Conditioning, Optical Biosensors

Disclosure: Nothing to disclose.

P64. 7T Proton Magnetic Resonance Spectroscopy Study of Neurometabolites in the Dorsolateral Prefrontal Cortex of Individuals With Posttraumatic Stress Disorder

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Background: Converging lines of evidence point to glutamatergic dysfunction in posttraumatic stress disorder (PTSD). Although a few in vivo magnetic resonance spectroscopy (MRS) studies have shown abnormalities in glutamate in individuals with PTSD, no PTSD MRS studies have used 7T, which has better signal-to-noise ratio and spectral resolution, thereby providing better spectral quality and higher sensitivity to detect more metabolites. We currently lack PTSD MRS studies of the lateral prefrontal cortex despite its role in emotion regulation and cognition, which are affected in individuals with PTSD. The objective of this study was to use 7T proton MRS to investigate glutamate and other neurometabolite concentrations in the left dorsolateral prefrontal cortex (DLPFC) of participants with PTSD, trauma-exposed participants without PTSD, and participants without trauma exposure. We hypothesized that individuals with PTSD would have lower glutamate levels compared to trauma-exposed individuals without PTSD and individuals without trauma exposure. Additionally, we explored the relationship between glutamate and psychiatric symptom severity as well as potential alterations in other neurometabolites.

Methods: The sample consisted of 27 participants with PTSD, 27 trauma-exposed participants without PTSD (TE), and 26 participants without trauma exposure (NT). PTSD Criterion A trauma exposure was determined from the Life Events Checklist for DSM-5 extended version. The severities of PTSD, depression, anxiety, and dissociative symptoms were measured using the PTSD Checklist for DSM-5, Beck Depression Inventory, Beck Anxiety Inventory, and Multiscale Dissociation Inventory, respectively. MRS data were acquired on a 7T Siemens MAGNETOM scanner at the Auburn University MRI Research Center. Three-dimensional structural images were acquired for anatomical reference and segmentation. Following FASTESTMAP shimming and voxel-based flip angle calibration, spectra were acquired from a voxel in the left DLPFC (25 x 25 x 25 mm) using an ultra-short TE STEAM sequence (TE/TR/TM = 5/10,000/45 ms, 32 averages, 4 kHz spectral bandwidth, 2048 points) with outer volume suppression and VAPOR water suppression. Spectra without water suppression (4 averages) were acquired for pre-processing and quantification. Spectra were processed and fit with Osprey and LCModel. Tissue- and relaxation-corrected molal concentration estimates (mmol/kg of tissue water) were calculated for each metabolite. Glutamate was the primary metabolite of interest. Other metabolites examined were glutamine, Glx (glutamate + glutamine), gamma-aminobutyric acid, N-acetylaspartate (NAA), N-acetylaspartylglutamate, total NAA, total choline, creatine, phosphocreatine, total creatine, glutathione, lactate, and myo-inositol. Statistical analyses were conducted in R/RStudio. Metabolites were compared between groups using ANCOVA controlling for age and sex. Statistical significance was set at Bonferroni-corrected $p < 0.0036$ (0.05/14 metabolites). Post hoc pairwise t-tests with Tukey-adjusted p-values were conducted if the overall group difference was significant. Pearson correlation coefficients were used to explore the association between glutamate and scores on the PCL-5, BDI, BAI, and MDI. Statistical significance was set at Bonferroni-corrected $p < 0.0125$ (0.05/4 symptom scales).

Results: The groups were comparable in age and sex. There was a significant group difference in glutamate ($F(2,71) = 6.17$, $p = 0.003$). Glutamate was significantly lower in the PTSD group compared to the NT group ($p(Tukey) = 0.005$, Cohen's $d = 0.92$) and significantly lower in the TE group compared to the NT group ($p(Tukey) = 0.02$, Cohen's $d = 0.80$). There was a significant group difference in NAA ($F(2,72) = 6.20$, $p = 0.003$). NAA was significantly lower in the PTSD group compared to the NT group ($p(Tukey) = 0.002$, Cohen's $d = 0.99$). There was a significant group difference in lactate ($F(2,68) = 6.18$, $p = 0.003$). Lactate was significantly higher in the PTSD group compared to the NT group ($p(Tukey) = 0.002$, Cohen's $d = 1.01$). There were no significant group differences for the other metabolites. Glutamate was negatively correlated with BDI scores in the combined sample ($r = -0.23$, $p = 0.046$), and this association was driven by the PTSD group (PTSD: $r = -0.47$, $p = 0.01$; TE: $r = 0.17$, $p = 0.42$; NT: $r = 0.19$, $p = 0.36$).

Conclusions: In this first 7T MRS study of PTSD, we observed that individuals with PTSD had lower glutamate, lower NAA, and elevated lactate compared to individuals without trauma. Glutamate was also reduced in trauma-exposed individuals without PTSD compared to those without trauma exposure. The results of our study add to the growing evidence of glutamatergic dysfunction in individuals with PTSD. We also provide evidence of abnormal levels of NAA and lactate, which is consistent with prior studies of PTSD and other psychiatric disorders. Further research is needed to unravel the precise mechanisms underlying glutamate alterations in individuals with PTSD and their implications for the development and treatment of the disorder. Interventions that enhance glutamatergic function and promote neuronal health are promising targets for drug development. High-field MRS offers insight into the neurometabolic abnormalities associated with PTSD and is a powerful tool to probe trauma- and stress-related neurotransmission and metabolism in vivo.

Keywords: PTSD, Magnetic Resonance Spectroscopy, 7T MRS, Glutamate, Dorsolateral Prefrontal Cortex (DLPFC)

Disclosure: Nothing to disclose.

P65. The Cross-Sectional Relationship Between Bed Nucleus of the Stria Terminalis (BNST) Grey Matter and Late-Life Worry and Cognitive Reappraisal

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Background: Worry is a core symptom present in late-life anxiety and mood disorders. However, the underlying neuroanatomy has been relatively understudied. The bed nucleus of the stria terminalis (BNST) plays an important role in sustained anxious apprehension and stress responses, but its relationship to worry in humans has received limited attention.

Methods: We recruited 131 older adults (> 50years) with varying worry severity to undergo MRI and assessments measuring anxiety phenotypes (worry, rumination, global anxiety), neuroticism, depression, stress, and tendency to use cognitive reappraisal and suppression. We applied an in-house automated segmentation approach to segment BNST, conducted manual quality control checks, and extracted BNST volumes with 0.1 threshold. We conducted multiple imputations on missing data and conducted pooled regression on total BNST volumes (sum of components, log10 transformed) with the following independent variables: age, sex, race, education, intracranial volume, cumulative illness severity (CIRS-G) and worry, rumination, anxiety, depression, reappraisal, suppression, stress, and neuroticism. We ensured that variance inflation factor < 5.

Results: We found that greater worry was associated with lower BNST volume [$B = -0.005$ (0.002), pooled $t(14) = -2.1$, p-value < 0.05] while greater habitual use of reappraisal was associated with greater BNST volume [$B = 0.007$ (0.003), pooled $t(14) = 2.3$, p-value < 0.05]. No association was found between BNST volume and rumination or global anxiety.

Conclusions: BNST volume is negatively associated with worry but positively associated with cognitive reappraisal. This highlights the complex association between BNST and severe worry in late-life, and the potential protective role of the habitual use of reappraisal.

Keywords: Worry, Human Neuroimaging, Functional MRI (fMRI), Anxiety, Geriatric

Disclosure: Nothing to disclose.

P66. Spatial, Temporal, and Circuit-Specific Activation Patterns of Basolateral Amygdala Projection Neurons During Stress

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Background: The basolateral amygdala (BLA) is reliably activated by psychological stress and can influence a wide range of behavioural and physiological changes evoked by stress exposure. However, there is poor understanding of the anatomical specificity of amygdala subregions, circuits, and cell types explicitly activated by stress, and of its role in regulating typical responses to stress such as activation of the hypothalamic-pituitary-adrenal (HPA) axis and subsequent release of corticosterone (CORT). Our overarching aim was to conduct a systematic investigation of the spatial, temporal, and circuit-specific activation patterns of BLA projection neurons during exposure to acute stress, and to explicitly test the role of the BLA in activation of the HPA axis. Additionally, we focused on two neuromodulatory systems, norepinephrine (NE) and corticotropin-releasing hormone (CRH), that may contribute to activation of the BLA during stress.

Methods: All experiments were performed in adult male rats or mice. We used immunohistochemistry for the activity-responsive protein c-fos to map spatial patterns of BLA activation to a range of stressors (n = 8-10 per group). We then used fiber photometry to map temporal patterns of activation (GCaMP6s) and norepinephrine release (GRAB:NE) in the BLA (n = 4-8 per group), and subsequently tested if systemically blocking ß-noradrenergic signalling altered temporal patterns of BLA activation during stress (n = 6-7 per group). Using restraint stress as a prototypical model for stress, we combined c-fos mapping with retrograde tracing (CTB) to map the topographical distribution of BLA projection neuron populations and identify circuit-specific patterns of activation during stress (n = 5-8 per group), focusing on BLA circuits targeting the nucleus accumbens (NAc), bed nucleus of the stria terminalis (BST), central amygdala (CeA), lateral hypothalamus (LH), prelimbic cortex (PrL), and ventral hippocampus (VH). To test the functional contribution of BLA projection neurons during stress, we used chemogenetic (n = 7-10 per group) and optogenetic tools (n = 7 per group) to inhibit or activate, respectively, a broad population of BLA projection neurons (CaMKii+) and measured subsequent release of CORT. We then used a dual-virus cre-dependent approach to restrict expression of the inhibitory DREADD to discrete projection neuron populations (BLA-NAc, BLA-BST, BLA-PrL, and BLA-CeA; n = 4-11 per group), allowing us to investigate the effects of inhibition of isolated populations on stress-induced CORT release. Finally, we used a transgenic CRHR1-tdTomato rat line in combination with c-fos immunohistochemistry to map the topographical distribution and stress-induced activation of CRHR1+ neurons in the BLA in both male and female rats (n = 5 per group). Group differences were compared using ordinary one-way ANOVA, 2Way RM ANOVA, and independent samples t-tests.

Results: All stressors reliably and specifically evoked a similar spatial pattern of activation in the BLA, with a bias in c-fos expression to the medial basal amygdala (mBA). In contrast, different stressors led to different temporal patterns of activation. In particular, we observed a robust initial calcium-related response to restraint stress, with a similar temporal pattern of NE release in the BLA. Additionally, we found that systemic administration of propranolol, a ß-noradrenergic antagonist, significantly reduced calcium-related response to restraint stress ($p = 0.0436$). Anatomical mapping revealed that 6 different BLA projection neuron populations were heterogeneously distributed throughout the BLA, but that all populations were activated by acute restraint stress, with a particular bias in activation of each population towards the mBA. Chemogenetic inhibition of CaMKii neurons prior to stress significantly attenuated stress-induced CORT ($p = 0.0167$), and optogenetic stimulation significantly increased CORT in the absence of any stressor ($p = 0.0104$). However, chemogenetic inhibition of isolated projection neuron populations did not significantly alter stress-induced CORT. Finally, mapping of CRHR1+ neurons in the BLA revealed that this molecularly defined population is largely expressed in the lateral amygdala and is significantly activated during exposure to restraint stress ($p = 0.0492$).

Conclusions: We identified the medial basal subdivision (mBA) of the BLA as a specific subregion that is specifically and robustly activated by a range of different stressors, and contributes to stress-induced activation of the HPA axis through projections to a range of downstream targets. Inhibition of a wide range of collective projection neuron populations (i.e., CaMKii+) reduced stress-induced CORT, but inhibition of isolated projection neuron populations had no effect on their own. Further, we found that the BLA is heterogeneously structured in topographical distribution of projection neuron populations (targeting the NAc, BST, PrL, BST, LH, and CeA) and molecular phenotype (CRHR1+). Together, this emphasizes the heterogeneity of BLA projection neurons, while providing evidence that a large, diverse population are activated by exposure to acute psychological stress to collectively drive the HPA response to stress. Our findings in particular point towards the mBA as an important subregion of the BLA involved in the response to stress, and further supports the role of the NE and CRH neuromodulatory systems in stress-induced activation of the BLA.

Keywords: Acute Stress, Neural Circuits, Basolateral Amygdala, Amygdala, Corticosterone

Disclosure: Nothing to disclose.

P67. Biological Markers of Ovarian Function in Women With PTSD

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Background: Posttraumatic stress disorder (PTSD) is associated with increased sympathetic nervous system reactivity and dysregulation of hypothalamic-pituitary-adrenal (HPA) axis function. There are known interactions between the HPA axis and the hypothalamic-pituitary-gonadal (HPG) axis. The HPA axis, which activates in response to stress, may impair ovulation by delaying or inhibiting the luteinizing hormone surge. The hypersecretion of stress hormones has been shown to impair oocyte maturity. While several studies have found an association between emotional

distress, anxiety, and depression with reproductive failure, little is known about the relationship between PTSD and ovarian aging.

This study examined the association between PTSD and markers of ovarian aging, including Anti-müllerian hormone (AMH), follicle stimulating hormone (FSH) and the reproductive hormones, including estradiol and androgens (testosterone, androstenedione). AMH is a glycoprotein hormone secreted by granulosa cells of preantral and small antral follicles and an indicator of ovarian function and reserve. AMH levels are highly correlated with follicle maturation, oocyte number, and ovarian volume. Abnormally low AMH levels have been associated with premature ovarian failure in young women and predictive of menopause. Another widely used indicator of ovarian function is follicle stimulating hormone (FSH), a gonadotropin that is secreted by the anterior pituitary gland which activates FSH receptors on granulosa cells to stimulate estradiol synthesis and promote oocyte maturation during the follicular phase of the menstrual cycle. The reproductive hormone estradiol is produced by the ovaries and contributes to maturation and release of an egg as well as thickening of the uterine lining. To date, evidence supporting the link between chronic stress and these outcome measures has been equivocal and limited in scope, but little is known about indicators of ovarian aging in women with diagnoses of PTSD. Given the prolonged and sympathetic and HPA axis alterations in PTSD, we examined whether there was effect of PTSD on indicators of ovarian aging. We hypothesized that women with PTSD would have lower AMH, FSH, and E2 levels than women without PTSD.

Methods: Participants included 26 medically healthy women aged 26-55 with a lifetime history of trauma exposure. Participants were evaluated for PTSD symptomatology using the Clinician Administered PTSD Scale for DSM-4 and screened for other current and lifetime Axis 1 disorders using the Structured Clinical Interview for DSM-IV Diagnosis-Non-patient Version. Blood samples were collected on menstrual cycle day 2 and enzyme-linked immunosorbent assays were performed to determine AMH and FSH levels. Plasma samples were assayed by Ultra-Pure Liquid Chromatography (UPLC) Mass Spectrometry (MS) to determine reproductive hormone levels.

Results: Correlational analyses revealed associations between older age and decreased AMH values. Additionally, a significant positive correlation was found between AMH level and androstenedione ($r(24) = 0.41$, $p = 0.04$). No other significant correlations between primary study variables were observed. Analysis of covariance analyses adjusting for age and BMI indicated that AMH levels were significantly lower in participants with PTSD compared to those without PTSD ($F(1,1) = [5.97]$, $p = 0.03$). No group differences were found for FSH or E2 levels in women with PTSD vs. controls.

Conclusions: Our results indicated that AMH levels were decreased in women with PTSD compared to women without PTSD. AMH is known to be a key marker of ovarian reserve and follicular development and has been shown to be highly correlated with follicle maturation, oocyte number, and ovarian volume, and decreased levels can predict premature ovarian failure in younger women and the development of menopause during aging. Our finding of lower AMH levels in women with PTSD suggests a potential mechanism through which trauma and psychological distress may impact reproductive health.

We also found a relationship between AMH levels and androstenedione. While these two hormones are distinct and serve different functions, androstenedione is converted to estrogen in the ovaries, therefore playing an important role in the regulation of the menstrual cycle and maturation and release of mature eggs during ovulation. High levels of both AMH and androstenedione may be explained by polycystic ovarian syndrome (PCOS), a common hormonal disorder characterized by high levels of androgens and commonly associated with infertility. While there were no other indications of PCOS in this current sample, future work may consider the role of PCOS in infertility amongst women with PTSD.

These findings suggest that trauma and psychological distress may have an impact on women's ovarian function. Alternatively, it is possible that reproductive hormones may have an influence on the HPA axis and stress responsivity. Further research is needed to disentangle the relationship between PTSD and ovarian function in women exposed to traumatic events. Clinical implications include the provision of appropriate support and interventions to address the mental and reproductive health needs of trauma-exposed women.

Keywords: PTSD, Ovarian Hormones, Women's Mental Health

Disclosure: Nothing to disclose.

P68. Effects of Estradiol Withdrawal on Neural Reactivity to Social Threat Cues

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Background: Preclinical evidence suggests that estradiol (E2), the primary ovarian hormone, facilitates plasticity in neural circuits involved in the adaptive regulation of fear. This may impact fear regulation in stress-related disorders. Moreover, women experience twice the risk for posttraumatic stress disorder (PTSD) following a traumatic event, relative to men. We hypothesized that changes in E2, such as the sharp decline of E2 immediately following ovulation, might promote a hyper-responsive state in the brain's threat regulation networks in women. We tested whether E2 withdrawal following ovulation would be associated with higher amygdala and lower vmPFC responses to threat cues, conducting fMRI scans under either natural withdrawal (placebo patch), or blockade of E2 withdrawal using a transdermal E2 patch in a randomized double-blind placebo-controlled crossover study.

Methods: $N = 143$ Black and African American women ages 18-35 participated, all with regular menstrual periods and not taking hormonal medications. Shortly after ovulation, LH + 3-7 days, $n = 50$ received 100ug E2 or placebo and completed fMRI neuroimaging with social threat, fear conditioning, and extinction tasks. Participants crossed over to the other condition following ovulation on a subsequent cycle ($n = 25$ began with estradiol, and $n = 25$ began with placebo). FMRI scans were collected using multi-echo multi-band echo-planar imaging (3 echoes; TR = 1100 ms, voxel size = 2.5mm3), which in pilot testing created a drastic increase in temporal signal-to-noise ratio in the amygdala, relative to comparable single-echo data. Data were preprocessed using fMRIprep 20.2.3 and analyzed in SPM12. We defined fMRI reactivity to social threat cues as the comparison of fearful>neutral face stimuli. PTSD was diagnosed using the CAPS-5, but analyses focused on symptom severity assessed at the first MRI visit using the PCL-5.

Results: $N = 3$ did not complete the social threat task during visit1 (omitted due to time constraints, or discomfort with the quick stimulus presentation), and $N = 5$ did not complete the task during visit2. Therefore, 90 total scan visits from $n = 48$ participants were included in the final analysis. $N = 37$ experienced a DSM-5 Criterion A trauma; $n = 8$ diagnosed with current PTSD. PCL-5 scores ranged from 0-60, $M = 14.42$. In the social threat fMRI task, the right amygdala and anterior hippocampus were more responsive to fearful than neutral faces ($pFWE < 0.05$) under both estradiol and placebo. Generalized linear modeling of regions-of-interest (ROIs) for the amygdala, hippocampus, and

vmPFC showed no significant E2 effects, controlling for whether E2 was administered on the first versus second visit. There was also no interaction of E2 with PTSD symptom severity. However, whole-brain voxel-wise analyses showed an E2 effect on reactivity to social threat cues in the right inferior frontal gyrus ($Z = 3.72$, cluster $pFDR = 0.037$, $k = 82$, $xyz = 46\ 20\ -4$), and right parahippocampal cortex extending into the right fusiform gyrus ($Z = 3.8$, cluster $pFDR = 0.037$, $k = 91$, $xyz = 34\ -58\ -6$). These regions are important for non-verbal memory encoding and adaptive emotion regulation. No region showed an E2 * PCL-5 interaction.

Conclusions: Importantly, the findings did not support the hypothesis that E2 might dampen amygdala reactivity to social threat cues, or facilitate vmPFC engagement. However, E2 did appear to increase the engagement of regions important for the encoding of configural, non-verbal information. During post-ovulation E2 withdrawal, distributed networks that encode emotional social information may be dampened, whereas blocking withdrawal allowed for a hormonal potentiation of these regions' activity. Further analysis of the emotional memory tasks (fear conditioning, extinction; not analyzed here) will provide additional insight into behavioral memory performance during E2 withdrawal.

Keywords: fMRI, Threat Reactivity, Estradiol, Neuroendocrine, Stress and Trauma

Disclosure: Nothing to disclose.

P69. The Indirect Effect of Early Life Stress on Metabolic Risk via Plasminogen Activator Inhibitor-1

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Background: Early life stress (ELS) is a well-known contributor to cardiometabolic risk. The mechanism through which ELS impacts the development of obesity and cardiometabolic disease is incompletely characterized and likely multifactorial in nature. One potential pathway is through Plasminogen Activator Inhibitor-1 (PAI-1), a serine protease inhibitor that acts within the fibrinolytic system and has been associated with poor cardiometabolic outcomes. In this study, we aimed to characterize the interplay between early life stress (ELS), metabolic syndrome (MetS), and plasminogen activator inhibitor-1 (PAI-1), an inhibitor of the fibrinolytic system implicated in cardiometabolic disease. We also examined the understudied intersection of ELS, exercise, and PAI-1.

Methods: Adults ages 18-40 ($N = 200$; 68% female) with no known cardiometabolic or thrombotic conditions were recruited from the community. Participants with ELS ($N = 118$) experienced childhood maltreatment and/or parental loss ($n = 92$). Controls ($N = 82$) had no history of childhood maltreatment or parental loss. Blood pressure and anthropometrics were measured, and fasting plasma samples were assessed for markers of metabolic risk. Total PAI-1 was assayed using the Bio-Plex Pro Human Diabetes Panel (Bio-Rad Laboratories). A composite metabolic risk Z score (MetS Z score) was computed from the mean Z scores of waist-to-height ratio, systolic and diastolic blood pressure, triglycerides, cholesterol (total, HDL, and LDL), glucose, and hemoglobin A1c.

Results: ELS was linked to a higher MetS Z score in adulthood via increased circulating PAI-1 levels (Average Causal Mediation Effect [ACME] $= 0.07$, $p = 0.036$). Conversely, ELS was also linked to higher PAI-1 levels via higher MetS Z scores (ACME $= 0.017$, $p = 0.008$). There was a significant interactive effect of ELS and exercise on PAI-1 levels ($p = 0.03$), such that engaging in higher levels of daily exercise was linked to lower PAI-1 levels in individuals with ELS.

Conclusions: These findings suggest that the association between ELS and MetS may be partially carried by circulating PAI-1 levels, and that this effect is likely bidirectional in nature. Among individuals with ELS, exercise is linked to lower PAI-1 levels, suggesting a potential direction for early intervention.

Keywords: Cardiometabolic Risk, Early Life Stress, Childhood Adversity

Disclosure: Nothing to disclose.

P70. Peripheral Mast Cells are Sufficient to Reduce Anxiety in a Mast Cell Knockout Model

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Background: Anxiety disorders are the most prevalent mental disorder in the US, but the available treatments have suboptimal efficiency and tolerability. Mast cells (MCs), innate immune cells best known for their role in allergic responses, could represent a novel therapeutic target for anxiety disorders. MCs are ubiquitous residents of tissues throughout the body, including the brain; they express a diverse array of receptors that allow them to respond to biological, physical and psychological stressors, and they release a plethora of mediators that could directly affect neuronal function, including cytokines, growth factors (NGF, VEGF), and neurotransmitters (histamine, serotonin, dopamine). Together, these suggest that MCs are uniquely positioned to coordinate the multisystem responses to environmental and internal cues driving behavioral adaptation to stress. Based on initial findings that MC knockout c-Kit w-sh (sash) mice show increased baseline anxiety (Nautiyal et al, 2008), here we used a combination of tissue-specific MC transplant, pharmacology, and behavioral analyses to uncover the underlying mechanisms.

Methods: 1) We first used the elevated plus maze, sucrose preference, and social interaction tests to compare the baseline anxiety and motivated behaviors of wild type (WT) and sash male and female mice. 2) To assess whether behavioral differences between genotypes were driven by a specific population of MCs, we next harvested bone marrow progenitor cells from WT mice, cultured them in media supplemented with stem cell factor and Il-3 for 6 weeks to generate bone marrow derived mast cells (BMMC), and used intraperitoneal (IP) or intracerebroventricular (ICV) injections to specifically reconstitute MCs in Sash peripheral vs. central tissues. Tissue-specific reconstitution was confirmed post-mortem using T-blue staining. 3) Eight weeks after MC reconstitution, we compared the behavior of WT, non-reconstituted sash, and IP or ICV MC reconstituted sash. 4) Based on previous findings that, compared to WT, sash show reduced hippocampal BDNF and serotonin, in a separate group we used a single dose of 1mg/Kg ketamine or a single or 14 daily doses of 20mg/Kg fluoxetine to test whether commonly used anxiolytics targeting NMDA receptors or serotonin transporter, respectively, could reduce exaggerated anxiety in sash. All experiments used a sample size of $n = 8$-16. After finding no sex differences in behavioral phenotypes, all behavior tests were analyzed using two-tailed t-tests (genotype) or one-way ANOVAS (genotype, treatment) followed by multiple comparisons.

Results: 1) We first found that, compared to wild type mice, MC knockout c-Kitw-sh (sash) mice show reduced time spent in the

open vs closed arms in the elevated plus maze ($p = 0.01$), sucrose avoidance ($p = 0.002$), and increased social vigilance ($p < 0.001$). 2) Next, we found that both, peripheral and central reconstitution with BMMCs "normalized" behavior in sash (open/closed arms WT vs. non reconstituted sash $p = 0.03$, no differences between WT and IP or ICV sash; sucrose preference WT vs. non reconstituted sash $p = 0.04$, no differences between WT and IP or ICV sash). 3) A single dose of Ketamine (open/closed arms sash veh vs ketamine $p = < 0.001$) as well as chronic (open/closed arms sash veh vs ketamine $p = < 0.001$), but not acute fluoxetine treatment, were sufficient to "normalize" behavior in sash.

Conclusions: Replicating previous results, here we found that sash, compared to WT, show a phenotype consistent with baseline increased anxiety. Surprisingly, we also found that in sash mice, IP administration of BMMCs, which reconstituted peripheral, but not central tissues, was just as effective at reducing anxiety as ICV administration of BMMCs. Finally, we found that the hyper anxious phenotype in sash can also be reversed by acute treatment with Ketamine as well as chronic treatment with fluoxetine. Together, these results suggest that MCs play a fundamental role in preventing exaggerated baseline anxiety possibly through modulation of BDNF/serotonergic signaling. Moreover, they support the intriguing idea that peripheral MCs can directly affect brain function and behavior, providing a novel mechanism for peripheral-central interactions. Current studies are using RNAseq analyses of multiple brain areas in sash and WT mice to identify pathways affected by peripheral MC reconstitution.

Keywords: Mast Cells, Neuroimmune Communication, Anxiety, Ketamine

Disclosure: Nothing to disclose.

P71. Microglial Acid-Sensing Regulates Panic-Relevant Outcomes Following Ethanol Withdrawal

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Background: Alcohol's effects on the body are complex, altering behaviors, emotions and physiology. These effects all contribute to the cycle of alcohol use and abuse that leads to alcohol use disorders (AUDs). They also contribute to the development of comorbid psychiatric disorders, like panic disorder (PD), which are common. AUD treatments are limited and comorbid AUD-PD results in worse treatment outcomes, pointing to a need for novel treatments. AUDs and PD have overlapping genetics and symptomology (e.g., alcohol withdrawal and panic attacks both involve extreme anxiety, hyperventilation and cardiovascular effects). This suggests AUD and PD share underlying molecular mechanisms and neurocircuitry. Despite unifying attributes and high comorbidity, shared mechanisms contributing to AUD, PD, and comorbidity are not well understood. Identifying these mechanisms and developing AUD-PD relevant rodent paradigms will improve our understanding of AUD and PD, and identify novel therapeutic targets.

Emerging evidence supports dysregulated acid-base homeostasis as a shared mechanism in AUD and PD. Maintaining physiological homeostasis (e.g., neutral pH) is critical for survival, and threats to homeostasis elicit behavioral, emotional and physiological responses directed toward this goal. Alcohol use induces acidosis which is positively correlated with withdrawal severity. Strong evidence supports dysregulated acid-sensing in PD, but its role in AUDs is not well understood. However, studies show individuals with PD or AUD are more sensitive to homeostatic threats like CO2 inhalation (non-hypoxic) which produce acid-base imbalance. CO2 inhalation elicits greater anxiety, hyperventilation, and cardiovascular effects (even panic attacks) in PD patients, in previously alcohol-dependent individuals, and during alcohol withdrawal. Thus, acid-sensing mechanisms likely contribute to PD and AUD pathophysiology and comorbidity.

Our lab recently found a role for a novel microglial acid-sensor T-cell death associated gene 8 (TDAG8) in panic-relevant behavior and physiology. TDAG8 mediates these effects through neuroinflammatory signaling within the subfornical organ (SFO). The SFO has a leaky blood brain barrier and helps maintain physiological homeostasis by regulating behaviors, respiration and cardiovascular function. Lesions of the SFO reduce ethanol consumption. There is also strong support for neuroimmune factors in AUDs. Therefore, TDAG8 within the SFO may provide a unique neuroimmune "body-to-brain" mechanism of relevance to both AUD and PD.

We previously showed that acid-sensing receptor TDAG8 regulates ethanol consumption and aversive outcomes (ataxia, respiratory depression) associated with alcohol use and that that these effects could be mediated through neuroimmune signaling within the SFO. Here we investigated whether TDAG8 contributes to the development of comorbid AUD-PD by testing the hypothesis that ethanol-evoked upregulation of TDAG8 exacerbates panic relevant physiological and behavioral outcomes during withdrawal from alcohol dependence.

Methods: Male and female TDAG8 knockout (TDAG8-/-) or wild-type (TDAG8 + /+) littermates were used (8-16 weeks old; n = 5-12). To determine if ethanol increases TDAG8 expression, we quantified TDAG8 mRNA by RTqPCR following fluorescence activated cell sorting in transgenic mice 24h after injection with 2g/kg ethanol. To determine the effect of ethanol dependence and TDAG8 on panic-relevant outcomes, we investigated the effect TDAG8 knockout on respiration (using plethysmography) and CO2-evoked defensive responding (panic-relevant model) following withdrawal (24h post EtOH) from alcohol dependence (9 day EtOH+ 4MP injections). Following ethanol exposure, we used immunohistochemistry to determine effects of TDAG8 on ethanol-evoked neuroinflammation. Fixed brain tissue was stained for microglial marker IBA-1 and microglia soma perimeter was then quantified. Statistical analysis was preformed using Students t test, ANOVA, 2-way ANOVA or 3-Way repeated ANOVA as needed.

Results: Ethanol increased TDAG8 within the SFO and peripheral monocytes ($p < 0.05$), but not another circumventricular organ expressing TDAG8, the OVLT. Preliminary evidence suggests increases in baseline respiration during withdrawal from alcohol dependence were attenuated in TDAG8-/- mice ($p < 0.05$). Further, responses to CO2 inhalation were blunted in TDAG8-/- compared to TDAG8 + /+ littermates ($p = 0.07$). TDAG8-/- mice also had reduced microglial soma perimeters compared to TDAG8 + /+ mice ($p < 0.05$) following ethanol exposure, which was specific to the SFO.

Conclusions: Together, these data point to acid-sensing receptor TDAG8 as a novel regulator of behavioral and physiological outcomes following withdrawal from alcohol dependence. Further, they suggest that these effects could be mediated through neuroimmune signaling within the SFO. Overall, these data support the SFO-TDAG8 and associated neuroimmune effectors as a unique and novel shared mechanism for AUD and panic disorder. They also suggest acid-sensing receptors may represent a novel treatment target for alcohol use disorders, particularly when co-occurring with panic disorder.

Keywords: Alcohol Withdrawal, Panic, Acid-Sensing

Disclosure: Nothing to disclose.

P72. C-Reactive Protein Expression and its Role in PTSD-Like Behavior 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: Wild-type and CRP null male and female C57BL6J mice were exposed to either predator stress (10 minutes roomed with a laboratory cat) or handled (stress control). After one week, mice were tested for avoidance behaviors by open field and light-dark box tests. Two weeks post predator stress, avoidance of trauma cues was assessed by measuring exploration of a cue scented with dirty cat litter. Following the trauma reminder paradigm, mice were tested for baseline fear extinction by pairing five separate twenty-second tones (75 dB, 4 kHz) with terminal shocks (0.7 mAmps, 1 second). After forty-eight hours, mice were exposed again to thirty-two tones (20 seconds) within chambers containing altered visual, tactile, and odor dimensions to minimize contextual fear.

Results: Loss of CRP function did not confer differences between either male or female mice in avoidance-like behaviors after predator stress exposure demonstrated reductions in PTSD avoidance-like behaviors. AAV8.CRP overexpression did not alter cued fear extinction in male ($F = 1.293$, $n = 17\text{-}19$, $p = 0.106$) or female mice ($F = 2.036$, $n = 17\text{-}19$, $p = 0.161$) after predator stress ($F = 1.293$, $n = 17\text{-}19$, $p = 0.106$). At baseline, however, CRP null female mice have at baseline enhanced recall fear extinction ($F = 1.794$, $n = 15$, $p = 0.0053$), though no difference was observed in male mice ($F = 1.051$, $n = 15$, $p = 0.3923$).

Conclusions: CRP does not affect enduring trauma response in either male or female mice after predator stress and does not alter fear extinction in males. CRP constitutive expression may disrupt fear extinction in females. Studies are also ongoing to determine how CRP expression alters cytokine signaling as part of the peripheral immune response to predator stress.

Keywords: CRP, PTSD, Immune Function, Genetic Mouse Model

Disclosure: Nothing to disclose.

P73. Suppressed Neuroimmune Function in PTSD: Evidence From PET Imaging

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Background: There is well known peripheral immune dysfunction among individuals with posttraumatic stress disorder (PTSD), but few studies have investigated brain immune function. Using positron emission tomography (PET) and the radiotracer [11C]PBR28, which binds to the 18-kDa translocator protein (TSPO), we recently reported that prefrontal-limbic TSPO availability was significantly lower in individuals with PTSD compared to controls by 14% and lower TPSO availability was associated with worse PTSD symptom severity (Bhatt et al., 2020). These findings suggest individuals with PTSD exhibit a suppressed neuroimmune state which is related to PTSD symptomatology. To date, it is unknown whether people with PTSD exhibit aberrant neuroimmune system function, i.e., abnormal response to an immune challenge, which was the goal of the present study. Using our previously validated imaging paradigm (Sandiego et al., 2015), we quantified the increase in TSPO availability induced by systemic administration of E. Coli lipopolysaccharide (LPS; endotoxin), a potent immune activator, among individuals with PTSD and demographically-matched healthy comparators to study neuroimmune function. Prior results using this model showed robust increases in brain TSPO availability by $46 \pm 8\%$ across regions, indicative of neuroimmune activation, accompanied by increases in pro-inflammatory plasma cytokine levels (e.g., IL-6, IFN-γ) and self-reported sickness symptoms (e.g., feelings of anhedonia) among healthy adults. We hypothesized that individuals with PTSD would exhibit a diminished LPS-induced TSPO increase compared to healthy individuals, suggestive of suppressed neuroimmune function, building on our previous work.

Methods: Thirty individuals ($N = 30$) participated in two PET [11C]PBR28 scans in one day: one before and one 3-hr after LPS (1.0ng/kg, IV). Fifteen individuals who met DSM-IV-TR or DSM-5 criteria for PTSD (60% male, 32 ± 10 years old, 40% white) and fifteen age-, sex-, and race-matched healthy comparators without PTSD (HCs; 73% male, 30 ± 8 years old, 46% white; 60% reported a traumatic event) completed this study. PTSD symptomatology was further characterized by Clinician Administered PTSD Scale for DSM-5 (CAPS-5). Individuals with significant medical or psychiatric conditions or low affinity for [11C]PBR28 (rs6971 T/T genotype) were excluded. Positron emission (PET) data were acquired with a High-Resolution Research Tomograph (HRRT) for 120 min following bolus injection of $584 + /-135$ MBq [11C]PBR28 with arterial blood sampling to measure the metabolite-corrected input function. Multilinear Analysis ($t^* = 30$ min) was used to estimate the outcome measure of [11C]PBR28 volume of distribution (VT), which is the ratio at equilibrium of radioactivity concentration in tissue to that in arterial plasma. The primary regions of interest were an a priori-defined prefrontal-limbic circuit that included the hippocampus, amygdala, insula and ventromedial prefrontal cortex, as used previously. The regions of interest were determined using an anatomic labeling (AAL) template co-registered to each subject's T1-weighted structural MRI (MPRAGE; isotropic voxel: 1mm3). Linear mixed-effects models evaluated group differences (PTSD vs. HC) in brain regional [11C]PBR28 VT pre- to post-LPS changes with rs6971 genotype as a fixed factor ('high' [C/C] vs. 'moderate' [C/T] affinity binders).

Results: Groups were well-matched and there were no significant group differences in imaging parameters, age, sex, race, smoking status, cannabis or alcohol use, or education. Linear mixed models indicated the PTSD group exhibited a significantly lower magnitude LPS-induced increase in TSPO availability relative to the HC group in the a priori prefrontal-limbic circuit ($p = 0.021$; Effect Size 0.89). Additionally, consistent with our prior findings, the PTSD group exhibited lower prefrontal-limbic TSPO VT at baseline ($p = 0.073$; Effect Size: 0.68 ml/cm3). Relative to controls, the PTSD group also had a significantly reduced granulocyte-macrophage colony-stimulating factor (GM-CSF) cytokine response after LPS ($p = 0.007$). In the PTSD group, worse baseline anhedonia symptoms ($\beta = -0.78$, $p < 0.001$) and greater LPS-induced increases in C-reactive protein ($\beta = -0.69$; $p = 0.001$) were associated with a more 'blunted' neuroimmune response to LPS.

Conclusions: Herein, we present the first-ever in vivo study of neuroimmune function among individuals with PTSD. Our data provide evidence that individuals with PTSD exhibit a suppressed neuroimmune response to LPS in prefrontal-limbic regions compared with matched controls. Additionally, individuals with PTSD exhibit lower baseline TSPO levels (prior to LPS), replicating previous reports in a new sample. These data also support a link between LPS-induced central nervous system changes (i.e., TSPO increase) with peripheral immune response (i.e., CRP) and PTSD symptomatology (i.e., anhedonia). Collectively, these findings

suggest important brain-periphery immune relationships that may inform further research into novel immunologic treatments for PTSD.

Keywords: PTSD, TSPO and [11C]PBR-28 PET, Neuroimmune Activation, Microglia

Disclosure: Nothing to disclose.

P74. Influence of Mindfulness-Based Cognitive Therapy on Trait Mindfulness and PTSD Symptoms in Trauma-Exposed Black Adults: Results From a Pilot Study

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Background: Despite high rates of trauma exposure and posttraumatic stress disorder (PTSD) in Black populations living in urban, under-resourced areas, limited access to behavioral health treatment and barriers to treatment remain. Mindfulness-based interventions are efficacious in improving a range of health problems, including depression. Evidence for the use of mindfulness-based cognitive therapy (MBCT) to treat trauma-exposed individuals with PTSD is growing. Thus, MBCT has strong potential as a transdiagnostic treatment in trauma-exposed groups. Importantly, Black adults are vastly underrepresented in mindfulness research. Integrating MBCT in settings where individuals receive regular medical care, like safety net hospital primary care clinics, has promise and may address serious treatment needs in communities with low access to behavioral health and trauma-focused treatment and high levels of stigma for engagement. The goal of this study was to evaluate initial outcomes from a pilot randomized controlled trial (RCT) of a trauma informed and culturally responsive 8-week MBCT group intervention (versus waitlist control [WLC]) for Black adults utilizing medical care at a safety net hospital in Atlanta, GA. Specifically, changes in trait mindfulness, depression, and PTSD symptoms by group were examined.

Methods: Participants (N = 80 consented and randomized; 86% female, mean age = 44.81) were recruited from medical clinics of an urban hospital serving predominantly Black adults with low socioeconomic resources. Inclusion criteria included presence of trauma exposure (3 or more traumatic events in lifetime), positive PTSD screen, positive depression screen, age 18-65, and self-identification as Black or African American. Individuals with active suicidality, psychosis, significant substance misuse, or significant cognitive impairment were excluded. Participants were assessed at pre-intervention, post-intervention (or 8-weeks after pre-assessment for WLC), and 1-month follow-up. Fifty-five percent were randomized to MBCT (n = 44). Although the intervention was originally developed for delivery in the primary care clinic, the majority participated in virtual intervention delivery that was started in the wake of COVID-19 (n = 46, 57.5%). Self-report measures were obtained at pre-, post-, and 1-month follow-up assessments. Trait mindfulness subscales, including observation, description, awareness, nonjudgment, and nonreactivity, were measured with the Five Facet Mindfulness Questionnaire. Depression symptoms were measured using the Beck Depression Inventory, II. The Clinician-Administered PTSD Scale was used to assess PTSD symptoms across clusters at pre- and post-assessments. Based on study completion rates, post-assessment data was available on 64% of participants (n = 51). Separate latent growth curve (LGC) models were run to examine trait mindfulness subscales and depression symptom change over time by group with the intercept modeled at T1 (pre-assessment). Regression analyses were conducted to predict post-assessment PTSD symptom by group, controlling for pre-assessment PTSD symptoms.

Results: Regarding trait mindfulness change, observation and nonreactivity increased over time (slope) in the MBCT group versus WLC (βslope = 0.39, SE = 0.15, p = .008 and βslope = 0.27, SE = 0.11, p = .001, respectively). The other subscales did not show significant change over time (p's > .05). Depression symptom change over time was not significantly different by group (βslope = -0.24, SE = 0.27, p = .38). Intercepts were not significant by group across any LGC models. Regression analyses showed that group assignment (MBCT vs. WLC) predicted PTSD avoidance symptoms at post-assessment, such that MBCT participation was related to lower avoidance symptoms at post-assessment independent of pre-assessment PTSD symptom level (R2change = .07, t = -2.51, p = .037). No other regression analyses by symptom cluster were significant, although overall PTSD severity was trending toward significance (R2change = .03, t = -1.78, p = .082).

Conclusions: This pilot study found initial evidence of improved mindful observation and nonreactivity and reduced PTSD avoidance symptoms following engagement in a group-based MBCT intervention designed for trauma-exposed Black adults with PTSD and depression symptoms. As efforts continue to diversify trauma treatment and enhance effectiveness in varied populations and settings, larger-scale trials powered to measure efficacy are needed to further understand the benefits of MBCT in multiply marginalized groups and to disentangle mechanisms of action in symptom reduction.

Keywords: Posttraumatic Stress Disorder, Mindfulness, Trauma Exposure

Disclosure: Nothing to disclose.

P75. Anxious and Irritable Behaviors in Children With 22q11.2 Deletion Syndrome: A Qualitative Interview Study and Development of a Conceptual Framework

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Background: 22q11.2 deletion syndrome (22qDS) is the most common microdeletion syndrome and causes a range of medical and behavioral problems. The most common behavioral/psychiatric diagnosis in children with 22qDS are anxiety attention-deficit/hyperactivity disorder and autism spectrum disorder occurring in over 40% of patients. The presence of anxiety in children with 22qDS is negatively correlated with adaptive function and impacts everyday living skills. To date, anxious and irritable behaviors and their impacts have not been qualitatively explored from a caregiver perspective. Recent FDA guidance underscores the importance of understanding patient perspectives on what is most important to them about how they experience a disease or condition and how they hope to benefit from a successful treatment in order to identify patient-centered endpoint measures that are fit-for-purpose. The goal of this study was to characterize the experience of anxiety in children with 22qDS to inform the selection of endpoint measures.

Methods: Twenty caregivers of a child with 22qDS between the ages of 6 and 17 years with at least moderate anxiety participated in a qualitative interview. Interviews followed a semi-structured interview guide which included questions on anxious and irritable behaviors and their impacts on daily functioning. Trained, experienced qualitative researchers conducted the interviews via video conference; interviews lasted approximately 90 minutes and were recorded and transcribed. Researchers analyzed de-identified transcripts using thematic (content) analysis with

features drawn from grounded theory. During the coding process words and phrases provided by caregivers were selected using a coding template and grouped into key themes, concepts, and relationships.

Results: All caregivers were female and had a mean age of 44 years (SD = 6.6). Children had a mean age of 12 years (SD = 3.6) and most were boys (60%) and Caucasian (75%). Caregivers reported a broad spectrum of anxious (total = 24) and irritable (n = 23) behaviors. Anxious behaviors reported by at least half the caregivers included: getting easily upset, repetitive questioning, refusal to do anxiety provoking activities, shutdown/withdrawal, avoidance of anxiety provoking situations, social anxiety, and worry. Irritable behaviors reported by at least half the caregivers included: grumpiness, tantrums/outbursts, non-compliance and yelling/screaming. Almost all caregivers reported negative impacts on daily life due to these anxious and irritable behaviors. Caregivers reported that small changes in either the frequency and/or severity of anxious and irritable behaviors would make a meaningful difference. A conceptual framework was developed illustrating anxious and irritable behaviors, negative impacts on daily life and their relationship to capture the patient/caregiver experience living with 22qDS. The conceptual framework can be used to guide selection of fit-for-purpose clinical outcomes assessments to measure anxious and irritable behaviors in clinical trials of new treatments for anxiety and behavior problems associated with 22qDS.

Conclusions: This qualitative study contributes to the limited qualitative literature and describes the patient/caregiver experience living with a child with 22qDS. The conceptual framework can inform the selection of fit-for-purpose patient centered assessments in future 22qDS clinical trials for the treatment of anxious and irritable behaviors for which there are no currently approved medications. Defining a path forward for trials will provide an opportunity to develop medicines that may reduce the negative impact of anxiety and behavioral problems in 22qDS that persist despite current empiric therapy.

Keywords: 22q11 Deletion Syndrome, Cannabinoid Receptors, Patient Centered Research Design, Novel Endpoints

Disclosure: Zynerba: Advisory Board (Self)

P76. Synaptic Plasticity Biosensors to Discover Novel Plasticity Modulators

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Background: Neuronal plasticity—the genesis and maintenance of dendritic spines—is crucial for brain development and function. One fundamental form of structural neuroplasticity is activity-dependent structural and proteomic changes at the dendritic spine. Neuronal plasticity is dysregulated in many neurological and neuropsychiatric disorders such as Major Depressive Disorder, PTSD etc. Recent studies suggest the potential role of different pharmacological agents such as ketamine and psilocybin in ameliorating altered neuronal plasticity and excitatory synaptic transmission, to treat brain disorders. Despite advances in basic neuroscience, the development of therapeutics for central nervous system disorders has been challenging. The absence of robust, simple, and scalable techniques to quantify neuronal plasticity is one major barrier for drug development.

Methods: In this study, we have developed genetically encoded biosensors where the translation of a reporter enzyme, Luciferase or Nanoluciferase, is subjected to the same activity-dependent regulation as synaptic activation under control of activity-dependent promoters. The readout of these sensors—a change in luminescence signal—is the quantitative measurement of local translation and potentiation of dendritic spines. We demonstrated dendritic spine compartment specificity and activity dependence of sensor expression in mature primary cortical neuron cultures (n = 3). All these experiments were performed in mature primary neuronal cultures harvested from E18 mice embryos of both sexes.

Results: We observed elevated luminescence response to known positive plasticity modulator FSK (Forskolin) in a dose dependent manner, with 0.1% DMSO as a negative control. We have optimized and scaled down the method to a semi-automated robust, and reliable high-throughput screening platform to discover compounds that can modulate excitatory synapse plasticity. We screened ~1,280 FDA-approved small-molecule and ~1,200 compound natural product libraries followed by a stringent statistical cut off analysis to nominate hits. From these libraries, we have identified a total of 20 hits (~1%) that positively affect neuroplasticity. We are combining orthogonal tools such as 2-photon glutamate uncaging-evoked dendritic spinogenesis and other complementary assays to validate the top hits and explore their action mechanisms.

Conclusions: Altogether, these plasticity-dependent sensors allow measurement of excitatory synapse potentiation through luminescence response and provide a flexible tool for high-throughput early-stage drug screening. This approach enables cell type-specific readout and allows screening for neuroplasticity in neurons harboring specific mutations for personalized medicine applications and mechanistic studies.

Keywords: Synaptic Plasticity, High Throughput Screening, Drug Discovery - New Approaches, Spinogenesis, Excitatory Synapses

Disclosure: Neuroplastica: Founder (Self)

P77. Differential DNA Methylation Related to Trauma Type in the PTSD Brain

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Background: Understanding the molecular basis of post-traumatic stress disorder (PTSD) is challenging due to significant variation in the type and chronicity of trauma experience and other life history variables that may impact the brain. Profiling of unique DNA methylation patterns within the PTSD brain may provide insights into the pathophysiology of this disorder. In addition to examining differentially methylated regions and genes, this profiling can give insight into biological aging that may have implications for disease pathways.

Methods: We conducted genome-wide DNA methylation analyses using Infinium MethylationEPIC arrays of dissected postmortem brain tissue. The current sample includes brains from subjects with post-traumatic stress disorder (PTSD, n = 101). Our analyses include DNA extracted from the central nucleus of the amygdala, hippocampus and medial prefrontal cortex (mPFC). Analysis was done on differentially methylated regions (DMRs) and differentially methylated probes (DMPs) as well as epigenetic age utilizing three epigenetic clocks (Horvath, Levine, and Hannum). Using medical records, we grouped individuals based on trauma history.

Results: Analyses of DNA methylation profiles indicate that there is significant variation in the ratio of neuronal/non-neuronal cells across different trauma categories, such as assaultive vs. non-assaultive trauma and the presence and absence of childhood trauma. We find differentially methylated CpG sites within the

PTSD brain when comparing those experienced assaultive vs non-assaultive trauma. A notable gene found differentially methylated in the amygdala between the assaultive vs non-assaultive trauma groups is protein kinase M zeta (PKMζ). This gene plays a critical role in memory and is involved in the stress response. Several DMRs and DMPs were found when comparing those with and without childhood trauma. The hippocampus had the highest number of DMRs and DMPs ($p < 0.001$). A notable gene found differentially methylated in the hippocampus between those with and without childhood trauma is SLC6A4, a gene that encodes for a serotonin transporter responsible for serotonin reuptake. Epigenetic age deceleration and acceleration were detected within individuals grouped by the trauma characteristics.

Conclusions: Our analyses suggest that there are unique DNA methylation signatures of PTSD that vary by types of trauma and implicate neurotrophin, cellular physiology and transcriptional pathways. Stress-sensitive genes have been hypothesized to play a critical role in psychiatric risk and here we find differential methylation in these genes within the amygdala. Epigenetic aging appears to vary by brain region and may be responsive to within-subject variation in life experience.

Keywords: DNA Methylation, PTSD, Postmortem Brain Tissue

Disclosure: Nothing to disclose.

P78. Safety Learning During Adolescence Augments Long-Term Fear Regulation

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Background: Stimuli associated with threat (fear cues) are highly salient. It is largely believed vigilance to potential threat in the surrounding environment informs appropriate behavioral responding and is adaptive for survival. Yet, the inability to disengage attention from threat, or attend to alternative indicators of safety, can contribute to fear-related psychiatric disease such as anxiety or PTSD. Using a mouse model, we have begun investigating how brief exposures to threat in the form of fear conditioning impact later acquisition and regulation of fear. While fear generalization to perceptually similar stimuli is common following threat exposure, less is known about how exposure to safety may scale generalization. We hypothesized that explicit safety training coinciding with threat exposure would diminish generalization and facilitate later fear extinction. The present experiments were carried out in both adolescent and adult cohorts of male and female mice to examine both age and sex differences in the generalization and persistence of fear following previous threat and/or safety exposure. Our overarching goal is to investigate how experiences with threat and safety impact later affective regulation in an effort to inform translation to the treatment of fear and anxiety disorders.

Methods: We used three groups of adolescent (30 days old) and three groups of adult (70 days old) C57bl6J mice. Group 1 ($n = 20$ adolescents, 10 female, and 19 adults, 10 female): Mice underwent four sessions of discriminative conditioning, consisting of intermixed presentations of a fear cue (tone paired with a mild footshock) and a safety cue (distinct tone, no footshock) (previous published protocol: Meyer and Gerhard, 2021). Conditioning was followed by a testing day, during which mice were presented with presentations of the fear cue, safety cue, or a compound presentation of both (i.e., summation test for conditioned inhibition). One month later, mice underwent fear conditioning (distinct tone paired with a mild footshock) followed by fear extinction. Group 2 ($n = 16$ adolescents, 8 female, and 16 adults, 8 female): mice underwent a training procedure similar to Group 1, including parallel presentations of fear cues but an absence of safety cues. Fear conditioning and extinction procedures were identical to Group 1. Group 3 ($n = 16$ adolescents, 8 female, and 32 adults, 15 female): mice of each age remained in the homecage undisturbed while Groups 1 and 2 underwent conditioning, until later fear conditioning and extinction were carried out, identical to Groups 1 and 2. Subsequently, all three groups underwent testing in an elevated plus maze.

Results: All mice in Group 1 exhibited discrimination, freezing more to fear than safety cues. Although, discrimination magnitude was significantly higher for adolescents relative to adults, $F(3, 102) = 5.10$, $p = 0.002$. For Group 2, fear conditioning was greater during adolescence, $F(3, 84) = 14.99$, $p < 0.001$. No sex differences in discrimination or fear conditioning were observed. Performance was similar in all mice during the test session, with freezing highest to the fear cue, lowest to the safety cue, and intermediate to the compound cue (indicating successful fear inhibition). Overall, females froze more, $F(1, 35) = 5.94$, $p = 0.002$. One month later, all mice acquired fear to a novel tone. During extinction of this novel tone, mice with previous conditioning in adulthood initially had higher fear to overcome, exhibiting greater freezing in Groups 1 and 2 than Group 3 controls for both sexes, $F(2, 64) = 3.58$, $p = 0.034$. In contrast, mice with previous conditioning in adolescence (both sexes) are protected from pitfalls of previous fear conditioning and show some evidence of enhanced extinction relative to controls. While freezing did not differ between fear conditioned (Group 2) and control (Group 3) mice, freezing was reduced in safety conditioned mice (Group 1) relative to fear conditioned ($F(1, 34) = 7.11$, $p = 0.012$) and marginally reduced relative to homecage controls ($F(1, 34) = 3.67$, $p = 0.064$). Moreover, while anxiety-like behavior in the elevated plus maze did not differ between adult groups, adolescents with previous safety conditioning (Group 1) exhibited greater exploration of the maze ($p = 0.026$) and lower freezing ($p = 0.002$) compared to other groups.

Conclusions: Intervention early in life, when the brain is still highly plastic, may provide a powerful opportunity to mitigate the adverse impacts of anxiety before it becomes a lifelong affliction. In line with this, our findings show initial support for safety learning during adolescence as a method to mitigate the detrimental impacts of aversive experiences for fear regulation in adulthood. Adolescent mice show a strong propensity to learn from safety cues during adolescence and retain these benefits into adulthood, generalizing to other encounters with threat (e.g., fear extinction). In adults, further investigation is necessary to identify mechanisms to address disproportionately higher fear responding by females. Together, this work emphasizes the impact that exposure to affective stimuli (both fear and safety) can have on later affective processing and suggest both age- and sex-specificity in the promise of safety signal-based treatments for fear-based psychiatric disease.

Keywords: Adolescence, Fear Conditioning, Anxiety

Disclosure: Nothing to disclose.

P79. Central Target Engagement and Pharmacodynamic Biomarker Profile for 2 Mg ENX-102, a GABA-A alpha2,3,5 PAM in Development for the Treatment of Generalized Anxiety Disorder

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Background: ENX-102 is a highly potent gamma-aminobutyric acid type A (GABA-A) positive allosteric modulator (PAM) that activates neurotransmission via GABA-A receptors containing alpha2, alpha3, or alpha5 subunits, while blocking alpha1 subunits. ENX-102 exhibited anxiolytic-like behaviors and demonstrated central target engagement evidenced by changes in quantitative electroencephalography (qEEG) measures when administered at doses that achieve ≥50% receptor occupancy in preclinical models. ENX-102 was safe and well-tolerated in Phase 1a and Phase 1b single- and multiple-ascending dose clinical studies in healthy volunteers. The Phase 1b study included pharmacodynamic biomarkers to inform dose selection for a Phase 2 study of ENX-102 in patients with generalized anxiety disorder (GAD). Summary data of those studies have been reported previously. Here we present the detailed pharmacodynamic biomarker profile of 2 mg ENX-102, the dose selected for Phase 2.

Methods: ENX-102 was evaluated in a randomized, double-blind, placebo-controlled, multiple ascending dose study in healthy male and female volunteers (N = 40; 5 cohorts of 8 volunteers each randomized 6:2 active:placebo). Doses ranging from 0.5 mg to 5.0 mg, administered once daily in the morning for 12 days, were evaluated.

Blood samples for pharmacokinetic (PK) analysis were collected pre-dose and then a full time-course after dosing on Day 1 and Day 12. Plasma concentration-time data for ENX-102 were analyzed using non-compartmental methods. Steady-state PK parameters included maximum plasma concentration (Cmax), time to reach Cmax (Tmax), area under the plasma concentration-time curve (AUC) to the last non-zero time point, and half-life (t1/2). Pharmacodynamic measures included qEEG, biomarkers assessing neurophysiological and neuropsychological function (NeuroCart test battery), and a declarative memory analyzed by mixed model analyses of covariance. The NeuroCart test battery assessed saccadic peak velocity, subjective alertness as measured by a visual analogue scale, sustained attention as measured by an adaptive tracking task, and psychomotor function as measured by body sway. Declarative memory was assessed by a Visual Verbal Learning Test. Sedation was measured by the clinician-administered Modified Observer's Assessment of Alertness/Sedation (MOAA/S) scale.

Results: Steady-state pharmacokinetic analyses indicated dose-related exposure. Of particular interest, a dose of 2 mg ENX-102 was associated with a mean Cmax value of 49.82 ng/mL and AUC of 873.17 h*ng/mL. Tmax was reached at 3 hours postdose. Mean t1/2 was 61 hours.

qEEG measures showed dose-related effects. Notably, ENX-102 at 2 mg decreased qEEG alpha-power and theta-power and increased beta-power and gamma-power indicating sustained reduced arousal with no sedative-like increase in delta-power. ENX-102 at 2 mg also decreased saccadic peak velocity with no impairment of alertness, sustained attention, or psychomotor function. There was no impairment of delayed recall. No sedation was observed on the MOAA/S.

Conclusions: ENX-102 exhibited a favorable PK profile with a long half-life consistent with once daily oral administration. Central target engagement was evidenced by qEEG changes consistent with sustained reduced arousal and decreased saccadic peak velocity. ENX-102 at 2 mg exhibited a favorable pharmacodynamic biomarker profile with no evidence of sedation, reduced alertness or attention, impaired psychomotor function, or impaired memory. The dose of 2 mg ENX-102 was selected for evaluation in a Phase 2 study in GAD and that clinical trial is ongoing.

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

Disclosures: Engrail Therapeutics, Inc.: Employee, Board Member, Stock/Equity (Self). Intra-Cellular Therapies, Inc.: Stock / Equity (Self).

P80. Preclinical Characterization of CYB004: A Novel, Deuterated N,N-Dimethyltryptamine (DMT) Analog for the Potential Treatment of Generalized Anxiety Disorder (GAD)

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Background: N,N-Dimethyltryptamine (DMT) is a naturally occurring psychoactive compound and the main active ingredient of ayahuasca, a traditional ceremonial beverage used for ritualistic purposes by several indigenous Northwestern Amazonian cultures. Placebo-controlled trials and observational studies in humans have demonstrated that ayahuasca and DMT can improve various mental health outcomes with positive effects on mood (Sanches et al., 2016; Palhano-Fontes et al., 2019; D'Souza et al., 2022). While DMT is efficacious and inherently safe, the central effects of DMT peak within 2-5 minutes and dissipate in about 30 minutes as it is rapidly metabolised by monoamine oxidase. This short half-life makes the therapeutic use of DMT challenging, e.g., limiting the psychological integration of the experience which may diminish its efficacy for specific treatment outcomes. Longer-acting psychedelic compounds, such as psilocybin, also appear to be effective pharmacotherapeutic agents, but their extended duration (6-8 hr.) makes scalability in the clinic complex. Deuteration of DMT can potentially improve its pharmacokinetic (PK) profile, allowing for effective and scalable psychological integration, while maintaining its desired pharmacodynamic (PD) effects. Cybin is developing CYB004, a novel deuterated analog of DMT that may offer these benefits over DMT. These preclinical studies aimed to compare the in vitro pharmacology, in vivo activity, and PK profile of CYB004 to DMT, to determine the impact of deuteration on the properties of CYB004.

D'Souza D.C., Syed S.A., et al. (2022) Exploratory study of the dose-related safety, tolerability, and efficacy of dimethyltryptamine (DMT) in healthy volunteers and major depressive disorder. Neuropsychopharmacology 47, 1854-1862.

Palhano-Fontes F., Barreto D., et al. (2019) Rapid antidepressant effects of the psychedelic ayahuasca in treatment-resistant depression: A randomized placebo-controlled trial. Psychol Med. 49, 655-663.

Sanches R.F., de Lima Osório F., et al. (2016) Antidepressant effects of a single dose of ayahuasca in patients with recurrent depression a SPECT study. J. Clin. Psychopharmacol. 36, 77–81.

Methods: Pharmacological profiles of CYB004 and DMT were compared using serotonin (5-HT) receptor binding and functional assays to evaluate potency, efficacy, and selectivity at serotonin receptors; both compounds were also screened for activity at over 160 proteins. Additionally, both compounds were evaluated in the mouse head twitch response (HTR) assay (a behavioral model of central serotonin 5-HT2A receptor activation). Finally, pharmacokinetic (PK) studies were completed in the mouse, rat, and dog to assess bioavailability via several routes of administration.

Results: The pharmacological and selectivity profile of CYB004 was similar to that seen with DMT. CYB004 binds and activates the 5-HT2A receptor (5-HT2A Ki: CYB004 = 180 nM; DMT = 130 nM) and induces the HTR in mice [Mean no. of HTR (5.6 mg/kg, SC): CYB004 = 8.2; DMT = 7.3]. The PK profile of CYB004 was characterized in C57BL/6 mice following subcutaneous administration, in Sprague-Dawley rats after intravenous, inhalation, and oral administration, and in Beagle dogs following intravenous bolus + infusion administration. Deuteration resulted in a decrease in metabolism of CYB004, compared to DMT, manifested as a longer half-life and a reduction in systemic clearance leading to an increase in plasma exposure in mouse, rat, and dog. Specifically, in

the mouse, systemic clearance of CYB004 was decreased by 38% (1098 vs. 1770 L/hr/kg) and the elimination half-life (t1/2) of CYB004 increased 2.5-fold (0.69 versus 0.28 hr). In the rat, the elimination half-life (t1/2) of CYB004 increased 2.6-fold (0.45 vs. 0.17 hr) and the brain:plasma ratio of CYB004 increased by 30% (12.3 vs. 9.5). In the dog, systemic clearance of CYB004 was decreased by 55% (622 vs. 1380 L/hr/kg) and the elimination half-life (t1/2) of CYB004 increased 2.9-fold (0.47 versus 0.16 hr).

Conclusions: Data from these studies confirm that deuteration did not impact the pharmacology and in vivo profile of CYB004, but deuteration potentially offers the ability to extend the PK profile of DMT and enhance treatment outcomes.

Keywords: Psychedelics, N,N-Dimethyltryptamine (DMT), Serotonin 5-HT2A Receptor, Preclinical Pharmacology, Pharmacokinetics

Disclosure: Cybin: Employee, Stock/Equity (Self). Daiichi Sankyo: Employee, Stock/Equity (Spouse/Partner).

P81. Prucalopride, a 5-HT4 Receptor Agonist, Induces Rapid Anxiolytic Effects in Mice

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Background: Anxiety disorders are among the most common psychiatric disorders, with a lifetime prevalence of over 25%. Benzodiazepines (BZDs) have been effective for most anxiety disorders and have been the standard treatment for years, as it has an over 80% response in reducing acute anxiety in patients. However, their long-term daily use has been associated with a risk of dependency and memory impairments. Consequently, they are often replaced by Selective Serotonin Reuptake Inhibitors (SSRIs) and other serotonergic agents, which suggest a key role of serotonin in anxiety. However, the delayed onset of action of several weeks combined with the fact that 40% of anxious patients are non-responders emphasizes the need to develop novel fast-acting anxiolytics. Over the past 10 years, it has been demonstrated that activation of the serotonin type 4 receptor (5-HT4R) may represent a new target for treating both depression and anxiety. Here, we evaluated whether prucalopride, a 5-HT4R agonist approved to treat chronic idiopathic constipation in humans, would induce fast anxiolytic-like activity.

Methods: To assess putative fast anxiolytic, prucalopride (0.5, 1.5, or 3 mg/kg) was administered intraperitoneally (i.p.) in males or females BALB/cJRj mice (Janvier Labs, Le Genest-St-Isle, France, 7 to 8 weeks old, weighed 25 to 30 g), 30 minutes before testing in two mouse behavioral tasks predictive of anxiolytic activity, the Elevated Plus Maze (EPM) or novelty suppressed feeding (NSF) paradigm, and compared to vehicle, diazepam (1.5 mg/kg), a classical BZD, fluoxetine, a SSRI or RS67333, a 5-HT4R agonist presenting fast anxiolytic properties.

Results: In the NSF, unlike fluoxetine but similar to diazepam, acute systemic injection of prucalopride at 1.5 and 3 mg/kg induced a fast anxiolytic-like effect when compared with vehicle in male and female BALB/cJRj mice. Prucalopride and diazepam decreased latency to feed [one-way analysis of variance (ANOVA) followed by Fisher's LSD posthoc test, $^{**}p < 0.01$ vs. vehicle group]. It is unlikely that this effect resulted from a change in locomotor activity since the ratio of ambulatory distance in the open arms divided by total distance was significantly increased for the four drugs (one-way ANOVA, $p < 0.01$ vs. vehicle group).

Conclusions: Overall, unlike fluoxetine but similar to classical BZD diazepam, prucalopride, the 5-HT4R agonist, induced robust anxiolytic-like effects like diazepam. We are currently testing whether a short treatment with prucalopride could decrease the onset of action of SSRI-induced anxiolytic activities.

Keywords: 5-HT4 Receptor, Anxiety, Anxiolytics, Balb/c Mouse

Disclosure: Nothing to disclose.

P82. Opioid Receptor Antagonism Facilitates the Anxiolytic-Like Effect of Oxytocin in Mice

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Background: Anxiety disorders are leading causes of disability worldwide. These disorders affect approximately 16% of the US population and disproportionately affect men and women, such that women are twice as likely to be diagnosed with an anxiety disorder compared to men. A previous study by our laboratory demonstrated that intracerebroventricular (ICV) administration of oxytocin (500 ng) reduced anxiety-like behavior in male and female mice, with increased efficacy in males. Additionally, others have shown that mu opioid receptor (MOR) activation can reduce anxiety-like behavior and early studies suggest that the opioid receptors regulate the oxytocin system in relation to stress responses. Thus, we hypothesized that modulation of the opioid system mediates the sex differences observed in response to oxytocin treatment.

Methods: To determine whether endogenous opioids mediated the effects of oxytocin, we systemically administered opioid receptor antagonists, naloxone (1-4 mg/kg subcutaneously), CTAP (1-3 ug ICV) and norbinaltorphimine (10-20 mg/kg intraperitoneally) prior to an effective dose of oxytocin (500 ng; ICV) in both males and females.

Results: Contrary to our initial hypothesis, our studies demonstrated that naloxone potentiated the anxiolytic-like effect of oxytocin. Using a MOR-selective antagonist, CTAP, and a kappa opioid receptor-selective antagonist, norbinaltorphimine, we further demonstrated that MOR blockade potentiated the anxiolytic-like effect of oxytocin, whereas kappa-opioid receptor blockade inhibited oxytocin-induced anxiolytic-like effects.

Conclusions: Our findings indicate that blockade of the opioid system may eliminate observed sex differences in the anxiolytic-like effects of oxytocin. Altogether these results suggest that endogenous opioids modulate the oxytocin system with respect to emotion regulation and have implications for the development of novel clinical treatments for anxiety disorders.

Keywords: Oxytocin, Opioid Receptor System, Anxiety

Disclosure: Nothing to disclose.

P83. Dopamine D3 Receptor Mediates the Synergistic Anxiolytic-Like Action of Cariprazine With Escitalopram in the Rat Foot Shock-Induced Ultrasonic Vocalization Model

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Background: Symptoms of anxiety are common in patients with schizophrenia, bipolar I disorder, and major depressive disorder, emphasizing the need for treatments that can address the

complex symptomatology characteristic of these disorders. Cariprazine is a dopamine D3-preferring D3/D2 and serotonin 5-HT1A receptor partial agonist approved by the US Food and Drug Administration to treat adults with schizophrenia as well as manic, mixed, and depressive episodes associated with bipolar I disorder. Recently, cariprazine has also been approved as an adjunctive treatment for major depressive disorder. A robust method for studying anxiety in rodents is with an ultrasonic vocalizations (USV) model, which we previously used to demonstrate that cariprazine exerts potent anxiolytic-like activity similar to the selective serotonin reuptake inhibitor (SSRI) escitalopram. Moreover, synergistic anxiolytic-like effects were observed for cariprazine applied in combination with escitalopram or diazepam at sub-effective doses of each compound. The present study sought to evaluate the possible involvement of D3 and/or 5-HT1A receptor mediations in the anxiolytic-like effects of cariprazine in rats.

Methods: Anxiety-like symptoms were tested using an electric foot shock-induced USV model in male Wistar Unilever rats (150–250 g). All experiments were performed in accordance with the European Communities Council directive (86/609 ECC) for the care and use of laboratory animals and with the approval of the Regional Animal Care Committee. The testing apparatus was a 20 cm x 20 cm x 25 cm chamber equipped with a grid floor, through which foot shocks (1 mA; 4 s) could be delivered. Day 1 consisted of two 10-min sessions, separated by 60 min, during which rats received 1–4 randomly distributed foot shocks until USV emission, i.e., detection of vocalization with frequencies >20 kHz. The following day, rats were placed in the chamber and received a single shock 30 min after injection of vehicle (saline with 10% 2-hydroxypropopyl-β-cyclodextrine, intraperitoneal). On day 3, the test was repeated 30 min after systemic injection of sub-effective doses of cariprazine (1 µg/kg, intraperitoneal) and escitalopram (0.2 mg/kg, subcutaneous) given alone or in combination. In a second experiment, animals received cariprazine and/or escitalopram injections plus the dopamine D3 receptor antagonist SB-277011A (6 mg/kg, intraperitoneal), the 5-HT1A receptor antagonist WAY-100,635 (0.1 mg/kg, subcutaneous) or vehicle 40 min before testing. Duration of USVs on each testing day was determined during a 5 min period beginning immediately after the first USV elicited by a single foot shock. USV durations were converted to a percentage of USV duration after vehicle treatment on day 2 and analysed using one-way ANOVA. When main effects were significant, treatment comparisons were further investigated using the Tukey post-hoc test, with the threshold for significant differences set at $P < .05$.

Results: Cariprazine and escitalopram given alone did not significantly change the duration of foot shock-induced USV; however, combined administration of cariprazine (1 µg/kg, i.p.) and escitalopram (0.2 mg/kg, s.c.), at doses inactive per se, induced a statistically significant decrease (42%) in the duration of foot shock–induced USV ($P < .01$), indicating a synergistic anxiolytic-like effect. When given alone or in combination with either cariprazine or escitalopram, the selective dopamine D3 receptor antagonist SB-277011A failed to change the duration of USVs. However, when SB-277011A was given with both agents, it prevented the cariprazine + escitalopram-induced reduction of USV ($P = .61$), indicating the role of dopamine D3 receptor mechanism in the synergistic action of cariprazine. On the other hand, the 5-HT1A receptor antagonist WAY-100,635 (0.1 mg/kg, s.c.) failed to alter the latter reduction of USV induced by cariprazine+escitalopram.

Conclusions: In a rodent foot shock-induced USV model, synergistic anxiolytic-like effects were demonstrated for cariprazine applied in combination with the SSRI escitalopram at sub-effective doses. Notably, the dopamine D3 receptor mechanism of cariprazine is involved in this synergistic activity. Cariprazine, in combination with a low-dose SSRI anxiolytic agent, may be effective in the treatment of patients suffering from anxiety disorders commonly associated with various psychiatric disorders.

Keywords: Cariprazine, Dopamine, 5-HT, Anxiety, Ultrasonic Vocalization (USV) Model

Disclosure: AbbVie: Employee (Self).

P84. Hypocretin Regulates Social Approach and Social Vigilance Behaviors in a Brain Region-Specific Manner

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Background: Best known for promoting wakefulness and arousal, the neuropeptide hypocretin (Hcrt), or orexin, also plays an important role in modulating stress response and anxiety-related behaviors. Central and systemic manipulation of the Hcrt system has produced mixed behavioral outcomes in preclinical research and emerging evidence suggests that the function of Hcrt varies across different brain regions. In this study, we examined the behavioral effects of Hcrt acting in the nucleus accumbens shell (NAcSh) and the anterior bed nucleus of the stria terminalis (aBNST) in male and female California mice (Peromyscus californicus), a species that is ideal for studying social stress. Both regions receive dense input from the Hcrt neurons, and have been reported to regulate social approach and social vigilance, a behavior in which an individual orients towards an unfamiliar conspecific while simultaneously avoiding it.

Methods: We microinjected vehicle, 30ng, or 300ng Hcrt1 bilaterally in the NAcSh in both male ($n = 30$) and female California mice ($n = 30$) and assessed the behaviors in a 3-phase social interaction test: an open field phase, an acclimation phase (a novel empty cage is introduced) and an interaction phase (a same-sex novel conspecific is placed into the cage). We also microinjected vehicle or 300ng Hcrt1 bilaterally in the aBNST in a different cohort of animals ($n = 20$ for male; $n = 18$ for female). To further investigate the behavioral effects of endogenous Hcrt, we microinjected vehicle or 30nmol Hcrt receptor2 antagonist in the NAcSh of female mice ($n = 16$) that were exposed to social defeat stress (placed into home cage of an aggressive same-sex resident). Previous research from our lab showed that social defeat stress induces prominent social withdrawal in female California mice.

Histology was used to confirm sites of injection. Two-way ANOVA (sex*treatment) followed by pairwise comparisons were used to analyze normally distributed behavioral data. For non-normal social vigilance data, Kruskal–Wallis test was used followed by Dunn's test.

Results: Microinjection of Hcrt1 in the NAcSh induced avoidance of a novel empty cage in both males ($p < 0.05$, $d = 0.90$ for 30ng; $p < 0.05$, $d = 1.43$ for 300ng) and females ($p < 0.01$, $d = 1.15$ for 30ng; $p < 0.01$, $d = 1.36$ for 300ng). Female but not male mice showed avoidance of a novel social target ($p < 0.001$, $d = 2.18$ for 30ng; $p < 0.001$, $d = 1.54$ for 300ng). Females treated with 30ng of Hcrt1 also exhibited increased social vigilance behaviors ($p < 0.05$, $d = 1.38$). On the other hand, 300ng Hcrt1 in the aBNST produced no significant behavioral effects in either male or female mice during the social interaction test. Stressed females that were infused with the antagonist showed increased social approach compared to stressed females infused with the vehicle ($p < 0.05$, $d = 1.52$).

Conclusions: Our current findings provide additional evidence that the behavioral effects of Hcrt are brain region-specific. There were also potential sex differences in how Hcrt regulates social behaviors. Hcrt1 acting in the NAcSh, but not the aBNST, induces

avoidance of a novel object in both male and female mice but avoidance of a novel social target only in female mice. Blocking Hcrt action in the NAcSh also attenuates social withdrawal in stressed females. Ongoing studies are using a genetically encoded Hcrt sensor to assess Hcrt release in the NAcSh during social interactions in threatening and non-threatening social contexts.

Keywords: Orexin/Hypocretin, Social Stress, Nucleus Accumbens, Bed Nucleus of The Stria Terminalis, Sex Difference

Disclosure: Nothing to disclose.

P85. Acute Sleep Restriction Enhances Fear Extinction Acquisition and Recall

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Background: Sleep has a reciprocal relationship with fear- and trauma-related disorders. Sleep problems including nightmares, difficulty falling or staying asleep, and restless or unsatisfying sleep are among the diagnostic criteria for conditions such as Generalized Anxiety Disorder (GAD) and Post-Traumatic Stress Disorder (PTSD). In addition, abnormalities in sleep patterns may contribute to, or exacerbate, other symptoms of these disorders. Existing research in rodent models demonstrate that fear learning produces significant alterations in sleep architecture that are alleviated by extinction training. However, studies on the inverse relationship—the impact of changes in sleep architecture on fear learning and extinction—have largely focused on disrupting the consolidation of memories immediately following learning. Here, we examined the effects of acute sleep disruption prior to fear extinction in male and female mice and observed corresponding effects on sleep architecture using a wireless telemetry system that enables sustained periods of continuous data collection in untethered, freely moving mice.

Methods: Adult male and female C57BL/6J mice were used for all experiments. Experimentally-naïve mice were habituated to conditioning chambers over for 15 minutes on two consecutive days. On Day 3, mice were returned to the conditioning chamber and presented with 5 tones that co-terminated with footshock. On the next morning at the start of the lights-on phase of the vivarium 12 hour light/12 hour dark cycle, mice were sleep-restricted (i.e., kept awake) for 6 hours using a gentle handling method while control mice were allowed to sleep ad libitum. Sleep restriction consisted of a cage change, introduction of novel objects to encourage voluntary activity, gentle handling and cage tapping, as necessary. After 6 hours of sleep restriction or control sleep, mice underwent a fear extinction paradigm, consisting of 15 presentations of the tone in a novel context. All mice were returned to their homecage and allowed to sleep ad libitum after extinction. The following day, mice were returned to the extinction context and tested for extinction recall. In parallel, a separate cohort of mice underwent surgeries to implant wireless telemetry transmitters (Data Sciences International [DSI]) that enable continuous, untethered recordings of EEG, EMG, as well as core body temperature and locomotor activity, before receiving the same series of sleep disruption, fear conditioning, and extinction and recall testing. Finally, another cohort of mice was sacrificed after 6 hours of gentle handling sleep restriction and quantitative polymerase chain reaction (qPCR) was performed examine expression of brain derived neurotrophic factor (BDNF) in brain regions known to be involved in fear learning and memory. Blood was also collected and used to measure plasma corticosterone (CORT) with an enzyme-linked immunoassay (ELISA).

Results: Sleep-restricted mice displayed enhanced extinction learning with reduced fear, as reflected by freezing behavior following exposure to cue presentations, during extinction ($P's < 0.01$) and recall testing ($P's < 0.05$). Analyses of males and females separately revealed that this effect appeared to be larger and more reliable in males ($P's < 0.05$), whereas sleep-restricted females only differed from control females on some trial blocks. Analysis of sleep architecture in mice implanted with wireless telemetry devices showed that sleep restriction led to rebound slow wave sleep (SWS) and rapid eye movement sleep (REM) during the period after extinction training ($P's < 0.001$). Across all mice, fear conditioning resulted in increased REM during the dark phase, when mice are typically awake and active. Acute sleep restriction resulted in a significant increase of BDNF in the amygdala ($P < 0.01$) and prefrontal cortex (PFC; $P < 0.05$) compared to control sleep conditions. In males, but not females, BDNF was also significantly increased in the bed nucleus of the stria terminalis (BNST) after sleep restriction ($P < 0.01$). There were no differences in plasma CORT levels in sleep-restricted mice compared to controls.

Conclusions: Acute sleep restriction reduced fear during fear extinction acquisition and recall. Increased neural plasticity in brain regions known to be involved in fear learning and anxiety (i.e., amygdala, PFC and BNST) may contribute to this effect. These data align with findings that acute sleep restriction or deprivation can have antidepressant-like effects in rodents as well as in humans. Rebound sleep—reflected by increases in both SWS and REM following periods of sleep restriction—may also contribute to enhanced consolidation of fear extinction-related memories. A more thorough understanding of interactions of sleep deprivation with fear and extinction memories is important for treatment of fear-and trauma-related disorders in humans, where impaired sleep is a common and often intractable symptom. Further, enhanced fear inhibition by acute sleep deprivation may lead to novel clinical treatment possibilities, which will be improved by an improved understanding of the neural and molecular mechanisms by which acute sleep deprivation improves fear extinction learning and recall.

Keywords: Fear Conditioning and Extinction, Sleep Deprivation, REM Sleep, Slow Wave Sleep, Amygdala

Disclosure: Nothing to disclose.

P86. Establishing Local dsHMGB1 Administration as a Pharmacological Model of Anxiety in C57BL/J6 Mice

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Background: High Mobility Group Box 1 (HMGB1), a non-histone chromosomal protein, has been recognized for its pivotal role in inflammation and immune responses. However, recent research has unveiled its significance in the context of neuropsychiatric disorders, particularly anxiety-related conditions. The aim of this study was to investigate the relationship between HMGB1 expression and stress exposure and to elucidate the involvement of HMGB1 in behavioral stress responses.

Methods: We first sought to determine if acute stress increases HMGB1 expression in the brain. C57BL\J6 male mice aged 12 weeks were subjected to an abbreviated Chronic Unpredictable Stress (CUS) for five days. Immediately following the cessation of stress tissue was collected from the hippocampus for ELISA.

In a much larger cohort of mice, male and female C57BL/J6 were assessed for behavioral responses to repeated dsHMGB1 administration via microinfusion to the medial prefrontal cortex

(mPFC). Free moving mice were infused with 75nl of aCSF or recombinant HMGB1 (dsHMGB1 certified LPS free (0.2ug/ul); HMGBiotech) using a 26G internal cannula (Plastics One, VA) once daily for five consecutive days. Following dosing mice completed a behavioral battery for social, anxiety-like, and despair behavior including open-field, elevated plus maze, novelty suppressed feeding, social preference, and tail suspension test. There were no significant effects of sex or estrus cycle thus male and female subjects were combined by treatment group for the final statistical analysis.

Results: Firstly, HMGB1 expression is increased by acute stress ($p < 0.05$, $t(1,13) = 2.326$).

dsHMGB1 treated animals demonstrated increased latency to feed in novelty suppressed feeding assay ($p < 0.05$, ($t(1,39) = 2.253$). Elevated plus maze behavior was also altered in the dsHMGB1 treated animals. dsHMGB1 administration is associated with decreased time in the open arms ($p < 0.01$, ($t(1,39) = 2.814$) as well as increased latency ($p < 0.05$,($t(1,39) = 1.836$).

Conclusions: These results suggest that HMGB1 may be a molecular marker of stress and predictive of behavioral alterations in response to stress. It is interesting that the behaviors affected are anxiety like behaviors with no significant alterations to social behavior, locomotion, or tail suspense test response. The mPFC projects to the basolateral amygdala and this circuit is well characterized in pre-clinical models of anxiety. Further studies are underway to better characterize the downstream signaling of HMGB1 in a cell type specific manner. A role for microglia is being investigated utilizing IHC methods and quantitative RNA and protein expression methods.

A very significant outcome of this study is the generation of a shared behavioral phenotype between male and female subjects. This convergence allows for subsequent studies to study anxiety with emphasis on shared etiology. Therefore, understanding the molecular mechanisms underlying HMGB1's influence on anxiety may pave the way for novel treatment strategies and the development of more effective and personalized therapeutic approaches for individuals suffering from anxiety disorders.

Keywords: Anxiety and Stress, Behavioral Pharmacology, Anxiety Circuitry, HMGB1, Acute and Chronic Stress

Disclosure: Nothing to disclose.

P87. Does the Bed Nucleus of the Stria Terminalis Mediate Circuit-Induced Relapse of Extinguished Fear?

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Background: Extinction learning is central to behavioral therapies for treatment of stressor- and trauma-related disorders including PTSD. We have recently discovered that the hippocampus (HPC) plays a critical role in the relapse of extinguished fear that occurs after pharmacological inactivation of the thalamic nucleus reuniens (RE) in rats. In particular, the HPC appears to encode contextual fear memories that are important for the "circuit-induced" relapse that occurs after RE inactivation. How these contextual memories drive increases in conditioned freezing behavior is unclear. However, there are abundant projections from the HPC to the bed nucleus of the stria terminalis (BNST) that may underlie relapse-induced freezing. Because the BNST has a prominent role in the expression of contextual fear memories, we hypothesize that it plays an important role in the circuit-induced relapse.

Methods: To test this idea, we explored whether pharmacological inactivation of the BNST would reduce the relapse of extinguished fear associated with RE inactivation. Adult male and female Long-Evans rats first underwent auditory fear conditioning followed twenty-four hours later by extinction. The next day, animals received intra-BNST infusions of either vehicle (saline) or the AMPA receptor antagonist, NBQX (10 ug/ul, 0.3 µl per side) ($n = 6$-8 per group) and intra-RE infusions of either vehicle (saline) or muscimol in a factorial design.

Results: Consistent with prior findings, RE inactivation caused a relapse of fear and increased freezing to the extinguished CS in the extinction context.

Conclusions: However, preliminary data suggest that concurrent BNST inactivation does not attenuate this circuit-induced relapse. Further work will explore alternate neural pathways by which RE inactivation drives relapse-induced increases in freezing behavior.

Keywords: Fear, BNST, Extinction, PTSD, Relapse

Disclosure: Nothing to disclose.

P88. Increasing Endocannabinoid Levels Blocks Predator Odor-Induced MAPK Activation in the Paraventricular Nucleus of the Hypothalamus in Rats

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Background: The neurobiology of stress-induced effects that may contribute to psychiatric disorders remains unclear. A potential modulator of the transition from stress to pathology could be Mitogen Activated Protein Kinase (MAPK), which is known to promote neural plasticity related to learning and memory impairments. While previous work indicates that brain MAPK is activated (phosphorylated) in response to standard lab stressors, its activity in response to trauma-like stress is not well characterized. Here, we examined the effects of exposing rats to fox urine odor, which is an ethologically valid model of trauma-like stress since foxes are natural predators of rats. Furthermore, we assessed modulation of stress-induced MAPK expression by cannabinoids, which are increasingly proposed as potential treatment targets for stress-induced psychiatric disorders. There are two endogenous cannabinoid ligands - anandamide (AEA) and 2-arachidonoylglycerol (2-AG), termed endocannabinoids (eCB). AEA and 2-AG have separate synthesis and breakdown mechanisms, allowing for distinct targeting of each endocannabinoid individually. This work identifies neural substrates through which eCBs could regulate the sequelae of trauma-like exposure.

Methods: Adult male Sprague Dawley rats ($N = 24$) were habituated to experimental procedures and handling for several days prior to testing. On the test day, animals received an intraperitoneal (IP) injection of either vehicle (DMSO; $N = 8$), JZL184 (16mg/kg; inhibitor of 2-AG catabolism; $N = 8$), or URB597 (0.3mg/kg; inhibitor of AEA catabolism; $N = 8$). Thirty min later, all subjects were placed into test cages. Half of the rats in each treatment group were assigned randomly to cages with fox urine pellets in the bedding (odor condition); the other half was placed into cages without odor pellets (control condition). Odor and control cages were located in separate rooms to prevent olfactory signals from the stress condition reaching the control animals. One hour after placement into test cages, rats were perfused transcardially with a paraformaldehyde solution and brains were removed. Brains were then cryoprotected in increasing concentrations of a sucrose solution and sliced into 40-micron sections for immunohistochemical staining of phosphorylated MAPK (primary antibody, Cat# 4370 Cell Signaling Technology). After immunohistochemistry, slices were mounted and slides were

cover-slipped for subsequent counting of MAPK-positive cells by an experimenter blind to the experimental conditions. We focused on the basolateral amygdala (BLA) and paraventricular nucleus of the hypothalamus (PVN) since both regions play key roles in responses to stress. Data were analyzed with multifactorial analysis of variance with stress condition (control vs. odor) and drug condition (vehicle, JZL194, or URB597) as between-subjects factors. Post-hoc tests were used for comparisons of means when indicated by significant main effects or interactions. The alpha level was set at $P < 0.05$.

Results: In the PVN, a significant increase in the number of MAPK-positive cells was seen in vehicle-treated odor-exposed rats versus control rats (vehicle-treated and NO odor exposure) ($P < 0.05$). Furthermore, a significant drug x stress condition interaction was seen [$F(2,42) = 6.46$, $P < 0.004$)]. Posthoc analyses indicated that the odor-induced MAPK increase was blocked by either JZL184 ($P < 0.001$) or URB597 ($P < 0.001$). However, JZL184 also significantly lowered MAPK levels in control animals ($P < 0.05$); URB597 did not (not significant). In contrast to the PVN, no significant changes to MAPK-labeling were observed in the BLA.

Conclusions: Plastic changes within the PVN, as evidenced by predator stress-induced phosphorylated MAPK activation, play a role in translating the effects of trauma-like stress. The prevention of this MAPK activation by the AEA breakdown inhibitor URB597 specifically in predator odor-exposed rats indicates that increased AEA tone can counteract this stress-induced effect in PVN cells. In contrast, the 2-AG breakdown inhibitor JZL184 decreased MAPK in both stressed and control animals, indicating that enhanced 2-AG tone causes an overall reduction in PVN MAPK that is not specific to stress. Taken together, these findings indicate differential functional roles for the two different eCB pathways in the regulation of stress-induced effects within the PVN. Finally, the lack of MAPK activation in the BLA indicates anatomical specificity of this intracellular signaling pathway's recruitment to the PVN following predator stress exposure. To the best of our knowledge, this is the first demonstration of eCB-mediated prevention of traumatic stress effects on PVN MAPK. These results indicate that increasing AEA could be a potential treatment strategy for reducing the long-term sequelae of trauma-like stress, particularly those involving trauma-induced plasticity in the PVN.

Keywords: ERK, 2-AG, Anandamide, Stress

Disclosure: Nothing to disclose.

P89. PTSD Symptoms are Associated With Increased Startle Magnitude in Pregnant Black Persons

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Background: Pregnant Black persons are at disproportionate risk for PTSD, with lifetime rates as high as 56%. PTSD is associated with dysregulated psychophysiology, including alterations in startle magnitude during a fear potentiated startle (FPS) task. For example, in military and non-pregnant civilian populations, people with PTSD show increased startle to all stimuli, as well as increased startle to a safety signal (impaired fear inhibition). With regards to pregnant persons, previous work from our group has shown that increased startle to a safety signal is associated with hyperarousal symptoms of PTSD in pregnant persons, though this study did not control for stage of pregnancy. Because pregnancy is associated with significant biological and social changes, further research is necessary to understand the associations between PTSD and fear-related psychophysiology at specific pregnancy stages. Thus, we examined the associations between PTSD symptoms and FPS in the late second and early third trimesters of pregnancy among pregnant Black individuals. We hypothesized that greater PTSD symptoms would be associated with higher startle magnitude across the FPS task, and that greater PTSD symptoms would be associated with higher startle to the safety signal.

Methods: Female Black participants ($N = 45$, mean age $= 27.7$) seeking prenatal care from a large publicly funded hospital in Atlanta, GA were invited to participate in the study. Participants underwent an interview in which basic demographic information and PTSD symptoms were assessed between 19 and 33 weeks of pregnancy (mean gestational age $= 26.2$ weeks). Trauma exposure was assessed with the Traumatic Events Inventory (TEI). PTSD symptoms were determined using the PTSD Symptom Checklist for DSM-5 (PCL-5). Startle magnitude was measured using the FPS task. The FPS task consists of an initial habituation phase where two conditioned stimuli (CS) are presented without an unconditioned stimulus (US). The habituation phase is immediately followed by the acquisition phase, consisting of three blocks in which one CS is followed by the aversive US (CS+) and the other CS is not followed by the US (CS-; safety signal). Startle magnitude was determined using the EMG eye blink response to a startle tone played independently (NA trials) or shortly after presentation of the CS (CS trials). A linear mixed effects model was used to determine the effect of PTSD symptoms, trial type, block, and their interactions on startle magnitude. Trial type and block were repeated measures and participant was a random factor. Startle data were log transformed because the raw startle magnitude data were positively skewed.

Results: Pregnant individuals had experienced on average 5.5 types of traumatic events. The linear mixed effects model revealed a significant effect of PTSD symptoms $F(1,34) = 4.77$, $p = .03$, where more PTSD symptoms were associated with greater startle magnitude. There was also a significant effect of trial type ($F(2,374) = 29.38$, $p < .001$) and block ($F(3,374) = 8.18$, $p < .001$) on startle magnitude. The interaction between PTSD symptoms and trial type was not significant ($F(2,374) = 2.14$, $p = .12$), nor were the interactions between PTSD symptoms and block ($F(3,374) = .42$, $p = .74$), trial type and block ($F(6,374) = 1.69$, $p = .12$), or PTSD symptoms by trial type by block ($F(6,341) = .71$, $p = .64$). Results were similar after adding gestational age as a covariate in the model; PTSD symptoms were associated with greater startle magnitude ($F(1,33) = 4.68$, $p = .04$). There was also a significant effect of trial type ($F(2,374) = 29.38$, $p < .001$) and block ($F(3,374)=8.18$, $p<.001$). The interaction between PTSD symptoms and trial type remained non-significant ($F(2,374)=2.14$, $p=.12$), as did the interactions between PTSD symptoms and block ($F(3,374)=.42$, $p=.74$), trial type and block ($F(6,374)=1.69$, $p=.12$), and PTSD symptoms by trial type by block ($F(6,341)=.71$, $p=.64$).

Conclusions: The current results indicate that PTSD symptoms are associated with greater startle magnitude across block and trial type in pregnant Black persons in the late second and early third trimesters. We did not find that greater PTSD symptoms were associated with greater startle magnitude to the safety signal as compared to the other trial types. These results suggest that PTSD symptoms are associated with dysregulated fear psychophysiology regardless of cue type within second and third trimester pregnant Black persons. Future longitudinal studies could assess how PTSD symptoms are related to changes in FPS over the course of pregnancy to determine if psychophysiology is more or less dysregulated at specific stages of pregnancy. Understanding how psychophysiology changes during pregnancy in individuals with PTSD symptoms may also inform intervention strategies.

Keywords: Fear-Potentiated Startle, PTSD, Pregnancy

Disclosure: Nothing to disclose.

P90. Combined Childhood and Adulthood Trauma is Associated With Lower CB1R Availability Compared to Adulthood Trauma Alone: A Positron Emission Tomography Study

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Background: There has been growing evidence from animal studies suggesting that early life adversity affects the endocannabinoid system in a different pattern compared to adulthood trauma. But the evidence from human studies is limited. Here, we present, for the first time, the preliminary results on the CB1 receptor availability among those who experienced both childhood and adulthood trauma compared with those with only adulthood trauma.

Methods: Adult individuals with a history of lifetime trauma underwent positron emission tomography (PET) using [11C]OMAR to measure the CB1R availability (the volume of distribution). The trauma questionnaire captured the specific trauma and the age at which each trauma was experienced. Based on the age reported, we classified participants as those with and without childhood trauma (≤18 years). All participants experienced at least one trauma during adulthood (>18 years). A mean composite measure of the CB1 receptor availability was calculated based on the average of all brain region measures to prevent possible collinearity. Non-parametric tests were used to compare the two groups regarding demographic characteristics and the PTSD diagnosis based on the 'Clinician-Administered PTSD Scale for DSM-5' (CAPS-5). The differences in CB1 receptor availability between the two groups were assessed using linear regression adjusted for age, sex, and body mass index. The study protocol has been approved by the Yale School of Medicine Institutional review board.

Results: Overall, data from 26 participants (80.8% men) were used for data analysis, with 11 individuals only reporting adulthood experience and 15 individuals experiencing both childhood and adulthood trauma. Only 3 individuals in each group met the diagnosis of PTSD. There was no significant difference between the study groups in terms of demographic characteristics and PTSD. We found that lower brain-wide CB1 receptor availability was associated with experiencing trauma both in childhood and adulthood compared to those without childhood trauma (adjusted beta coefficient: -0.20; 95%CI: -0.36, -0.038; p-value: 0.018; mean percent difference: -13%).

Conclusions: We found that co-occurring childhood and adulthood trauma is associated with altered endocannabinoid system regulation with lower CB1 receptor availability. Future studies are warranted to explore the implications and the possible role of the frequency of the trauma and its type.

Keywords: Endocannabinoids, Childhood Trauma, CB1 Receptor

Disclosure: Nothing to disclose.

P91. Additive and Interactive Effects of Traumatic and Physiologic Stress: Evidence for a Common Biologic Mechanism of Persistent Central and Autonomic Symptoms After COVID-19 Infection and Prior Traumatic Stress

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Background: A significant proportion (10% or more) of individuals infected with COVID-19 report symptoms that persist for weeks, months, or years after infection [1]. Many of these persistent symptoms are similar to those experienced after a traumatic stressor or a traumatic brain injury (TBI), for which increased central and peripheral adrenergic signaling has been linked to symptom expression [2]. In an ongoing clinical study, we are testing the hypothesis that one mechanism by which COVID-19 infection results in persistent symptoms is the activation of persistent increases in central and peripheral adrenergic signaling, a mechanism that overlaps and can interact with the effects of traumatic stress and traumatic brain injury.

Methods: Results are drawn from the baseline assessment of an ongoing longitudinal, observational, self-report online assessment. Participants are 389 United States adults with (N = 265) and without (N = 124) a self-reported history of COVID-19 infection. Self-report measures covered autonomic symptom burden (Composite Autonomic Symptom Score [COMPASS 31]), lifetime traumatic stressors (Life Events Checklist), post-traumatic stress disorder (PTSD Checklist-5), neurocognitive functioning (Neuro-QoL), insomnia (Insomnia Severity Index), and fatigue and pain (PROMIS Fatigue and Pain Interference measures). Objective neurocognitive functioning was assessed using the unsupervised Test My Brain online assessment battery from the Many Brains Project, and included assessments of processing speed, visual short-term memory, sustained attention, working memory, cognitive control, response inhibition, verbal memory, and nonverbal reasoning.

Results: Here, we present initial results from the self-report and neurocognitive-testing data from the first N = 389 participants (N = 265 with a history of COVID-19 infection, N = 152 with self-identified "Long COVID"). We present 3 main initial findings from this work. First, we found that autonomic symptom burden is significantly elevated in the group with a history of COVID-19 infection compared to the group without a history of COVID-19 infection ($p < 1e\text{-}8$), and that these peripherally driven somatic symptoms are strongly associated with and a strong statistical mediator of self-reported impairment in neurocognitive functioning ($R = -.62$ $p < 1e\text{-}25$), consistent with the potential for a co-regulated common mechanistic pathway underlying both peripheral and centrally generated symptoms.

Second, we found that both history of COVID-19 infection and cumulative lifetime trauma burden were associated with higher autonomic symptom burden. Consistent with our specific hypothesis that a prior history of traumatic stress would increase the risk of persistent symptoms after COVID-19, a multivariable linear regression model exploring the relative contributions of COVID-19 infection and history of traumatic stress to autonomic symptom burden found a statistically significant interaction between these two factors ($p = .03$).

Finally, drawing from 6 independent self-administered online neurocognitive tests, we found that tests that depended on processing speed (e.g. Choice Reaction Time) showed a significantly different relationship to self-reported neurocognitive functioning for those with versus without a history of COVID-19 infection: for those without a known history of COVID-19 infection, objective performance was not significantly related to self-reported cognitive functioning ($R = -.04$, $p = .77$), while for those with a history of COVID-19 infection, objective test performance was significantly and positively associated with positive self-reported cognitive functioning ($R = 0.28$, $p = .001$). These findings are consistent with self-reported cognitive symptoms in individuals with persistent symptoms after COVID-19 demonstrating a meaningful relationship to objective performance on neurocognitive testing, particularly in the domain of processing speed.

Conclusions: Prior history of traumatic stress has a positive and interactive effect on symptoms of autonomic dysregulation following COVID-19 infection, independent of PTSD symptoms.

This raises the possibility that exposure to traumatic stress may result in persistent changes in stress-threat response systems that can affect the response to future stressors, including physiologic stressors such as COVID-19 infection. The relationship may help to explain prior observations that baseline anxiety prior to COVID-19 infection is associated with increased likelihood of persistent symptoms following COVID-19 infection.

Keywords: PTSD, Stress and Trauma, COVID-19, Noradrenaline, Autonomic Nervous System

Disclosure: Nothing to disclose.

P92. High CRP is Associated With Deficits in Learning Late in Fear-Potentiated Startle Extinction

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Background: Despite effective therapies for posttraumatic stress disorder (PTSD), a significant minority of patients do not respond. Translational models have improved the understanding of the etiology and treatment of PTSD and other fear-based disorders. In particular, fear extinction paradigms provide an analogue to exposure-based treatments for PTSD such as prolonged exposure therapy (PE). Investigating potential mechanisms and biomarkers through such paradigms allows not only for increased understanding of etiology, but also potential prognostic markers and intervention targets to better personalize care. High inflammation, as marked by C-reactive protein (CRP), has been associated with trauma exposure as well as development of PTSD. Additionally, previous work from our group has shown high CRP to be associated increased startle to a safety signal during fear acquisition in a fear-potentiated startle (FPS) paradigm. What remains to be determined is the association between CRP levels and fear extinction and subsequently the link between inflammation and PE outcomes.

Methods: As part of a large epidemiological study of PTSD in urban, under-resourced, and chronically traumatized populations, 231 participants (65.7% female, ages 18-65) approached in outpatient general medical and OB/GYN clinics returned for a follow-up psychophysiology study. Participants completed the Modified PTSD Symptom Scale (MPSS) to assess PTSD symptom severity and provided a blood draw, which underwent subsequent hsCRP assay. Participants underwent a validated FPS paradigm consisting of three blocks of fear conditioning and four blocks of fear extinction, the latter of which is the focus of the current project. Electromyographic (EMG) recordings to an acoustic startle probe were measured by Ag/AgCl electrodes placed on the orbicularis oculi muscle 1 cm below the pupil and the lateral canthus. Dichotomous categories were created for inflammation in line with previously published cutoffs (high = ≥ 3 mg/L and low < 3 mg/L) based on serum hsCRP and extinction (success = ≥ 50% decreases in FPS from block 1 to block 4, poor < 50% FPS decrease).

Results: Continuous measurements of serum CRP were predictive of reduction of fear, such that higher inflammation predicted impaired learning ($F[6,207] = 1.88$, $p = < .01$, $R2 = .052$). Compared to those with low inflammation, participants with high inflammation had increased odds of being categorized into the poor extinction group (OR = 1.92, 95% CI [1.03-3.61]; $p = .04$). This finding increased in magnitude when examining just participants with PTSD (OR = 5.12, 95% CI [1.34-19.62]; $p = .02$).

Conclusions: Higher inflammation, measured both continuously and dichotomously, was associated with impaired extinction learning in a FPS paradigm amongst trauma-exposed individuals. This finding was amplified in the subset participants with PTSD. The lack of safety signal learning seen among those in the high inflammation group is not only important for the understanding of learning mechanisms and behavior, but also in the context of exposure therapy. PE includes both in-vivo and imaginal exposures, which in part rely on learning that the feared stimulus is not a threat. Thus, one potential hypothesis may be that those with high inflammation may not respond as well to a typical dose of exposures. Alternatively, novel approaches to facilitate learning may be needed in those with high inflammation. Beyond PTSD, this has application to other fear-based disorders such as phobia and obsessive-compulsive disorder. These results also build off of prospective research showing that high CRP pre-deployment to combat is associated with development of PTSD post-deployment. Findings suggest that future work should be conducted using inflammation as both a prognostic indicator for treatment success as well as a potential target to engage as an adjunctive to facilitate learning.

Keywords: Inflammation, Fear Extinction, Post Traumatic Stress Disorder

Disclosure: Nothing to disclose.

P93. Higher Arterial Stiffness and Blunted Autonomic Control of the Heart in Young Women With PTSD Compared to Controls

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Background: Post-traumatic stress disorder (PTSD) is associated with significant cardiovascular disease (CVD) risk. Women are twice as likely as men to develop PTSD after a traumatic event. Although premenopausal women are thought to be protected from CVD, a diagnosis of PTSD increases CVD risk by up to threefold. Available data in predominantly male cohorts point to autonomic nervous system dysfunction as a potential underlying mechanism. Therefore, the aim of this study was to determine if premenopausal women diagnosed with PTSD, compared to those without, present with greater arterial stiffness and a blunted parasympathetic control of the heart compared to trauma-exposed women without PTSD.

Methods: Fifty-eight otherwise healthy young women (18-40 years) with a history of trauma exposure were included in this study, 26 with PTSD and 32 without PTSD diagnostic. The study took place during two visits. At visit one, we collected anthropometric measures, assessed PTSD symptom severity with the PTSD checklist for DSM 5 (PCL5) and depression severity using the Beck Depression Inventory (BDI). At visit two, we measured resting pulse wave velocity (PWV), augmentation pressure and augmentation index (AI) via pulse wave analysis using applanation tonometry. Additionally, heart rate variability (HRV) was assessed using the finger volume pulse waveforms obtained from a peripheral arterial tone signal. We compared the two groups with independent t-tests and we report one-tailed p-values.

Results: As expected, women diagnosed with PTSD had higher PCL5 (45 ± 14 vs 28 ± 15 a.u, $p < .001$) and BDI (24 ± 12 vs 15 ± 8 a.u, $p = 0.002$) scores compared to women without PTSD. Resting brachial arterial blood pressure [systolic, 116 ± 10 vs 110 ± 8 mmHg, $p = .007$; diastolic, 72 ± 7 vs 68 ± 7 mmHg, $p = .017$] and heart rate (73 ± 10 vs 67 ± 8 bpm, $p = .008$) were higher in women with PTSD compared to women without PTSD. Our primary outcome measures of PWV (6.1 ± 0.8 vs 5.6 ± 0.8 a.u., $p = 0.007$)

was also higher in women with PTSD. Likewise, aortic augmentation index (24 ± 12 vs 17 ± 10 a.u., p = 0.016) and augmentation pressure (8 ± 4 vs 6 ± 3 mmHg, p = 0.010) were higher in women with PTSD. Finally, when examining HRV, standard deviation of NN [SDNN, 44 ± 22 vs 57 ± 18 ms, p = 0.018] and root mean square of successive differences between normal heartbeats [RMSSD, 36 ± 28 vs 57 ± 23 ms, p = 0.015] were lower in women with PTSD compared to women without PTSD.

Conclusions: In sum, our results show that among premenopausal women with a PTSD diagnosis is associated with higher resting arterial blood pressure and heart rate, increased arterial stiffness and blunted parasympathetic control of the heart. These findings suggest that PTSD may impose a high allostatic load on the heart in young women, who are typically thought to be protected from CVD. These data also provide mechanistic insight into links between PTSD and hypertension prior to menopause.

Keywords: PTSD, Women, Cardiovascular Function, Arterial Stiffness, Heart Rate Variability

Disclosure: Nothing to disclose.

P94. A Crh+ Amygdala Cell Activity Signature for Initiation of Aggression

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Background: Physical aggression is typically a last resort for settling a dispute; this implies that the transitions between behaviors leading up to an attack represent sequential checkpoints for either escalating or deescalating a confrontation. The likelihood of an aggressive attack increases if the opponent is perceived as threatening; as such, we examine threat-encoding corticotropin-releasing-hormone-expressing central amygdala (Crh+ CeA) neurons during the escalation of aggressive behaviors using single-cell calcium imaging, chemogenetics, and localized knockout strategies.

Methods: Male CRH-ires-Cre mice received intra-CeA Cre-dependent adeno-associated-virus (AAV) to drive expression of the calcium-dependent-fluorescent indicator, GCaMP, in Crh+ CeA cells. Mice were implanted with a gradient index lens over the CeA to record calcium-dependent fluorescence during low- or high-threat encounters with a submissive or aggressive conspecific, respectively. Additional CRH-ires-Cre males expressed control fluorescent protein, inhibitory Designer Receptors Exclusively Activated by Designer Drugs (iDREADDs) in all Crh+ CeA neurons, or iDREADDs in Crh+ CeA-ventral tegmental area (VTA) projections. DREADD agonist was injected prior to aggression tests to inhibit Crh+ CeA cells. For localized CRH knockout, aggression-naïve or aggression-experienced floxed-Crh mice and wild-type controls received intra-CeA AAV-Cre; two weeks later, mice underwent repeated low-threat aggression testing (2-3 tests/wk for >4 wks).

Results: Crh+ CeA cells were classified into ensembles during low-threat offensive aggression tests– 41% were active before attack initiation, 32% were active during attacks, and 27% were inactive during aggression. Chemogenetic inhibition of all Crh+ amygdala cells selectively prevented offensive attacks without affecting social approach, contact, or adaptive self-defensive bites toward a high-threat aggressive intruder. Chemogenetically inhibiting Crh+ CeA-VTA projections neurons suppressed – but did not eliminate – offensive attacks. Localized Cre-mediated CRH knockout in the CeA prevented the development of agonistic behaviors in aggression-naïve mice without affecting fighting in experienced aggressors.

Conclusions: Amygdala CRH signaling is necessary for the initiation of offensive aggression, but not for self-defense, suggesting distinct neural circuitry and signaling substrates for offensive vs. self-defensive aggression. All-optical closed-loop approaches are being used to block attack initiation using Crh+ CeA cell activity to trigger real-time inhibitory optogenetics.

Keywords: Amygdala, Aggression, Corticotropin-Releasing Hormone, Threat Reactivity, Closed-Loop Control

Disclosure: Nothing to disclose.

P95. Altered Belief Updating and Visual Prediction Error in Psychedelics Users

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Background: Serotonergic psychedelic compounds, such as psilocybin or LSD, have recently come into focus in neuropsychiatry for their therapeutic potential for individuals experiencing mood and anxiety disorders. Improvements in symptoms and feelings of well-being after just one dose of such drugs last for weeks and are comparable with first line antidepressant medications. The neurobiological and neuropsychological mechanisms underlying these therapeutic effects are not well-understood. One hypothesis, based in the predictive processing framework, suggests that psychedelic drugs loosen ingrained beliefs and biases that have become pathological, leading to shifts in bottom-up vs top-down processing of sensory input that alter perception, cognition, and mood.

At the level of early sensory processing, an established paradigm for studying how bottom-up sensory inputs are integrated with top-down expectations in the brain is the "oddball" paradigm. Herein, predictable stimulus sequences are randomly interrupted with rare, unexpected "deviant" stimuli. Deviant stimuli evoke increased brain responses, as indexed by e.g. the mismatch negativity (MMN) and P300 scalp potentials (as recorded with e.g. electroencephalography (EEG)). In a predictive processing framework, MMN/P300 have been conceptualized as neural "prediction-errors" which signal a mismatch between internally held beliefs and incident sensory data. Therefore, increased flexibility in belief systems should, in theory, affect MMN/P300 response magnitudes. Further, our prior work in mice has shown that frontal brain regions modulate sensory brain regions during oddball paradigm in a "top-down" manner, biasing local processing of incident stimuli to produce MMN-like responses to unexpected inputs.

Methods: Here we tested the hypothesis that use of serotonergic psychedelics alters how sensory information is processed against internal expectations in the brain, producing changes in predictive processing and cortical prediction error-like responses. We recorded EEG (32 channel) in fifteen human participants who had used serotonergic psychedelics in the prior 30 days (psychedelics users; psilocybin or 5meO-DMT) and fifteen age and gender-matched control participants. Male and female participants viewed a visual oddball task involving guided saccades to target stimuli at i) predictable locations (80%), ii) unpredicted locations. For the latter, the locations could be informative (surprise update -10% of trials) or not-informative (surprise no-update; 10% of trials) in that they either predict (update) or do not predict (no-update) the future stimulus locations. This distinction allowed segregation of brain processes related to "novelty detection" and "belief updating" in the visual

event-related potentials, which were compared between groups with mixed ANOVA. Further, guided by our rodent research, we examined fronto-visual coherence (1-circular variance of inter-electrode phase-lag from 1- to 40-Hz) across the scalp as an index of predictive modulation during the oddball paradigm – a theoretical substrate of internally held beliefs.

Results: Control participants displayed similar visual MMN/P3A-like potentials to both surprise trial types (global field power from 200-275ms post-stimulus onset), suggesting that these responses reflect cortical "prediction errors" in response to novel stimuli. This vMMN-like activity was notably weaker in psychedelics users, regardless of surprise-type (Cohen's $d = 0.94$). Later sustained global field power in the 400 to 700ms time range showed a P3b-like distribution and was different between surprise-update and surprise no-update trials in control participants ($d = 1.19$), suggesting a potential an index of "belief-updating". Interestingly, this condition-wise difference was not present in psychedelics users (cohen's $d = 0.006$), and coincided with weaker scalp-wide coherence in the alpha-band.

Conclusions: Individuals reporting recent use of psychedelics displayed evidence of altered predictive processing in cortical networks, including smaller indices of prediction error, altered belief-updating, and weaker predictive modulation. Consistent with past behavioral and computational research, this suggests that serotonergic psychedelics may effectively weaken priors – or lead to greater flexibility in expectations and beliefs – even in sensory processing cortical circuits. Together with basic studies in mice during visual oddball paradigms, this work may point to potential cortical cell and circuit mechanisms mediating the effects of psychedelics for future research.

Keywords: Psychedelic Medicine, Visual Processing, Mismatch Negativity, Predictive Coding, Self-Medication With Psychedelics

Disclosure: Nothing to disclose.

P96. Neural Correlates of Safety Signaling and Conditioned Inhibition of Fear in the Rat Ventromedial Prefrontal Cortex

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Background: The ability to accurately discern between safe and threatening situations is challenged in stress disorders, often leading to maladaptive fear expression. Many individuals with stress disorders fail to show conditioned inhibition of fear, the downregulation of fear during the simultaneous presentation of a learned fear and safety cue (fear+safety cue). The ventromedial prefrontal cortex (vmPFC) of humans and rodents plays a key role in modulating fear, anxiety, and stress behaviors. Our lab has a well-validated safety-fear-reward cue discrimination task, where we have previously shown that the prelimbic subregion (PL) of the vmPFC is necessary for fear expression, while the infralimbic subregion (IL) of the vmPFC is necessary for conditioned inhibition of fear.

Methods: We adapted our safety-fear-reward cue discrimination task to produce a greater range in fear suppression in male and female Long Evans rats ($n = 8$/sex), such that approximately half of males and females showed "good" fear suppression, while the other half did not. During this behavior we collected longitudinal single cell calcium activity within the prelimbic (PL) or infralimbic (IL) regions of the ventromedial prefrontal cortex (vmPFC) in freely behaving male and female Long Evans rats using miniscopes and GCaMP6m expressed via AAV1.GCaMP6m.W-PRE.SV40 from Inscopix ($n = 2$-3/sex).

Results: Our preliminary data in the IL indicate that there is an ensemble of neurons highly correlated in its activity to the fear +safety and fear cues ($p = 6.16e\text{-}24$) that is separate from an ensemble of neurons highly correlated in its activity to the fear +safety and reward cues ($p = 0.0007$). Preliminary results show that we can also follow approximately 25-30% of these same cells for at least 12 days. Current analyses are examining cue-evoked calcium transients against expressed safety, fear and reward behaviors across the entirety of the 15 day task.

Conclusions: During conditioned inhibition of fear, there appears to be separate neuronal ensembles within the rat vmPFC that are active during the fear+safety cue which are also active during either the fear cue or reward cue. This indicates that separate ensembles may be encoding the fear suppressing properties of the safety cue versus the rewarding properties of the safety cue.

Keywords: Discriminative Conditioning, Safety Learning, Conditioned Inhibition of Fear, Ventromedial Prefrontal Cortex, Calcium Imaging

Disclosure: Nothing to disclose.

P97. An Amygdala-Cortical Circuit for Encoding Generalized Fear

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Background: A debilitating symptom of nearly all anxiety and trauma- and stress-related disorders is the overgeneralization of fear. This is characterized by the expression of fear in new, safe, or ambiguous environments and can disrupt normative functioning. For example, people with PTSD overgeneralize traumatic experiences, develop fear of harmless stimuli, and experience traumatic memories in safe places. Our previous work demonstrated that the anterior cingulate cortex (ACC), a prefrontal cortex region, and its projections to the basolateral amygdala (BLA) control generalized fear when rodents experience new contexts in which threat is uncertain. However, the role of the ACC in context fear learning is poorly understood. Here, we used neuronal activity analysis, pharmacological inactivation, NMDA receptor blockade, and circuit-specific manipulations of inputs to the ACC to determine its role in context fear learning.

Methods: All experiments used male and female F1 B6129 hybrids derived from crossing C57BL/6J males with 129S1/SvImJ females. All mice were generated in a breeding colony at the University of South Carolina School of Medicine. Mice were unilaterally cannulated over the anterior cingulate cortex (ACC), counterbalanced for side for pharmacological experiments ($N = 23$-27 per experiment). For virus infusions, mice were bilaterally infused in the ACC (0.8mm AP, +0.7mm ML, -1.75mm DV) and the basolateral amygdala (BLA) (-1.6mm AP, +3.4mm ML, -4.9mm DV) for BLA-ACC circuit manipulations using a retrograde Cre AAV infused into the ACC and a Cre-dependent hM4Di AAV into the BLA ($N = 25$-30). To activate the BLA-ACC circuit an AAV expressing hM3dq we infused into the BLA or ACC and mice were cannulated over the target region (ACC or BLA) to either activate BLA terminals in the ACC or BLA neurons themselves using intracranial infusions of CNO.

Mice were trained using contextual fear conditioning in four identical conditioning chambers containing two Plexiglas walls, two aluminum sidewalls, and a stainless-steel grid-shock floor. The training context consists of the conditioning chamber with a polka-dot insert attached to the rear Plexiglass wall, white noise (70dB), dim illumination, and the stainless-steel grid floors cleaned

with 70% ethanol. Animals received 2 days of pre-exposure for 5 minutes and then, the following day underwent 9 minutes of fear conditioning by pairing the training context with a series of 5 un-signaled foot-shocks (1.0mA) separated by 90-second ISI. Measures of fear were recorded using the Freezeframe5. Mice were then tested for fear expression by examining freezing across the testing period. The test consisted of a 5-minute exposure to the training context and a novel context. The novel context consists of no background, flat floor, fan (60db), IR lights, and cleaned with 2% quataricide (Pharmacal). Mice underwent testing in both contexts in a counterbalanced design.

All data were analyzed using GraphPad Prism statistical software (GraphPad 9.5.1). All experiments were analyzed using unpaired t-tests, between subjects', or repeated measures two-way Analysis of Variance (ANOVA). Statistically significant ANOVAs were followed with either Tukey's or Sidak's post-hoc analyses where appropriate.

Results: Experiment one demonstrated that the ACC is active in response to strong fear conditioning that produces generalized fear to a novel context but is not responsive to shock only.

Experiment two examined post-training inactivation of the ACC using lidocaine. After training, mice were tested in the training or novel contexts. We found that inactivation of the ACC significantly reduced fear in the novel context but had no effect on specific fear in the training context (main effect of context and treatment ([$F(1, 19) = 64.52$, $p < 0.0001$], [$F(1, 19) = 11.58$, $p = 0.0030$] and a significant interaction [$F(1, 19) = 7.206$, $p = 0.0147$]).

Experiment three examined pre-training infusions of APV to assess the necessity of NMDA-dependent plasticity in the ACC during fear learning. Infusions of APV also significantly reduced fear in the novel context but again did not affect specific fear in the training context (Main effect of context and treatment ($F(1, 24) = 243.4$, $p < 0.0001$], [$F(1, 24) = 9.660$, $p = 0.0048$], and significant interaction [$F(1, 24) = 4.986$, $p = 0.0351$]). These data suggest that NMDA receptor-dependent plasticity in the ACC is necessary during learning for mice to generalize their fear to new contexts.

Experiment four inactivated BLA projections to the ACC during fear learning. Mice were injected i.p. with CNO (5 mg/kg) 30 minutes before training. Chemogenetic inhibition of BLA projections to the ACC significantly reduced fear to the novel context but left fear to the training context fully intact (Context effect [$F(1, 25) = 230.7$, $p < 0.0001$] virus effect [$F(1, 25) = 10.10$, $p = 0.0039$]. These data suggest that BLA projections to the ACC are necessary during fear learning to drive generalization but play no role in fear to specific contexts associated with a threatening stimulus.

We next activated the BLA-ACC pathway during weak fear training to demonstrate that this pathway is sufficient to produce generalization. Activating the BLA-ACC circuit significantly increased fear to the novel context but had no effect on fear to the training context.

Conclusions: Together, our findings implicate the ACC in encoding highly salient experiences to enable generalized responses. Further, BLA-ACC projections are both necessary and sufficient to produce generalized fear.

Keywords: Fear Conditioning, Contextual Fear, Emotion Circuitry

Disclosure: Nothing to disclose.

P98. Fear of Heights: A Novel Translational, Ethological Threat Paradigm

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Background: The detection of threat is essential for survival and involves specialized neural circuits which may contribute to anxiety- and trauma-related disorders. A major barrier in developing new treatments for these disorders lies in our inability to induce the same emotional experience of life-threatening danger in animal models and humans. The visual cliff task has been used widely to study human innate behavioral preference for avoiding high places in infants and to study visual depth perception in animals. Recently, a virtual reality heights task has been used in humans with chronically implanted electrodes and found to potently elicit subjective fear, peripheral physiological arousal, and anterior insula oscillations. Despite this history and recent advances in human neurophysiology of heights, threat from physical height has not been adapted to circuit and quantitative behavioral investigation with systems neuroscience approaches. Thus, we developed a virtual reality height threat task in which height threat can be manipulated to investigate neural circuits underlying threat-based decision-making. The medial prefrontal cortex (mPFC) is known to be activated by spatial proximity to threat and hypothesized to coordinate escape behaviors. Thus, we hypothesized mPFC neurons may be responsible for coordinating threat-based decisions.

Methods: Both male and female C57BL/6J mice (8-12 weeks in age) were injected with 500 nL of AAV1.Syn.GCaMP8f in mPFC (AP + 1.6, ML + - 0.3 DV -1.3). Two weeks later, a GRIN lens (4.0 x 0.5 mm, Inscopix) was implanted above the mPFC. Animals were fitted with a baseplate to allow for miniaturized microscope (nVoke, Inscopix) attachment. For optogenetic inactivation experiments, animals were injected bilaterally with AAV1.CamKII.stG-tACR2-FusionRed and simultaneously implanted with 200 uM ferrules (Neurophotometrics) for optogenetic stimulation. Optogenetic inhibition was performed using 5uW of constant stimulation at 450 nM. Prior to experiments, animals received two, ten minute handling sessions as well as 10 minutes of acclimation to wearing the miniscope or fiber optic patch cord in a clean cage. For behavioral experiments, animals were placed at the top of a 20" pole in an enclosed metal box. Visual stimuli were presented on a monitor beneath the mice and included checkerboard visual stimuli where one quadrant was visually close to the mouse, and the other three quadrants were projected as close (5cm) or far (20cm) from the fourth quadrant. The pole ends in a cone at which point mice must decide which quadrant to exit onto a Plexiglas surface. Animals receive 48 randomized trials where the target quadrant is rotated as well as the stimulus depth. "Correct" responses are those in which the mouse exits to the close, target quadrant.

Results: When the difference between the target quadrant and remaining quadrants is small, mice choose the target quadrant at chance levels, while mice choose the target quadrant more often when the visual discrepancy is large ($p < 0.001$, paired t-test). To test the necessity for mPFC in threat-based decision making, we optogenetically inhibited mPFC in half of the trials and find that compared to no-light trials, inhibition of mPFC reduces preference for the target quadrant in the far condition ($p = 0.004$, RM One-Way ANOVA; $p = 0.03$ Tukey's multiple comparisons for far condition light vs no light). We next recorded calcium activity of single neurons while mice performed this task using Inscopix and found that the population activity in mPFC is significantly higher when mice make correct decisions in the far stimulus condition compared to all other choice-stimulus combinations (221 neurons, $p < 0.0269$).

Conclusions: We developed a novel, virtual height threat task in which neural circuits underlying threat-based decision making can be investigated. We demonstrate the necessity of mPFC for optimally performing threat-avoidance behavior in this task and

that population level mPFC is highest when mice make correct decisions in more threatening environments. Current analyses are focused on determining the properties of individual neurons in this population and which ensembles might underly decision making. Importantly, this task is ethologically relevant and has high potential for translation to humans in virtual reality, providing the opportunity to more carefully characterize the circuits underlying threat processing in health and disease.

Keywords: Threat Reactivity, Neural Circuits, Optogenetics, In Vivo Calcium Imaging, Norepinephrine

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

P99. Older and Wiser? The Neural Basis of Worry and Worry Reappraisal in Older Adults

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Background: Worry is a transdiagnostic, highly prevalent symptom in late life. Worry has been associated with significant morbidity and is highly resistant to treatment. Understanding the neural basis of worry and worry reappraisal is important to develop novel treatments. Our objective was to understand the neural basis of worry and worry reappraisal and then evaluate how these are related to clinical morbidity.

Methods: We recruited older adults from the Pittsburgh area in a cross-sectional study. We recruited 124 older adults (≥50years) with varying worry severity and clinical comorbidity (27% generalized anxiety, 23% depression, and 18.5% mild cognitive impairment). Participants completed the worry induction task during functional magnetic resonance imaging (fMRI) including participant-specific worry induction, worry reappraisal, and neutral conditions. Participants rated their worry after each condition. We compared conditions using voxel-wise paired t-tests and conducted k-means clustering on regional activation to identify two groups. We predicted cluster groupings using elastic net using demographic and clinical data.

Results: Worry ratings were higher than neutral and reappraisal ratings in-scanner. Worry induction activated regions in default mode network, executive control network, anterior salience network, visual processing areas, basal ganglia, and thalamus. Reappraisal activated these regions with broader activation of the cingulate and prefrontal cortex.

Reappraisal showed greater activity (compared to worry) in the visual cortex, inferior/middle frontal gyrus, and bilateral parietal/angular gyrus. Worry showed greater activation (compared to reappraisal) in parts of the visual cortex, cerebellum insula, precuneus, and parts of the anterior cingulate and orbitofrontal gyrus.

Activity clustered into two groups that largely hypoactivated or hyperactivated across the cortex. Compared to hypoactive group, the hyperactive group showed hyperactivity in default mode and salience network during worry induction, but also greater posterior cingulate and supramarginal gyrus activity during reappraisal. The hyperactive group showed greater activity in the dorsal cingulate and insula during worry, while the hypoactive group showed higher activity in the dorsal cingulate during neutral condition.

We accurately predicted clusters using elastic net regression and found that hypoactive group exhibited higher reflection, lower in-scanner worry, and greater cumulative illness compared to hyperactive group.

Conclusions: We validate our previous work of the neural basis of worry showing three components related to emotion generation, subcortical relay, and implicit regulation in a large sample of older adults. We identify a group of individuals with hyperactivation and high acute worry and another group with relatively lower worry and higher reflection. These results identify potential target networks in chronic worriers that may be amenable to neuromodulation.

Keywords: Worry, Older Adults, Reappraisal, Generalized Anxiety Disorder, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P100. Fear and Safety Circuits are Topographically Organized in the Medial Prefrontal Cortex

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Background: The medial prefrontal cortex (mPFC) has been implicated as a critical brain structure for both promoting and dampening defensive responses in states of fear and anxiety. A canonical model has been established, implicating the dorsomedial prefrontal cortex (dmPFC) in the expression of defensive behavior, while the ventromedial prefrontal cortex dampens this expression. Yet, the extent to which these functional distinctions are regional or a gradient along the dorsoventral axis is unknown. Likewise, there have been limited investigations to variations in function along the anteroposterior extent of the mPFC.

Methods: Cellular activity was tracked in awake, behaving mice using in vivo two photon calcium imaging in the mPFC of mice differential fear conditioned to a shock-paired aversive cue and an explicitly unpaired neutral cue. Water restricted mice exhibited defensive behavior by suppressing their licking to the aversive cue and a cessation of spontaneous movement. Pupil dilation, pulse, and respirations were also tracked to examine differential physiological responses to the aversive and neutral cues. The functional roles of the anterior and posterior dmPFC were assessed using optogenetics, silencing these subdivisions in a temporally precise way using the inhibitory opsin stGtACR2 in behaving mice. Cue-evoked activity was evaluated along the anteroposterior and dorsoventral axes of layer II/III mPFC. Differences in the afferent and efferent connectivity of the anterior and posterior dmPFC were characterized using viral tracing, brain clearing, and light sheet microscopy.

Results: The canonical model was supported by a distinct pattern of activity along the dorsoventral axis of the mPFC during fear expression and subsequent fear extinction. However, patterns of activity during discrimination of the paired and unpaired cues demonstrated that discrimination of both the aversive and non-aversive cue was strongest more dorsally. Within the dmPFC, there was a gradient along the anteroposterior axis such that anterior dmPFC most strongly encoded the non-aversive cues, while the posterior dmPFC most strongly encoded the aversive cues. Optogenetic silencing of the anterior and posterior dmPFC revealed a dissociation of function, such that silencing posterior dmPFC exhibit the effect predicted by the canonical model, a decrease in defensive behavior and autonomic response to the aversive cue without effect on the non-aversive cue, while silencing anterior dmPFC did not affect responses to the aversive cue but promoted inappropriate defensive responding to the non-aversive cue. Viral tracing approaches reveal distinct patterns of

afferent and efferent connectivity of the anterior and posterior dmPFC that likely underlie their functional distinction.

Conclusions: Our findings both support and extend on the canonical model of dorsal and ventral mPFC function. While the vmPFC supports fear extinction, the dmPFC both supports defensive responding to aversive cues and suppresses responses to learned non-aversive cues. These functions are spatially segregated into the posterior and anterior poles of the dmPFC, respectively, which exhibit contrasting patterns of connectivity with afferent and efferent brain structures.

Keywords: Fear Conditioning, Medial Prefrontal Cortex, Discriminative Conditioning, Connectivity Gradients

Disclosure: Nothing to disclose.

P101. Hippocampal Area CA2 Neuronal Activity Supports Defensive Behavior During Acute Social Stress in Mice

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Background: In mice and humans, only a subset of individuals will go on to develop lasting behavioral changes in response to an environmental stressor. For example, exposure to an aggressive mouse results in about half of tested mice developing a socially avoidant phenotype, while a more resilient population exhibits no overt lasting behavioral effects of the experience (Nasca, Menard et al. 2019, Colucci, Marchetta et al. 2020). The hippocampus is critical for acquisition and retrieval of memory and influences an animal's response to stress. Moreover, neurons in area CA2 are required for social recognition memory and aggression (Hitti and Siegelbaum, 2014; Dudek, 2016; Leroy, et al., 2018). Given the high concentration of mineralocorticoid receptors in area CA2 (Vázquez, et al., 1998; Herman, et al., 1999; McCann, et al., 2021), this region is also positioned to play an important role in the greater hippocampal stress response. Therefore, we sought to determine whether CA2 plays a role in regulating behavioral responses to social stress in mice.

Methods: To determine whether neuronal activity in CA2 plays a causal role in an animal's response to an acute, socially derived stressor, we chemogenetically inhibited CA2 neuronal activity in vivo during an acute social defeat and tested whether memory for the defeat was influenced (Rosenhauer, Beach et al. 2019). In this model, C57/B6J male mice undergo a five-minute bout of social defeat in the home cage of a large male CD1 aggressor. The mice are then tested 24 hours after the defeat for social preference or avoidance. We and others find that defeated mice spend, on average, significantly less time investigating a novel CD1 mouse when compared to control mice that had not been exposed to the aggressor mice. Furthermore, we found that the mice showing the avoidant phenotype could be observed to show this behavior for up to one month following a single defeat. Neuronal activity of the pyramidal cells in CA2 was inhibited during the defeat using cell-type specific expression of the inhibitory Gi-DREADD receptor using a mouse expressing cre recombinase under the Amigo2 promoter and administration of clozapine-n-oxide (CNO) 5 minutes prior to the defeat. Animals were also assessed for behavior during defeat, together with total aggression received, defensive submission (e.g., rearing, defensive attacks), and fleeing behavior. Changes in neuronal activity during social investigation were measured by assessing immunofluorescence for the immediate early gene (IEG) cfos, with a focus on the hippocampal subfields and downstream corticolimbic regions.

Results: When CA2 activity was inhibited with CNO during the defeat, subject mice (DREADD-expressing, cre-positive animals) exhibited a significantly greater amount of social avoidance one day later when compared to defeated littermates not expressing DREADDs (cre-negative animals). CA2 inhibition during the defeat also resulted in reduced neuronal activity following social investigation, as measured by Fos staining. Lastly, given CA2's known role in aggression, we asked whether inhibition of CA2 also modulated the behavior of subjects during the defeat itself. We observed a significant reduction in the amount of defensive submission behavior (vs. fleeing) during defeat when CA2 was inhibited.

Conclusions: Taken together, these results indicate that CA2 neuronal activity supports submissive defensive behavior during social defeat, which is correlated with less avoidant behavior in subsequent days. This CA2-dependent behavior during the defeat is a predictor of future 'resilience' to social stress.

Keywords: Aggression, Sociability, Hippocampus-mPFC Pathway

Disclosure: Nothing to disclose.

P102. Accumbal Single Cell Ensembles Bidirectionally Respond to Experienced Versus Observed Aversive Stimuli

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Background: Empathy is the ability of adopting others' sensory and emotional states and is an evolutionary conserved trait among mammalians. In rodents, empathy manifests itself as social modulation of aversive stimuli such as acknowledging and acting on conspecifics' distress. The neuronal network underlying social transmission of information is known to overlap with the brain regions that mediate behavioral responses to aversive and rewarding stimuli.

Methods: In this study, we recorded single cell activity patterns of nucleus accumbens (NAc) core neurons using in vivo optical imaging of calcium transients via miniature scopes. This cutting-edge imaging methodology not only allows us to record activity patterns of individual neurons but also lets us longitudinally follow these individual neurons across time and different behavioral states. Using this approach, we identified NAc core single cell ensembles that respond to experienced and/or observed aversive stimuli.

Results: Our results showed that experienced aversive stimuli evoked a strong response in NAc core single cell ensembles. We showed that most NAc core single cells positively responded to experienced aversive stimuli, however, there was also a smaller group of cells exhibiting a negative response suggesting that the NAc core single cell ensembles are heterogeneous in their response to aversive stimuli. Interestingly, observed aversive events also elicited a significant positive response, however this response was weaker. The size of the positively responding single cell ensembles was greater for experienced aversive stimuli compared to observed aversive events. Importantly, we discovered a sub-population of NAc single cells that shows bidirectional response patterns to experienced versus observed aversive events. That is, a group of NAc core cells negatively respond to experienced and positively respond to observed aversive events (or vice versa).

Conclusions: Together, these results suggest that the NAc plays a role in differentiating somatosensory experience from social observation of aversion at a single cell level. This has important

implications for psychopathologies where social information processing is maladaptive, such as autism spectrum disorders.

Keywords: Nucleus Accumbens, Aversive Learning, Social Behavior

Disclosure: Nothing to disclose.

P103. Chemogenic Maniplation of Prefrontal-Periaqueductal Gray Circuits in Female and Male Rats During Pavlovian Fear Conditioning

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Background: In Pavlovian fear conditioning, darting is an escape-like conditioned response. Darting occurs more frequently in females and is reliably accompanied by a distinct behavioral phenotype: Darters have heightened unconditioned responses, such as shock and post-shock response, which emerge before the first dart occurs, and also show enhanced extinction retention, as measured by decreased freezing. Although much has been studied about the brain regions and circuits associated with conditioned freezing, the regions and circuits responsible for driving darting behavior is unknown. The dorsal periaqueductal gray (dPAG) is active during escape-like threat responses and receives direct input from the infralimbic cortex (IL). The IL plays a role in emotionally regulated behavior and is critical for extinction learning. The neural circuitry underlying darting is unknown, but the IL-dPAG circuit is a potential mediator of this and associated behavior. In these two studies, we tested this hypothesis.

Methods: In our first experiment, male and female Sprague Dawley rats underwent a 7 CS-US auditory, cued-fear conditioning paradigm. Animals were exposed to a 0.3mA (N = 18 females, 13 males), 1mA (N = 14 females, 13 males), or no shock (N = 10 for both males and females). Ninety minutes after completion of fear conditioning, animals were perfused and tissue was immunostained for cFos+ cells. cFos+ cells were quantified in the medial prefrontal cortex and the dorsal, ventral, and lateral columns of the periaqueductal gray. We quantified cFos+ cells across shock intensities, between Darters and non-Darters, and between males and females. For the second experiment, we used a cre-based intersectional DREADDs approach to activate or inhibit the IL-dPAG pathway before Pavlovian fear conditioning. Male and female Sprague Dawley rats (N = 14/group) underwent stereotaxic surgery to deliver a retro-cre virus into the dPAG and a Gq/Gi/mCherry control cre-dependent virus into the IL. After five weeks, allowing for sufficient viral travel and expression, animals went through fear conditioning, extinction learning, and extinction retention tests on separate days. One hour before fear conditioning, animals were given an injection of clozapine-N-oxide to activate the DREADD. Using ScaredyRat, a custom Python tool designed in our lab to analyze raw Ethovision data files, we quantified conditioned (freezing, darting) and unconditioned responses (shock and post-shock response), as well as behavior during extinction learning and retention.

Results: In experiment 1, we found that males and females did not differ in the level of cFos expression in the dPAG, but females showed higher levels of cFos expression in the IL than males. This effect was partially driven by higher cFos expression in female Darters when compared to female non-Darters. There was also a female-driven effect of shock intensity in the dPAG. There was an "inverted-U "effect of shock intensity in the IL in males, with 0.3mA males having the highest levels of cFos expression. In experiment 2, a sex-dependent effect was seen when exciting or inhibiting the IL-dPAG circuit: Excitation of the circuit led to increased conditioned freezing in males and inhibition led to decreased shock response, while having no effect on conditioned responding during fear conditioning.

Conclusions: The results of experiment 1 suggest that activity in the IL and dPAG during fear conditioning is potentially a driver of the behavioral phenotype associated with darting as a conditioned response and seen primarily in females. The results from experiment 2 suggest that the role of the IL-dPAG circuit in fear conditioning is sex dependent. Taken together, these data suggest a novel, sex-dependent role of the IL during fear conditioning for both conditioned and unconditioned behaviors.

Keywords: Sex Differences, Fear Conditioning and Extinction, Chemogenetics, Medial Prefrontal Cortex, Periaqueductal Grey (PAG)

Disclosure: Nothing to disclose.

P104. Amygdalostriatal Transition Zone Neurons Encode Sustained Valence to Direct Conditioned Behaviors

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Background: In order to respond appropriately to threats in the environment, the brain must rapidly determine whether a stimulus is important and whether it is positive or negative, and then use that information to direct behavioral responses. The amygdalostriatal transition zone (ASt) is anatomically poised to play a critical role in these processes by providing a shortcut between corticolimbic and basal ganglia circuitry to mediate behavioral responses to stimuli in parallel with the amygdala. Like the amygdala, the ASt receives converging sensory input from the thalamic and cortical pathways. However, the projections of the ASt are distinct from canonical outputs of the amygdala complex, and are integrated with striatal circuits involved in action selection. Despite this intriguing circuit connectivity, the function of the ASt is almost completely unknown.

Methods: To investigate the role of the ASt in associative learning and motivated behaviors, we first used single-nucleus RNA sequencing to generate a comprehensive profile of gene expression in ASt neurons and adjacent GABAergic brain regions (n = 18-25 mice, >15000 cells per brain region, >50k unique molecular identifiers per cell). We then quantified the genetic identity of neurons in the ASt, dorsal striatum (DS) and tail of striatum (TS) using RNAscope labelling targeted to dopamine receptor 1 (drd1a) and dopamine receptor (drd2) (N = 8 mice, 16 sections ASt, 8 mice, 12 sections DS, 8 mice, 12 sections TS). We used in vivo electrophysiology to examine changes in ASt neuron responses to conditioned stimuli predicting aversive and rewarding stimuli (N = 19 mice paired, 4 mice unpaired, n = 228/88 neurons ASt, 291/261 neurons TS, 55/65 neurons CeA, paired/unpaired). ¬We examined the behavioral affects of activating ASt neurons using optogenetic stimulation (n = 8 mice ChR2, n = 10 mice eYFP). We also used in vivo miniscope calcium imaging to record changes in GCaMP7f fluorescence in Drd1a+ and Drd2+ ASt neurons in response to cues predicting aversive and rewarding stimuli (N = 6 mice 83 neurons, Drd1a+ paired. N = 6 mice, 34 neurons Drd1a+ unpaired. N = 4 mice, 60 neurons Drd2+ paired, 4 mice, 53 neurons Drd2+ unpaired). Finally, to determine if Drd2+ ASt neuron activity was necessary for

behavioral responses to conditioned stimuli, we targeted Drd2+ neurons with the inhibitory opsin NpHR, and examined the effects of reversibly inhibiting these neurons on responses to aversive and rewarding stimuli during a two-tone discrimination task (N = 8 mice NpHR, 9 mice eYFP).

Results: Our RNA sequencing data showed that ASt neurons formed a distinct cluster from neurons in the tail of striatum (TS), dorsal striatum (DS) and central nucleus of the amygdala (CeA) based on their transcriptional profiles. We found that the ASt has a significantly greater proportion of D2+ ASt neurons than both the dorsal striatum and tail of striatum (Chi-square test, $p = 0.0010$ ASt v. DS, $p = 0.0081$ ASt v. TS). Our in vivo cellular resolution electrophysiology recordings showed that ASt neurons have robust sustained responses to negative valence stimuli (Two-tailed t-test, $p = 0.00136$, paired v. unpaired), which are distinct from responses observed in adjacent amygdala and striatum (One-way ANOVA, $p = 0.0008$). We further show that photostimulation of the ASt is sufficient to drive freezing and avoidance behaviors (Two-way ANOVA, group x laser interactions, $p = 0.0023$ freezing, $p = 0.0186$ avoidance). Additionally, our calcium imaging show that that this Drd2+ ASt neurons robustly encode stimuli of negative valence (Two-tailed t-test, $p = 0.0026$). Finally, in loss-of-function experiments we found that optogenetic inhibition of D2+ ASt neurons caused a striking reduction in conditioned fear responses to a shock-predicting cue (43% decrease in freezing, $p = 0.0145$, paired t-test).

Conclusions: Our study demonstrates that ASt neurons are sufficient to drive robust freezing and avoidance behaviors, and undergo conditioned changes in responsiveness to cues which predict aversive stimuli. Additionally, we show that the ASt contains a higher proportion of Drd2+ neurons than other regions of the striatum, and that inhibition of these Drd2+ neurons results in a significant reduction in fear response (conditioned freezing) to cues predicting aversive stimuli. Consequently, we believe that the ASt may be an overlooked and critical structure of the amygdala complex that contributes to behavioral responses to conditioned stimuli.

Keywords: Amygdala, Striatum, Valence, Learning, Fear

Disclosure: Nothing to disclose.

P105. Valence-Specific Gating of Behavioral Flexibility by Medial Prefrontal Cortex Projections to the Ventral Tegmental Area

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Background: In order to efficiently and safely traverse an environment, one must learn to distinguish between cues that predict outcomes of different modalities. In a dynamic environment where cues predicting rewarding or aversive outcomes may unexpectedly change, it is critical to flexibly update behavioral responding while retaining the ability to recall previous associations. Impairments in the ability to flexibly update responses to cues according to the outcome are evident in a number of psychiatric disorders. Dopamine and γ-aminobutyric acid (GABA) neurons in the ventral tegmental area (VTA) are involved in appetitive and aversive learning. In rodents, these neurons receive projections from the medial prefrontal cortex (mPFC), which has been implicated in behavioral flexibility. In the current study we determined if flexible behavioral responding to changes in learned associations is valence-specific, and whether this is modulated by mPFC projections to the VTA. We addressed this by manipulating mPFC-VTA projections and recording from VTA dopamine and GABA cells during a behavioral task that involved reversal of learned appetitive and aversive associations.

Methods: Adult male and female Long-Evans rats (TH::Cre, GAD::Cre, or wild type) were trained on 24 sessions of a flexible contingency learning (FCL) task developed in our laboratory. During the first 8 sessions, three distinct auditory cues (tone, white noise, and click) are paired with either an appetitive outcome (sugar pellet reward), an aversive outcome (mild foot shock), or no outcome. The cue-outcome pairings were counterbalanced across subjects. After learning, the appetitive and aversive outcomes reverse such that the cue previously paired with a shock will instead precede reward delivery and vice versa for 8 sessions. The cues reverse back to the initial associations for the final 8 sessions. Pathway-specific inhibition of the mPFC-VTA projection during FCL was performed by infusing an inhibitory chemogenetic (AAV5-hSyn-DIO-hM4D(Gi)-mCherry) or control (AAV5-hSyn-DIO-mCherry) virus into the mPFC (targeting both prelimbic and infralimbic subregions) and retrograde CAV-cre into the VTA. All rats received i.p. injections of 5mg/kg CNO 30 minutes prior to the sessions in which the cue contingencies reversed, and the same volume of saline prior to all other sessions.

Fiber photometry recordings were conducted to measure changes in calcium activity from VTA dopamine or GABA neurons during FCL. We infused a calcium indicator (AAV1-Syn-Flex-GCaMP6f-WPRE-SV40; GCaMP) into the VTA, followed by implantation of an optic fiber into TH::Cre or GAD::Cre rats.

Results: Rats discriminated between the three different cues across initial training sessions in the FCL task, demonstrating increased conditioned responding to the reward-paired cue (cue effect $p < 0.0001$; cue x session effect $p = 0.001$; n = 5 males, 6 females). Conditioned responding updated accordingly following the reversal of the appetitive and aversive associations (cue effect $p = 0.0003$; cue x session effect $p < 0.0001$). There was no effect of sex on behavioral responding during initial (sex effect on initial appetitive cue $p = 0.71$, initial aversive cue $p = 0.25$, neutral cue $p = 0.11$) or reversal sessions (sex effect on initial appetitive cue $p = 0.27$, initial aversive cue $p = 0.27$, neutral cue $p = 0.63$).

Chemogenetic inhibition of the mPFC-VTA projection during the reversal of associations in the FCL task enhanced conditioned responding selectively to the appetitive-paired cue that was previously associated with the aversive outcome (chemogenetic effect on initial appetitive cue $p = 0.37$, initial aversive cue $p = 0.03$, neutral cue $p = 0.74$; chemogenetic virus n = 12; control virus n = 9). This manipulation did not affect the latency to respond to the food port for any cue (chemogenetic effect on initial appetitive cue $p = 0.52$, initial aversive cue $p = 0.21$, neutral cue $p = 0.59$), or the total number of head entries (chemogenetic effect $p = 0.48$). Of note, preliminary data in which chemogenetic inhibition of the VTA-mPFC projection was performed during the reversal of associations in the FCL task demonstrates a trend towards no effect on conditioned responding (chemogenetic virus n = 5; control virus n = 3).

Our fiber photometry recordings indicate a dissociation between responses of VTA cell groups during the FCL task. The VTA dopamine cell population increases calcium activity in response to initial learning and reversal of reward associations (n = 5), whereas the VTA GABA population encodes initial learning and reversal of both appetitive and aversive associations (n = 4). Ongoing experiments involve recording VTA calcium activity in combination with chemogenetic inhibition of the mPFC.

Conclusions: These data identify mPFC-VTA projections as a critical pathway for valence-specific flexible associative learning. Our findings further highlight distinct roles for VTA dopamine and GABA neurons in updating the reversal of previously learned associations.

Keywords: Behavioral Flexibility, Medial Prefrontal Cortex, Dopamine

Disclosure: Nothing to disclose.

P106. Interpeduncular Nucleus GABAergic Function Controls Threat Processing and Innate Defensive Inhibitory Learning

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Background: Anxiety disorders are the foremost contributor to global mental illness, impacting an estimated 10-20% of adults in the United States. Among the clinical heterogeneity of anxiety disorders, a core domain involves the abnormal processing of threat-related information, impairing the inhibition of innate defensive responses in the absence of real threats and leading to difficulty in assessing and responding to potential threats. Identifying the neuronal circuits/mechanisms underlying these innate defensive behaviors, and how they are disrupted in anxiety disorders is fundamental for the design of improved therapeutic strategies.

Methods: We used the overhead dark visual looming stimulus (VLS) paradigm to investigate innate defensive responses in rodents. The VLS apparatus consisted of a plexiglass box (40x30x30cm) with a rectangular shaped nest (10x12cm) located in one corner and a projector screen (30x20cm) above the arena. A video-camera was used to record and track animals' behavior. Each VLS involved 15 consecutive dark expansions of 0.5s length and each mouse received 5-7 looming per session, repeated over the course of three days. To measure neural responses to VLS, we synchronized animals' behavior with fiber photometry recordings. Specifically, we virally expressed a Cre-dependent Ca2+ biosensor GCaMP in GABAergic neurons of the interpeduncular nucleus (IPN), a region previously associated with anxiety. Activity dynamics of IPN neurons (N = 7 males and N = 5 females) and its projections to the laterodorsal tegmental area (LDTg) (N = 6 males) were time-locked to VLS presentations. To functionally assess the role of the IPN-LDTg circuit in defensive behaviors, we used closed loop optogenetic stimulation during VLS events (N = 6 – 9 mice/group).

Results: Our results indicate that mice learn to inhibit defensive behavior after consecutive exposures to VLS, manifested as spending less time inside the shelter upon VLS events (One-way repeated measures ANOVA $F(2,101) = 12.51$, $p < 0.0001$). Importantly, this adaptive process is associated with progressive reductions of VLS-evoked activation of IPN GABAergic neuronal activity (One-way repeated measures ANOVA $F(2,26) = 5.037$, $p = 0.0275$) and its projections to the LDTg area (One-way repeated measures ANOVA $F(2,17) = 12.38$, $p = 0.0088$). Optogenetic silencing IPN GABAergic function with halorhodopsin during VLS presentations reduced the time that mice spent inside the shelter upon VLS, indicating that these neurons are necessary for threat processing and innate defensive inhibitory learning.

Conclusions: GABAergic neurons in the IPN and particularly, those that project to the LDTg area are engaged by potential VLS threats. With repeated VLS presentations, animals learn to inhibit innate defensive behaviors and this correlates with reductions in IPN-LDTg circuit recruitment. The present results provide new insights into the etiology and neurocircuitry underlying anxiety behaviors and reveal new circuit-level interventions to ameliorate symptoms of the disease.

Keywords: Anxiety, Interpeduncular Nucleus, GABA Neuron

Disclosure: Nothing to disclose.

P107. Defining the Function of the Cholinergic Basal Forebrain During Acquisition of Complex Threat Associations

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Background: Stress and trauma-related mental illnesses such as post-traumatic stress disorder (PTSD) are highly prevalent and severely diminish quality of life. Differences in attention and salience encoding during trauma result in debilitating symptoms of PTSD including maladaptive defensive behavior and hyperarousal. The cholinergic basal forebrain is vital for regulating attention and salience encoding in the healthy brain. This system may be dysregulated in PTSD because differences in sustained attention are a predictor of PTSD vulnerability, and attention deficits are observed in PTSD patients. The basal forebrain is also connected to and controls neuronal plasticity in brain areas associated with PTSD dysfunction, such as the basolateral amygdala. However, the role of the cholinergic system in regulating salience encoding during acquisition of complex threat and trauma associations is unknown. The goal of this project is to determine the function of the cholinergic basal forebrain during acquisition of complex fear memories and the impact this has on expression of defensive behavior.

Methods: To investigate aversive salience assignment, we developed a threat conditioning paradigm that uses a serial compound stimulus composed of a tone, followed by white noise, which is followed by footshock. After learning, mice exhibit freezing behavior to the distal tone cue, but then transition to intense running and jumping responses to the noise, which is proximal to the shock. This conditioned flight paradigm is ideal for investigating how stimulus salience during learning impacts defensive response intensity because subjects escalate behavior in response to the more proximal and important auditory cue.

Chemogenetic inhibition of cholinergic basal forebrain neurons was achieved through injection of AAV-hSyn-DIO-hM4D(Gi)-mCherry into the basal forebrain of male and female ChAT-Cre mice (n = 4) and systemic application of DREADD agonist deschloroclozapine via i.p. injection. Chemogenetic excitation of this neuronal population was achieved through injection of AAV-hSyn-DIO-hM3D(Gq)-mCherry into the basal forebrain of ChAT-Cre mice (n = 5) and systemic application of DREADD agonist as above.

Population activity of cholinergic neurons was examined by injection of AAV5-CAG-FLEX-GCaMP6f in the basal forebrain of ChAT-Cre mice and implantation of an optical fiber (400nm) for photometry. Acetylcholine release in the BLA was measured via injection of AAV9-hSyn-Ach4.3(3.0) a GRABAch3.0 sensor that fluoresces in the presence of extracellular acetylcholine.

Results: Chemogenetic inhibition of cholinergic basal forebrain neurons during serial compound stimulus fear conditioning had no significant effect on freezing to the tone, but did result in significant ($p = .011$, unpaired t-test) reduction in flight compared to controls. Similarly, chemogenetic excitation of cholinergic neurons during conditioning had no significant effect of freezing to the tone, but did result in significant ($p = .013$, unpaired t-test) potentiation in flight compared to controls.

Fiber photometry data suggest that population activity of cholinergic neurons shows differential pattern of activation to the tone and white noise epochs following fear conditioning. Additionally, acetylcholine release in the BLA mirrors this differential pattern of activation.

Conclusions: The chemogenetic manipulations link the cholinergic basal forebrain to salience encoding during acquisition of complex fear conditioning, which subsequently affects high intensity fear responses. The neuronal activity data demonstrate that basal forebrain cholinergic neurons are activated during the flight paradigm and that acetylcholine is released in the basolateral amygdala during conditioning. Based on these data, we hypothesize that salience assignment during fear learning is controlled by an integrated brain network that includes the basal

forebrain and the basolateral amygdala. Further, functional alterations of this network are expected to change behavioral responses to threat following trauma.

Keywords: Fear Conditioning, Acetylcholine, Basal Forebrain, Defensive and Motivated Behaviors, Basolateral Amygdala

Disclosure: Nothing to disclose.

P108. Dissociable Contributions of the Amygdala and Ventral Hippocampus to Stress-Induced Changes in Defensive Behavior

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Background: Acute severe stress is able to produce an array of long lasting changes in fear and anxiety-related behavior, including defensive responses to stress-associated stimuli, heightened reactions to novel aversive events, and increases in anxiety-related behavior. While much is known about the circuits supporting associative fear responses, how circuit plasticity supports the broader changes in defensive behavior observed after severe stress remains unclear. Here, we find that stress-induced plasticity in the ventral hippocampus (vHC) and basolateral amygdala (BLA) support doubly dissociable defensive behavioral changes.

Methods: Experiment 1 examined the role of stress-induced protein synthesis in the vHC and BLA on subsequent defensive behavior. Mice were exposed to 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-related behavior was assessed in a light-dark test, and sensitivity to 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 projection-specific chemogenetic approach to directly examine the contributions of reciprocal BLA-vHC connections, as well as their connections with down-stream targets, to stress-induced changes in defensive behavior. n = 8-15/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-related behavior of the same animals. Conversely, blockade of stress-induced protein synthesis in the vHC attenuated changes in anxiety-related 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 find that inhibiting projections from BLA and vHC to hypothalamic targets also produce dissociable effects on defensive behavior.

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 microcircuits within these structures. Critically, these results suggest that the impacts of severe stress are biologically divergent. If true, this might indicate that interventions geared towards one 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 projection neurons within these networks, we might better be able to mitigate post-stress phenotypes.

Keywords: Amygdala, Hippocampus, Fear

Disclosure: Nothing to disclose.

P109. A Protein Interaction Landscape of Autism Spectrum Disorder Risk Genes Reveals Convergent Biology

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Background: Over 100 genes that, when disrupted, impart large risk for ASD have been identified (high-confidence ASD risk genes, hcASD). However, this success in gene discovery has not yet translated to a mechanistic understanding of ASD. A major challenge is that hcASD are pleiotropic – meaning they have multiple biological functions that vary based on developmental and cell-type context – and only a subset of these functions contribute to ASD pathobiology. This challenge can be overcome by using a convergent approach to study many risk genes in parallel and identify points of overlap, which likely indicate core aspects of etiology. Systematically generated functional data of ASD risk genes remains strikingly limited. The development of large-scale biological data sets, particularly those that derive from direct experimental evidence, is essential for using convergent approaches to identify core biological features of ASD. Here, we systematically defined the molecular interaction landscape of proteins encoded by hcASD genes and leverage this data to identify molecular pathways that may be involved in ASD pathobiology.

Methods: We generated protein-protein interaction (PPI) data for 100 hcASD by individually overexpressing tagged hcASD in HEK293T cells and performing affinity purification-mass spectrometry (AP-MS). To confirm that the identified interactors are brain- and ASD-relevant, we analyzed human brain transcriptomic datasets to evaluate whether interactors are co-expressed with hcASD and enriched in spatiotemporal contexts previously associated with ASD (prenatal forebrain, adult cortex and cerebellum). Additionally, we analyzed genetic data from ASD whole-exome/genome sequencing studies to evaluate whether interactors are enriched for damaging variants from ASD individuals. We analyze single-cell RNAseq datasets from developing human brain tissue to identify cell types with significantly higher or lower ASD-PPI connectivity. We conducted a network proximity analysis to identify subnetworks of proteins that cluster in network space and are therefore more likely to have overlapping molecular functions. We functionally characterized a molecular complex involving several hcASD proteins in vitro in iPSC-derived neural progenitor cells and in vivo in Xenopus tropicalis.

Results: We generated an ASD-PPI network that consists of 100 bait (hcASD risk genes), 1074 interactors, and 1778 unique bait-interactor interactions, of which 87% are novel. ASD-PPI interactors are highly expressed in human brain tissue ($p < 0.001$), highly expressed in spatiotemporal contexts previously associated

with ASD (prenatal forebrain, p.adj < 0.001; adult cortex and cerebellum, p.adj < 0.05), co-expressed with hcASD ($R2 = 0.81$, $p < 0.001$), and significantly enriched for ASD-associated de novo mutations (burden test, OR 2.34, $p < 0.001$). We find that ASD-PPI connectivity is significantly higher in early-stage neural progenitors (p.adj < 0.001) and significantly lower in mature neurons and microglia (p.adj < 0.001). We identified a molecular complex that involves several hcASD. We found that members of this molecular complex colocalize to the mitotic spindle of dividing cells, and that knockdown of individual complex members phenocopy one another in vitro (altered neural progenitor cell proliferation, p.adj < 0.05) and in vivo (reduced telencephalon size in Xenopus tropicalis tadpoles, p.adj < 0.05).

Conclusions: We integrate human genetics, interaction proteomics and systems biology approaches to understand how ASD risk genes identified through rare variations converge at a molecular and cellular level. We find that proteins encoded by high-confidence ASD risk genes interact with many previously unknown molecular partners and highlight cell types and molecular complexes that are likely to contribute to ASD pathobiology and which may point to future therapeutic targets.

Keywords: Autism, Systems Biology, Neurodevelopmental Disorders, Proteomics

Disclosure: Nothing to disclose.

P110. Cell Type-Specific Effects of High-Risk Neuropsychiatric CNVs in Postmortem Human Brain

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Background: Copy number variants (CNVs) are duplications or deletions of specific portions of genomic DNA. Several large recurrent CNVs have been shown to increase risk for a variety of neuropsychiatric disorders with high penetrance. While CNVs have been studied using various cellular models, little is known about their impact on the human brain. It is also unclear how apparently disparate CNVs lead to convergent patterns of psychopathology. Here we examined transcriptional effects of neuropsychiatric CNVs in postmortem human brain with cell type resolution.

Methods: We identified 15 brain samples carrying high-risk neuropsychiatric CNVs across three brain banks, along with 28 non-carriers matched on brain bank, age range, sex, ancestry, and psychiatric diagnosis. We performed bulk and single-nucleus RNA-sequencing on dorsolateral prefrontal cortex and anterior cingulate cortex, whenever available (total $n = 75$). Samples included deletions and corresponding duplications on chromosomes 22q11.2, 16p11.2, 1q21.1, 15q11.2 (BP1-BP2), and the 7q11.23 deletion. Bulk data were quantified via RSEM, and differential expression analysis was performed using a linear mixed effects model that accounts for repeated measures and nested matching design (dream). In the single-nucleus data, CellRanger was used to align reads, generate feature matrices, and perform clustering, Seurat was used for doublet removal, quality control, and filtering, and Azimuth was used for annotation of 9 major cell types (upper and deep layer excitatory neurons, medial and central ganglionic eminence-derived inhibitory neurons, astrocytes, oligodendrocytes, oligodendrocyte precursor cells, microglia, and vascular cells). Differential gene expression analyses were performed within each cell-type, using a pseudobulk approach to identify genes that are differentially expressed across samples while accounting for repeated measures and nested matching design (dreamlet).

Results: As expected, genes within duplications showed increased expression while genes within deletions showed decreased expression. However, most genes whose expression was altered in CNV carriers lay outside the CNV. Transcriptome-wide analysis of both bulk and single-nucleus data showed a greater number of differentially expressed genes (defined as FDR < 0.05) in brain samples with deletions compared to the corresponding duplications. Single-nucleus data exposed cell type-specific differences in differential gene expression. In the 22q11.2 deletion, astrocytes had 5 times more differentially expressed genes than other cell types. In the 7q11.23 deletion, the most prominent effects were seen in upper and deep layer excitatory neurons. Gene ontology and pathway enrichment analyses on the differentially expressed genes showed CNV-specific enrichments along with some convergent themes. Terms shared across multiple CNVs were related to energy metabolism, oxidative phosphorylation, and synaptic function.

Conclusions: We present the first transcriptome-wide study of high-risk neuropsychiatric CNVs in postmortem human brain. The findings suggest that deletions exert widespread effects on gene expression that converge on energy metabolism and synaptic pathways. Broadly consistent with previous in vitro studies, these results suggest that diverse CNVs may lead to convergent neurobiological defects in brain.

Keywords: Copy Number Variants, Human Postmortem Brain Tissue, Brain Transcriptomics, Single-Nucleus RNA-seq

Disclosure: Nothing to disclose.

P111. The Long-Term Impact of Early Life Stress on Serotonin Circuits and Behavior

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Background: Chronic childhood stress is a prominent risk factor for developing mood disorders, yet mechanisms underlying this association remain unclear. Serotonin plays a crucial role in neurodevelopment and vulnerability to mood disorders. Maintenance of optimal serotonin levels during early postnatal development is critical for the maturation of brain circuits. Developmental stress can alter the serotonin system, leading to chronic behavioral deficits. Yet, our understanding of the long-term impact of early life stress (ELS) on serotonin connectivity remains incomplete.

Methods: Female and male mice were exposed to a limited bedding and nesting chronic stress paradigm during the first postnatal week. When mice reached adulthood, we performed behavioral analysis to determine anxiety-like behavior, sociability and stress-coping strategies ($n = 7$-11 per group per sex). Using control and ELS FosTRAP mice, we cross-correlated regional c-fos density across all mice within each group and used raphe nucleus as a seed region to determine its functional connectivity with 60 other brain regions. We next performed in vivo fiber photometry to determine the differences in the activity of serotonin neuron population during acute stress between control ($n = 5$ per sex), and ELS ($n = 6$-7 per sex) ePet1::Ai148 mice. Following this, we determined the c-fos based activity levels on the downstream serotonin-modulated circuits and performed in vivo fiber photometry in selected brain regions to determine ELS induced changes

in serotonin dynamics. Finally, we optogenetically stimulated selected circuits to overcome ELS-induced deficits in anxiety-like behavior in mice exposed to ELS.

Results: We first established that adult female and male mice exposed to ELS during the first postnatal week show heightened anxiety-like behavior. Using in vivo fiber photometry and c-fos dependent activity mapping, we found that ELS enhances susceptibility to acute stress by disrupting the brain-wide functional connectivity of the raphe nucleus and the activity of dorsal raphe serotonin neuron population, in conjunction with a profound increase in the orbitofrontal cortex (OFC) activity. We further identified that serotonin release in the medial OFC during environmental challenge is disrupted in mice exposed to ELS. Optogenetic stimulation of 5-HT terminals in the medial OFC elicited an anxiolytic effect in ELS mice in a sex-dependent manner.

Conclusions: Our findings highlight the potential of combining targeted stimulation and pharmacotherapies to improve serotonin neurotransmission as a promising approach for treating emotional dysregulation that arises from childhood stress.

Keywords: Serotonin, Early Life Stress (ELS), Functional Connectivity, In Vivo Fiber Photometry, Circuit Optogenetics

Disclosure: Nothing to disclose.

P112. Early Life Trauma Disrupts Prefrontal Cortex Circuit Development and Adolescent Social Behavior in Male Mice

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Background: Experiencing early life trauma (ELT) can greatly increase the likelihood of developing psychiatric disorders as an adult. These disorders often have social cognitive deficits that can negatively impact health outcomes and quality of life. The medial prefrontal cortex (mPFC) undergoes a prolonged maturation process, making it vulnerable to early life stress. Studies have shown that ELT can hinder the maturation of the PFC in adolescents who have experienced abuse. This can lead to reduced PFC excitability and decreased top-down control over emotional regulation in areas such as the amygdala in adulthood. Establishing a reliable rodent model of ELT is essential in identifying any disruptions in molecular and circuit functions that may persist into adulthood. Our study aims to examine how ELT shapes PFC circuit development, which is essential for social behaviors in mice.

Methods: Juvenile male C57 mice (postnatal day 29) were exposed to a 10-day defeat paradigm by placing them into the home cage of an aggressive male CFW mouse. Each session consists of physical interactions and side-by-side 24-hour housing (using a plastic partition) with the aggressor (chronic stress). Control mice were housed with other control mice but separated by a plastic partition to ensure consistency across housing conditions and to control for effects of social isolation. The average number of bites - $26 +/- 0.78$, and duration of attack $84 +/- 6.5$ s in the juvenile chronic social defeat stress (jCSDS) mice. Social interaction testing was done 24 hours after the last defeat and brains were collected for transcriptomics and in situ hybridization. A separate cohort of animals were defeated as juveniles then injected with axonal tracer as adults and sacrificed 10 days later. The McLean Hospital Institutional Animal Care and Use Committee approved all animal care and experimental procedures.

Results: jCSDS-exposed mice showed no change in body weight after the ten days; however, there was a significant decrease in the adrenal weights (t-test; $p = 0.04$) compared to control mice. jCSDS-exposed mice showed a socially avoidant phenotype in the social interaction tests (t-test $p > 0.01$; $n = 10$-12). Furthermore, there was a significant change in the prelimbic axons density in brain regions such as the NAc ($p < 0.01$; $n = 4$ mice per group and 24 sections per mice) using a viral-based anterograde tracing system.

Conclusions: Our data demonstrate that jCSDS induces social deficits, alters prelimbic projections to areas involved in social behaviors, and changes the transcriptomic profile of the mPFC. Our results suggest that ELT could contribute to PFC dysfunction, altering the development of the PFC and its projections to areas implicated in social behaviors and psychiatric disorders.

Funding support: T32MH125786 and Eric Dorris Memorial Research Award (EH), US-Israel Binational Science Foundation Grant #2019021 (OF)

Keywords: Early Life Stress (ELS), Chronic Social Defeat Stress, mPFC

Disclosure: Nothing to disclose.

P113. Serotonin Signaling During Development: Impact on Response to Threat Across Species

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Background: Fear is the human emotion that is elicited when danger or threat is perceived or recognized. Maladaptive fear is common in anxiety disorders. Fear responses are orchestrated by the activation of stimulus-specific neural circuits that converge in the periaqueductal gray (PAG) of the midbrain. In humans a risk factor for increased fear is increased 5-HT exposure during early life, for example, due to early life trauma or SSRIs use in pregnancy. In rodent models postnatal day 2 to 11 (P2-11) of fluoxetine treatment causes enduring changes in 5-HT function and decreased 5-HT innervation of certain brain structures (mPFC and hippocampus). However, it remains unknown if changed developmental serotonin signaling alters adult 5HT PAG circuit function to increase innate fear.

Methods: We adopted a cross-species approach to show how increased 5HT signaling in early life is responsible for changes in innate fear behavior and underlying neural circuitry including the PAG. In this approach: 1) we employed chemogenetic (Pet1-Cre::Hm3d) or pharmacologic (fluoxetine, 10mg/kg) methods to increase 5-HT signaling during P2-11 in mice; 2) we identified in utero SSRIs exposed or unexposed humans from the Adolescent Brain and Cognitive Development (ABCD) study. We utilized functional MRI imaging and innate fear responses during predator odor exposure in mice and the BOLD response to fearful vs neutral faces, and CBLC scores in humans. Using projection-specific excitatory and inhibitory optogenetics (Pet1Cre::Ai32 or Ai39) we explored the necessity of adult DR(5-HT)-to-dlPAG input to harness innate fear responsivity in mice.

Results: In mice, increased developmental 5-HT signaling exacerbated innate fear responses and activated fear brain regions (amygdala, PAG, putamen). Human adolescents exposed to SSRIs in utero showed hyper-activation of fear brain structures (amygdala, putamen, thalamus) and higher anxiety and depressive

symptoms than unexposed adolescents. Optogenetics confirmed the necessity of DR(5-HT)-to-dlPAG in harnessing innate fear responses.

Conclusions: Increased 5HT levels, i.e., SSRI's, in early life enhance innate fear responses and fear brain circuit activation that are conserved across species. Understanding the modulation of PAG by 5-HT will provide insight into pathophysiology and etiology of anxiety disorders.

Keywords: Periaqueductal Grey (PAG), Sensitive Period, Developmental Trajectory, Adolescent Anxiety, Anxiety Circuitry

Disclosure: Nothing to disclose.

P114. Attempt to Develop Therapeutic Drug for Autism by Targeting IGF-1: Exploration of Small Compounds for IGF-1 Gene Expression

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Background: Autism Spectrum Disorder (ASD) is a disorder with a wide variety of symptoms, including impairment of social interaction, repetitive and restricted behaviors. The core symptoms of ASD are difficult to treat, and therapeutic agents are still in development. Although each drug candidate in clinical trials has its own set of problems, improving these problems may lead to the development of more effective and safer treatments. We focused on the human insulin-like growth factor (IGF-1), which is one of the most promising therapeutic candidates. The immature form of human IGF-1 consists of 195 amino acids (a.a.) and is converted to 70 a.a. after maturation. This single-chain polypeptide is highly conserved across species and acts as the endogenous ligand of IGF-1 receptor (IGF-1R). IGF-1 has multiple functions including stimulation of cell growth and division, regulation of glucose metabolism, inhibition of apoptosis, and promotion of protein synthesis. In addition, IGF-1 also has been shown to have a number of functions in the CNS such as a neuroprotective effect, promotion of neurogenesis, neural plasticity, and increase in the release of dopamine. Consequently, IGF-1 contributes to CNS homeostasis and is a molecular target for the treatment of ASD, Rett syndrome, and Phelan-McDermid syndrome. IGF-1 that have shown satisfactory results in various animal experiments and clinical trials, but there are some problems, such as expensive cost, and short half-life time, which reduce the efficiency due to antibody production. However, one of the solutions to this problem is to find a compound that promotes the production of IGF-1 by regulating its endogenous expression. Since IGF-1 is mainly produced by microglia in the brain, we aimed to find compounds that promote IGF-1 by screening using the mouse microglia cell line, BV-2.

Methods: A luciferase assay system was established as a convenient method to detect IGF-1 production. A luciferase vector containing the regulatory region of the human IGF-1 gene was transfected into BV-2, and a stable cell line was established by puromycin selection. Using this stable cell line, we performed screening by luciferase assay, and further conducted reproducibility, dose-dependency, and examine the toxicity of compounds that showed high activity values. In addition, transcriptome and pathway analyses were performed to find compounds that are less likely to affect genes other than IGF-1.

Results: The high-throughput screening was performed using a library of 9600 compounds, and 92 were selected as first-hit compounds. Among them, we found three compounds with good reproducibility and dose dependency. MTT assay revealed that these compounds showed low toxicity and abnormal cell growth. The results of Western blot analysis and ELISA showed that the production of IGF-1 was also increased at protein level. In addition, transcriptome analysis was performed to examine gene expression specificity and a compound was excluded from the candidates because it caused expression changes in many genes. Pathway analysis was also performed, and a compound was also excluded because it was suggested to have an increased pathway leading to inflammation and disease. Finally, one compound that promoted IGF-1 gene expression, had few nonspecific responses and showed no toxicity was selected as a true hit compound.

Conclusions: A true hit compound from a library of 9600 compounds induced human IGF-1 expression without non-specific gene expression and toxicity. In this study, there are two issues: (1) the study was conducted only in cultured cells, and (2) the structure-activity relationships of the hit compounds were not investigated. In the future, we hope to overcome these issues and put the compound to practical use as a potential therapeutic agent for ASD.

Keywords: Autism, IGF-1, Microglia, High-Throughput Screening

Disclosure: Nothing to disclose.

P115. Stress During Puberty Leads to Lasting Disruptions in Epigenetic, Transcriptomic, and Behavioral Outcomes in Peripartum Mice

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Background: Two potent risk factors for the development of mood disorders in women are adversity experienced during puberty and later becoming pregnant, although we understand little about the mechanisms underlying these risk factors individually or when they are combined. A key endophenotype observed in mood disorders is a disrupted hypothalamic-pituitary-adrenal (HPA) axis. We have previously shown that pubertal adversity is associated with a blunted glucocorticoid response in both peripartum humans and mice. We have used this translationally-relevant mouse model to probe the mechanisms of risk for negative peripartum outcomes in pubertally stressed mice, with a particular focus on the underlying epigenetic and transcriptional mechanisms. Based on our previous pharmacological and transcriptomic findings, we have identified a pubertal stress-induced transcriptomic signature in the paraventricular nucleus (PVN) of the hypothalamus that we hypothesize is uncovered in pregnancy by the increased allopregnanolone levels to produce the blunted HPA axis response. We further sought to extend our understanding of the potential negative outcomes when pubertal stress exposure and pregnancy interact in the same individual by characterizing postpartum behavior.

Methods: Mice were exposed to chronic variable stress (CVS) from postnatal day (PN) 21-34 using our previously established paradigm. CVS consisted of two stressors (tactile, olfactory, or auditory) per day. Brains were collected either during CVS (PN21, PN28, PN35) or in adulthood. Both male and female mice were used for all outcomes, unless those outcomes were postpartum behavior. To assess the role of pregnancy in negative outcomes, mice were either bred in adulthood or were given either allopregnanolone (100ng/200nl per side) or vehicle (25% w/v HP-β-CD) via intra-PVN cannulae 2h before brain collection. The epigenetic regulators histone deacetylase and histone acetyltransferase were examined in nuclear extracts from the pubertal

and peripartum brains by ELISA-based assays (n = 7-10/group). Gene expression was measured using quantitative real-time PCR (n = 4-8/group). The stress-induced transcriptomic signature was examined by measuring expression of six immediate early genes (IEGs) that were previously found to be increased in the PVN of pubertally stressed, pregnant females. Postpartum and nulliparous female mice were assessed for behavior in a grooming assay, open field, pup retrieval, and social preference (n = 5-10/group).

Results: Epigenetic regulators were altered by age, sex, and pubertal stress. Histone acetyltransferase (HAT) concentration in the PVN was altered by pubertal stress, age, and sex of the mice. There was a significant increase in HAT concentration in CVS animals at PN35 ($F(1,27) = 7.48$, $p = 0.01$). A linear regression revealed a significant decrease in HAT concentration amongst female controls from PN21 to PN35 ($F(1,14) = 12.95$, $p = 0.003$). There was no change in HAT concentration in the male controls from PN21 to PN35. Further testing revealed that males and females had significantly different slopes of change during this developmental window ($F(3,38) = 2.96$, $p = 0.04$). There was no effect of age of pubertal stress on histone deacetylase concentration in the PVN. Allopregnanolone delivered directly to the PVN of pubertally stressed, virgin female mice was sufficient to recapitulate the previous transcriptomic findings of increased IEG expression. Of the six IEGs tested (Arc, Egr1, Fos, Fosb, Junb, Jund), we replicated the transcriptomic signature in Arc, Egr1, and Fos. For Arc expression, there was a significant interaction between pubertal stress and intra-PVN allopregnanolone treatment ($F (1, 18) = 8.682$, $P = 0.0086$). Similarly, for Fos expression, there was a significant interaction between pubertal stress and intra-PVN allopregnanolone treatment ($F (1, 18) = 4.842$, $P = 0.0411$). The same pattern was observed for Egr1 (pubertal stress x allopregnanolone interaction, $F (1, 18) = 7.538$, $P = 0.0133$). Finally, there was heightened anxiety-like behavior due to both parturition and pubertal stress. There was a significant interaction between pubertal stress and parity on the amount of time spent in the center of an open field ($F(1, 22) = 8.454$, $p = 0.0082$).

Conclusions: We found that stress during puberty led to increased anxiety-like postpartum behavior, altered transcription in hypothalamic brain regions that regulate maternal and stress responses, and altered the trajectory of important epigenetic regulators. Further, we directly linked allopregnanolone to the pubertal stress-induced transcriptomic phenotype. These findings provide further support for a role of allopregnanolone in the epigenetic changes that occur in the brain during pregnancy. These results provide novel insight into the impact of adversity during puberty on the risk for negative molecular, physiological, and behavioral outcomes in the peripartum window.

Keywords: Early Life Stress, Epigenetics, Pregnancy, Adolescent Brain, Developmental Trajectory

Disclosure: Nothing to disclose.

P116. Preliminary Data Indicating Lower Positron Emission Tomography Derived [18F]SynVesT-1 Binding in Autism Versus Non-Autistic Mental Health Help Seeking Youth

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Background: Autism Spectrum Disorder (autism) is a neurodevelopment disorder (NDD) affecting 1-2% of children and young adults in the US (CDC) and associated with significant social, sensory and cognitive impairments. Converging evidence indicates that synaptic alterations may contribute to the neurobiology of autism. This report presents preliminary data using positron emission tomography (PET) imaging of the synaptic vesicle membrane protein (SV2A) radioligand [18F]SynVesT-1, providing an indirect in vivo measure of synaptic density, in youth and emerging adults with autism compared to an age-matched sample of mental health help seeking individuals without neurodevelopmental disorders. The main hypothesis is that compared to mental health help seeking youth without neurodevelopmental disorders, autistic youth will have reduced [18F] SynVesT-1 binding within specific regions of interest (ROIs) that have been implicated as altered in autism (prefrontal cortex, fusiform gyrus, cerebellum, amygdala and striatum).

Methods: Seventeen autistic participants (9M/8F, Age M: 24.22) and 12 mental health help seeking youth without an NDD diagnosis, recruited as part of the Toronto Adolescent and Youth (TAY) Study, (3M/9F, Age M: 20.452) completed an 120-minute PET scan after injection of 187 ± 10MBq of [18F]SynVesT-1. Arterial blood was collected during the scan to generate an arterial input function. All participants completed a T1-weighted MRI image for ROI delineation in Pmod 4.2.Volume distribution (VT) was estimated using a 1-tissue compartment model.

Results: Preliminary VT data was analyzed in 11/14 autistic participants and 8/12 non-NDD mental health help seeking youth (processing challenges limited ability to extract initial high quality VT data in remaining participants). All non-NDD youth/emerging adults had diagnoses of anxiety and/or mood disorders (81% of autistic participants were also diagnosed with co-occurring mood and/or anxiety disorders). Preliminary findings indicate that [18F] SynVesT-1 binding was, on average, ~7% lower across ROIs in autistic versus non-NDD participants. Autism-nonNDD differences in [18F]SynVesT-1 binding across ROIs ranged from 5-10% lower in autism (PFC: -5.1 ± 0.7%, fusiform gyrus: -9.9 ± 1.4%, cerebellum:-9.6 ± 1.6%, amygdala: -4.0 ± 0.1 and striatum: -4.62 ± 0.7%; smallest coefficient of variation in the fusiform gyrus and cerebellum).

Conclusions: Here, we provide preliminary data indicating that [18F]SynVesT-1 binding is lower across a number of brain regions in autistic youth and emerging autistic adults compared to a group of youth and emerging adults with anxiety or mood disorders but without a neurodevelopmental disorder diagnosis. While this preliminary report is limited by the small sample included, younger overall age in the non-NDD group, and the lack of a control group without mental health diagnoses, ongoing recruitment and characterization with an expanded sample will ultimately provide more definitive indication of whether [18F] SynVesT-1 binding is lower in autistic youth/emerging adults and correlates with behavioural/cognitive measures.

Keywords: Autism, Positron Emission Tomography Imaging, Synaptic Density

Disclosure: Nothing to disclose.

P117. Efficacy and Safety of JNJ-42165279, a Fatty Acid Amide Hydrolase inhibitor, in Adolescents and Adults With Autism Spectrum Disorder: A Randomized, Phase 2, Placebo-Controlled Study

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Background: Autism Spectrum Disorder (ASD) is a heterogenous disorder characterized by the core symptoms of deficits in social communication and the presence of repetitive and restrictive behaviors. Current first line treatment is through behavioral modification, supportive therapy, and off-label use of medications. There are currently no medications approved for the treatment of core symptoms of ASD. Given the increasing incidence of ASD, and the long-term associated morbidity, there is significant unmet need for novel, effective pharmacotherapies.

JNJ-42165279 is a potent, selective, and orally bioavailable inhibitor of enzyme Fatty Acid Amide Hydrolase (FAAH). This enzyme is primarily responsible for the degradation of a variety of fatty acid amides (FAAs), including the endocannabinoid N-arachidonoylethanolamine, or anandamide (AEA), the first identified endogenous cannabinoid receptor agonist. The endocannabinoid system is believed to play important roles in the regulation of the immune system, pain perception, affect, motivation, emotion, fear, and anxiety responses. As such, the endocannabinoid system and FAAH inhibition could modulate the core symptoms associated with social communication deficits, and restrictive and repetitive behaviors. Thus, this study assessed the therapeutic effect of JNJ-42165279 in participants with ASD.

Methods: This was a randomized, multicenter, double-blind, placebo-controlled, parallel-group, outpatient study conducted in the United States that evaluated the efficacy, safety, pharmacokinetics, and tolerability of JNJ-42165279 during 12 weeks of treatment in adolescent and adult participants with ASD. The target population consisted of participants (aged 13 to 35 years, inclusive) with a definitive diagnosis of ASD according to DSM-5 criteria, and an IQ of ≥60 as measured by the KBIT-2. Diagnosis was confirmed using the ADOS-2 (minimum score of 8 [autism spectrum]).

Results: During the study, 78 participants across 8 sites in the United States were randomized in a 1:1 ratio to receive double-blind treatment of either JNJ 42165279 ($n = 40$) or placebo ($n = 38$). The median age was 20 years (ranging from 13 to 35 years), there were 26 participants (34.2%) in the 13-17 age range. In the study population, a higher proportion of participants were male (76.3%), and white (80.3%). The demographic characteristics were generally comparable across treatment groups.

Conclusions: This is the first study to evaluate the efficacy, safety, and tolerability of JNJ-42165279, a modulator of the endocannabinoid system, in participants with ASD. Study enrollment completed in Q4 of 2022. This poster will present safety and efficacy results of this phase 2a study.

Keywords: Autistic Spectrum Disorders, Endocannabinoid System, Clinical Trial

Disclosure: Janssen Pharmaceuticals: Employee (Self)

P118. Psychopathological Impact of Maternal Levels of Omega-3 Fatty Acids and Fish Oil Supplementation During Pregnancy: A Randomized Controlled Trial in the COPSAC2010 Cohort at Age 10

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Background: Omega-3 fatty acids obtained from intake of fish oil, specifically docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are pivotal parameters for early brain development, and epidemiological data has indicated that dietary fish oil intake during pregnancy is beneficial for neurodevelopment. However, there is still lack of data on the association between maternal blood levels of DHA + EPA in pregnancy and mental health in children, and causality needs to be established in randomized controlled trials (RCT).

In an RCT of fish oil supplementation during the last trimester of pregnancy, we aimed to investigate:

1) Whether pre-intervention maternal whole blood levels of EPA and DHA during pregnancy, were associated with psychopathological outcomes in the children at age 10.

2) Whether fish oil supplementation during the last trimester of pregnancy improved psychopathological outcomes at age 10 in an RCT design.

Methods: The COPSYCH study is nested in the COPSAC2010 cohort consisting of 700 mother-child dyads followed prospectively since gestational week 24. The pregnant women were randomized to 2.4 g daily fish oil (37% DHA and 55% EPA) or placebo (olive oil) in the third trimester and one week postpartum. Maternal whole-blood levels of EPA and DHA were assessed prior to randomization. Psychopathology was assessed at age 10 with trained clinicians assessing diagnostic psychopathology using the Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS). Dimensional psychopathology measured as symptom counts in the entire cohort was assessed using parental rated questionnaires: ADHD Rating Scale (ADHD-RS) and Social Responsiveness Scale (SRS-2). Statistical inferences were based on linear and logistic regression models.

Results: 604 (86%) of 700 children participated in the COPSYCH evaluation. Eighty-eight (14.5%) children fulfilled diagnostic criteria for any neurodevelopmental disorder. The most common diagnosis was Attention Deficit Hyperactivity Disorder (ADHD) with 65 (10.8%). Of these, 29 had ADHD predominantly inattentive and 36 had the combined presentation. Sixteen children (2.6%) fulfilled diagnostic criteria for autism spectrum disorders (ASD).

Higher maternal EPA + DHA blood levels in pregnancy was associated with lower risk of a diagnosis of ADHD (OR 0.71 [95%CI: 0.52; 0.95], $p = 0.03$). Similarly, higher maternal EPA + DHA blood levels were associated with a lower load of ADHD symptomatology ($\beta = -1.21$, $p < 0.001$) across the entire cohort. EPA + DHA blood levels were not significantly associated with a diagnosis of ASD (OR 0.70 [95%CI: 0.36; 1.29], $p = 0.27$), but higher EPA + DHA blood levels were associated with as lower load of autistic symptomatology ($\beta = -1.96$, $p = 0.01$).

The RCT showed no overall effect of fish oil supplementation in risk of ADHD; OR 0.79 [0.47; 1.33], $p = 0.37$. However, secondary analyses indicated a protective effect of fish oil supplementation against the ADHD predominantly inattentive presentation; OR 0.41 [95%CI: 0.17; 0.89], $p = 0.03$. Fish oil supplementation did not affect the risk of other psychiatric diagnoses or levels of dimensional symptomatology in the entire cohort.

Conclusions: Our prospective birth cohort thoroughly assessed at age 10 suggest that higher prenatal maternal levels of omega-3 fatty acids are associated with lower risk of ADHD. Findings were corroborated by associations with lower levels of ADHD and ASD symptom load in the entire cohort. Although our RCT of fish oil supplementation in the last trimester of pregnancy was overall negative, secondary analyses indicated that fish oil during pregnancy may protect against inattentive presentation of ADHD.

Overall, our findings support the notion that maternal levels of omega-3 fatty acids have impact on children's levels of psychopathology at age 10.

Keywords: Omega-3 Fatty Acid, Neurodevelopmental Disorders, Randomized-Controlled Trial, Pregnancy, Psychopathology

Disclosure: Lundbeck Pharma: Honoraria (Self)

P119. Target Engagement Evidence for a Novel Executive Working Memory Training Intervention for Attention-Deficit/ Hyperactivity Disorder

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Background: Over the past decade, considerable effort has been expended to develop cognitive training therapies for patients with Attention-Deficit/Hyperactivity Disorder in hopes of validating a non-pharmacological intervention that alters brain function in ways that reduce ADHD symptoms and associated impairments. Many such clinical trials have yielded modest, mixed, or ambiguous clinical efficacy evidence, prompting investigators to devise alternative training interventions with different treatment targets. As the first stage in NIMH's exploratory experimental therapeutics pipeline, this R61-funded study examined a novel intervention involving intensive practice of 4 specific "executive working memory" (EWM) operations – selective attention abilities that promote flexibility or lend stability to working memory representations. The study goal was to learn if training engages theorized cognitive and neural treatment targets.

Methods: 36 male and 26 female ADHD-diagnosed adolescents ages 12-18 volunteered for a blinded, randomized clinical trial consisting of 20 virtually coached training sessions completed within 4-6 weeks from home via internet. Half were assigned to practice progressively challenging training exercises using verbal and nonverbal versions of Shifting, Updating, Filtering, or Suppression EWM tasks. The others engaged in plausible placebo game exercises with low working memory demands. After examining trial retention rates and tolerability, the data analyses characterized a) training exercise responsiveness, b) study group differences at trial endpoint on both trained and untrained tests of each EWM operation, c) changes in both brain activity and d) functional connectivity for two a priori lateral prefrontal cortex treatment targets – the superior frontal sulcus (SFS) and inferior frontal junction (IFJ). fMRI data were prepared for analysis using Human Connectome Project pipelines and cortical atlas parcellation. Endpoint treatment group differences were tested using robust regression models of 90% trimmed means. Each dependent measure was regressed on dummy-coded active vs. placebo membership and participants' pre-treatment measurements as a covariate. Because this was an early phase target engagement study, both uncorrected p values and Cohen's d effect sizes were examined for effects of at least "medium" size.

Results: The overall trial retention rate was 92%. Adolescents in the active intervention group rated it as moderately tolerable (6.3 on a 1-10 Likert scale). All but 3 of the 30 active intervention completers (90%) showed treatment engagement by consistently advancing to more challenging exercises. diceR consensus clustering found 47% of the active treatment group were practicing the hardest exercises by the 20th session, whereas 43% reached moderate difficulty trials. Analysis of accuracy improvements on trained tests was hampered because many scores already were at training performance criterion levels before treatment. Nonetheless, the treatment group's verbal Shifting accuracy improved from 69% to 79% ($p = .05$, $d = 0.58$) and nonverbal Suppression accuracy from 53% to 65% ($p = .06$, $d = 0.54$). Reaction time was faster for verbal Shifting ($p = .06$, $d = -0.55$), Updating ($p = .08$, $d = -0.53$), and Suppression ($p = .005$, $d = -0.83$) trained tasks. Treated ADHD also showed "near-transfer" reaction time changes on alternative, non-trained tests of Shifting ($p = .005$, $d = 0.79$), Filtering ($p = .05$, $d = 0.56$), and Suppression ($p = .006$, $d = 0.78$). Conventional fMRI timeseries analyses of SFS and IFJ activity elicited when performing the fMRI tasks found sparse and inconsistent evidence that the intervention altered how strongly the target regions engaged. In contrast, there was credible, widespread, medium-to-large effect size evidence at $p < .05$ for SFS and IFJ functional connectivity changes in the active EWM training group vs. placebo. Each EWM operation showed a different profile of connectivity change. The effect of EWM training on Updating was a reduction of connectivity in regions not already strongly integrated into the frontoparietal network engaged for task performance (i.e., enhanced segregation and specificity of assumed neural communication). For Shifting, connectivity was enhanced between SFS and IFJ targets as well as with other frontoparietal, sensory and motor cortices. Connectivity changes during the Suppress/Filter fMRI task were the most diverse observed; stronger or weaker connectivity depended on which SFS or IFJ seed was examined.

Conclusions: This novel experimental intervention not only was tolerated by ADHD-diagnosed adolescents, but it also engaged the study's treatment targets as seen by improved EWM test performance, near transfer effects, and altered brain functional connectivity. The latter was especially intriguing because results support the idea the intervention changes how SFS and IFJ brain regions dynamically reconfigure well-described, hierarchically-organized intra-prefrontal and frontoparietal systems that influence other brain regions/networks by biasing information processing. This positive target engagement evidence suggests future intervention development research should be fruitful. Subsequent proposed R33 phase work will attempt to replicate findings in a larger, independent ADHD sample, compare ADHD to non-ADHD controls, and characterize predicted links between target brain function and ADHD symptom severity.

Keywords: ADHD, Cognitive Training, Working Memory, Clinical Trial, Target Engagement

Disclosure: Nothing to disclose.

P120. Alterations in Amygdala Subregion Volumes in Youth at High Risk for Bipolar Disorder and Their Potential to Predict Acute Treatment-Related Arousal Outcome

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Background: Youth with a family history of bipolar disorder (BD) and symptoms of depression/anxiety may be at high risk for developing BD. Although antidepressants are commonly used to treat depression and anxiety, predicting their treatment response and potential to induce treatment-emergent mania and related adverse events remains challenging. The amygdala, a key brain region implicated in BD, plays a critical role in emotion processing and regulation and may be a key hub for circuit abnormalities associated with treatment-emergent mania. Here, we examine amygdala subregion volumetric changes among high-risk (HR) youth to advance understanding of BD pathophysiology and to identify early predictive markers for treatment-emergent mania.

Methods: We recruited 105 youth (12-17 years old) with at least one first-degree relative with BD-I and current moderate or severe depression and/or anxiety disorders. We also enrolled 51 age- and sex-matched healthy controls (HCs) without any first or second-degree relative with a mood or psychotic disorder. All participants received baseline structural MRI scans at the University of Cincinnati or Stanford University. HR youths were randomized to

either psychotherapy plus escitalopram (n = 66) or psychotherapy plus placebo (n = 39) for up to 16 weeks. Adverse events were conceptualized across multiple behavioral and imaging units of analysis as part of the Research Domain Criteria Arousal construct, and related outcome measures were serially assessed using the Pediatric Adverse Events Rating Scale (PAERS) and Treatment-Emergent Activation and Suicidality Assessment Profile (TEASAP) at baseline and weekly or every other week for up to 16 weeks. To evaluate treatment-related arousal outcomes, we employed linear mixed models to estimate individual-specific slope coefficients for each participant's PAERS and TEASAP scores, representing their individualized average trajectory across the follow-up period. Anatomic images were automatically segmented using FreeSurfer software (V7.0). Then, total amygdala volume and volumes of nine bilateral subfields were obtained. We employed ComBat harmonization to remove site-related variations in volume measurements. In the initial analysis, baseline volume alterations in amygdala subregions in HR youth were explored using two-sample t-tests, with age, sex, and estimated total intracranial volume (eTIV) as covariates. Significance thresholds were set at $p < 0.05$ with False Discovery Rate (FDR) correction. In addition, partial correlations were used to investigate the associations between altered amygdala subregion volumes and clinical arousal symptoms at baseline, with age, sex, and eTIV as covariates. Finally, we employed linear regression models to investigate whether altered baseline amygdala subregion volumes could predict treatment-emergent mania or arousal outcomes.

Results: Compared to HCs, HR youth had significantly reduced volumes in the left lateral nucleus, left medial nucleus, bilateral central nucleus, bilateral cortical nucleus, and bilateral corticoamygdaloid transition area, while showing increased volumes in the bilateral basal nucleus and accessory basal nucleus (all FDR-corrected $P < 0.05$). Within the HR group, there were significant negative correlations between the volumes of the left medial nucleus ($r = -0.25$, $P = 0.011$), left cortical nucleus ($r = -0.29$, $P = 0.003$), left corticoamygdaloid transition area ($r = -0.30$, $P = 0.002$), and PAERS scores at baseline.

Patients receiving either escitalopram or placebo had significant negative change rates for PAERS scores (escitalopram: $r = -0.38$, placebo: $r = -0.28$, all $P < 0.001$) and TEASAP scores (escitalopram: $r = -1.56$, placebo: $r = -1.68$, all $P < 0.001$), indicating improvements in symptom severity over time.

Finally, the linear regression analyses revealed significant volume-by-treatment interaction effect on PAERS scores for right cortical nucleus ($t = 2.50$, $P = 0.014$), left medial nucleus ($t = 2.17$, $P = 0.033$) and left basal nucleus ($t = 2.13$, $P = 0.036$). In the escitalopram group, smaller baseline volumes in right cortical nucleus ($r = 0.27$, $P = 0.027$) and left medial nucleus ($r = 0.41$, $P = 0.001$) predicted better PAERS outcomes. Conversely, larger baseline volumes of left basal nucleus were linked to poorer treatment-related arousal outcomes ($r = 0.27$, $P = 0.035$) in the escitalopram group, while no such correlation was seen in the placebo group.

Conclusions: Differences in amygdala subregion volumes between HR youth with depression and/or anxiety disorders and HCs suggest dysregulation in emotion processing. These findings provide insights into the neurobiological mechanisms underlying the development of affective or anxiety disorders in HR youth. Importantly, baseline volumes of specific amygdala subregions predicted clinical arousal in HR youth who received escitalopram, but not in those who received placebo, raising the possibility that pre-treatment amygdala subregion volumes may represent a biomarker for SSRI-related arousal.

Keywords: Bipolar Disorder, Amygdala, Structural MRI, SSRI

Disclosures: Otsuka: Consultant (Self). Cerevel, Intracellular Therapeutics: Advisory Board (Self) Myriad: Grant (Self). Cambridge, UpToDate, Springer: Royalties (Self). MedScape Live, Neuroscience Education Institute: Honoraria (Self).

P121. Altered Trajectories of Cortical Development Among Youth in the Adolescent Brain and Cognitive Development (ABCD) Study Following the Exposure to the COVID-19 Pandemic: A Repeated Measures MRI Study

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Background: Through childhood and into adolescence, the brain undergoes rapid development, including age-related thinning of the cerebral cortex. Accelerated cortical thinning during childhood and adolescence has been associated with risk for emergent psychiatric illnesses including psychotic and mood disorders. The COVID-19 pandemic imposed substantial social stressors, particularly for adolescents, who have experienced increased incidence of psychiatric disorders on a population level during and after this time. Here, using one of the largest available longitudinal data sets of brain development – including scans conducted within the same individuals before and after the onset of the pandemic – we examined whether exposure to the pandemic was associated with changes in the trajectory of age-related cortical thinning.

Methods: The Adolescent Brain Cognitive Development (ABCD) Study is a large-scale, population-based study collecting longitudinal brain development and health data from 11,878 children enrolled at baseline between the ages of 9-10 at 21 sites around the country. Children are being followed longitudinally for 10 years, and structural and functional MRI brain images are collected at baseline and every two years thereafter. As of the 5.0 release in June 2023, complete imaging data from baseline and year 2 (Y2) visits are currently available. Importantly, while all baseline scans and approximately 85% of Y2 scans occurred prior to the pandemic, approximately 15% of Y2 scans were conducted after the pandemic onset, enabling a within-subject "natural experiment" design. Minimally processed scan volumes were downloaded from the NIMH Data Archive, bias-field corrected, and reconstructed in Freesurfer (version 7.0). We limited the analysis to participants with available baseline and year 2 (Y2) scans, scan quality of "C" or better (i.e., < 63 surface holes; see Elyounssi et al., BioRXiv, 2023), and no clinical concerns, for a final sample of 6,111 participants. Images analysis used the FreeSurfer longitudinal processing pipeline. Cortical thickness values for each region of interest were extracted and entered into a linear mixed repeated measures analysis, controlling for the fixed effects of sex, age, intracranial volume, surface hole number at each scan, and income-to-needs ratio (INR); and the random effects of scanner, site, and subject ID. Group differences in the slope of cortical thickness change over time were compared between participants whose Y2 scans were collected before (up to 3/20) versus after (7/20 and following) the COVID-related freeze in ABCD scan acquisition. Statistical analysis was completed in RStudio using the lme4 package and controlled for multiple comparisons across 68 cortical regions (Desikan-Killianey segmentation) using the false discovery rate.

Results: Exposure to the pandemic was associated with significantly faster cortical thinning in the left and right superior frontal cortices (left $t = 4.17$, Wald Chi-squared $= 17.4$, $p = 0.020$, right $t = 4.86$, Wald Chi-squared $= 23.6$, $p = 0.003$). Across these regions, individuals who were exposed to the pandemic underwent cortical thinning at 180% of the rate of the non-exposed control group, with brain thinning accelerated by 4.39 months relative to non-exposed controls.

Conclusions: Exposure to the COVID-19 pandemic was associated with accelerated cortical thinning in the dorsomedial prefrontal cortex, an area of the brain associated with executive

function, planning, and depression symptoms. The mechanism by which increased cortical thinning has occurred remains unclear, however, in animal models, chronic stress leads to dendritic spine loss and dendritic retraction in the medial prefrontal cortex. Altered development of the prefrontal cortex has critical implications for risk for psychiatric diseases and long-term functional trajectories of these adolescents. Consideration of vulnerability factors and resilience factors will be important to understand how these changes in cortical thinning influence onset and worsening of symptoms. Future analyses will include subsequent time points in the ABCD study to examine whether impacted trajectories revert to expected patterns as the world recovers from the COVID-19 pandemic.

Keywords: COVID-19 Pandemic, Adolescent Brain and Cognitive Development Study, Cortical Thinning, Medial Prefrontal Cortex

Disclosure: Nothing to disclose.

P122. Negative Symptom Clusters in Youth With Psychosis

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Background: Negative symptoms have been found to affect approximately 60% of SZ patients, and are associated with poor socio-occupational functioning and quality of life. Furthermore, negative symptoms have been shown to more strongly predict poor long-term functional outcomes compared to positive symptoms such as delusions and hallucinations. In particular, negative symptom severity early in the course of illness is important for predicting functional outcomes, including both social and role functioning. Psychosis with early onset is particularly challenging to diagnose because diagnoses show instability over time, in part because patients with younger onset tend to show less differentiated presentations, which can make diagnosis challenging. For example, individuals with early onset psychosis may show more overlap of psychotic and affective symptoms. Thus, as symptom profiles emerge across time, diagnoses may shift. Negative symptoms are particularly relevant for adolescent psychosis and early onset psychosis. In comparison to adult-onset patients and consistent with a less differentiated presentation, adolescent onset patients show proportionately more negative symptoms. The potential for negative symptoms to predict outcome may be particularly important for adolescent onset psychosis, which has highly variable outcomes that are difficult to foretell. Furthermore, negative symptoms may have an important impact on some key developmental processes during adolescence and early adulthood. The ramifications for symptoms such as anhedonia, affective flattening and avolition during this time may be widespread, resulting in decreased development of social skills and decreased engagement in the age-appropriate activities necessary to develop independence. However, there is little data on anhedonia or on other negative symptoms in youth; for example, adolescent-appropriate measures for indexing anhedonia have only recently been developed. Given the known variability in symptom expression in psychotic spectrum disorders, and the particular diagnostic instability in early onset psychosis, a DSM-based categorical approach may not most optimal in this population. Here, we used a cluster approach to parse heterogeneity based on profiles of negative symptoms.

Methods: Our analyses utilized a longitudinal sample of 89 youth (age 15-25) with early psychosis, recruited at the Zucker Hillside Hospital and the Feinstein Institute for Medical Research. Participants had DSM-V diagnoses on the broadly defined psychosis spectrum, were within two years of illness onset, and were being treated with atypical antipsychotics. We used baseline data to employ a hierarchical cluster analysis in SPSS based on subscores of the Scale for the Assessment of Negative Symptoms (SANS). We assessed stability of the clusters over time, and association with reward related cognitive tasks (the Balloon Analogue Risk Task; BART) and with diffusion tensor imaging measures of white matter integrity in key regions of interest previously associated with schizophrenia.

Results: The cluster analysis yielded three clusters with different symptom profiles on the SANS. One cluster had minimal negative symptoms, the second had high scores across negative symptom domains, and the third had moderate symptoms with the most severe domains being anhedonia and affective flattening. We found that within the clusters, negative symptoms were stable across one year follow-up. For affective flattening, there was a significant effect of cluster across time ($p < .001$) but no cluster x time interaction; for avolition there was a significant effect of cluster ($p = .004$) but not of the interaction; and for anhedonia an effect of cluster ($p < .001$) but no significant interaction. Further, the clusters were associated with differences in performance on the BART, specifically, on mean adjusted pumps ($p = .008$). Finally, while no differences in white matter integrity were noted between cluster groups at baseline, in preliminary analyses, there was a difference in change across time in the superior longitudinal fasciculus such that the least symptomatic cluster showed an increase in fractional anisotropy (FA) over time, while the other two clusters did not (interaction: $p = .034$).

Conclusions: In summary, this research aims to serve as preliminary work to shed light on the relationship between negative symptoms, reward processing, and neural changes in youth with psychosis. In particular, we aim to determine whether a within-patient symptom-based cluster approach might be particularly powerful in populations with substantial diagnostic heterogeneity such as young and early psychosis patients.

Keywords: Psychosis Spectrum Symptoms, Negative Symptoms, Developmental, Early Onset Psychosis

Disclosure: Nothing to disclose.

P123. Examining Subcortico-Cortical Dysconnectivity in Attention Deficit/Hyperactivity Disorder: A Comprehensive Voxelwise Meta-Analysis of Multiple Datasets

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Background: Extensive research has been conducted to investigate the differences in resting-state brain connectivity between individuals with attention deficit/hyperactivity disorder (ADHD) and unaffected controls, with much of this work guided by cortico-striato-thalamo-cortical models of the disorder. Such models propose that dysregulation centered on the caudate nucleus, a brain region involved in various cognitive processes, may contribute to difficulties in attentional focus, impulse inhibition, and task motivation, all of which are characteristic symptoms of ADHD. However, the findings have been inconsistent, partly due to the use of underpowered sample sizes. By analyzing very large, mega-analytic samples, the present study aimed to improve overall statistical power, and to perform well-powered sensitivity analyses.

Methods: Data were included from the Adolescent Brain Cognitive Development (DOI: 10.15154/1519007. NDA identifiers: #2573 #3165), Lifespan Human Connectome Project Development

(release 2.0. NDA identifiers: #2846), Healthy Brain Network (release 9.0), enhanced NKI-Rockland (DOI: 10.1038/s41597-022-01329-y) and National Consortium on Alcohol and Neurodevelopment in Adolescence (DOIs: 10.7303/syn22216455, 10.7303/syn11605291, 10.7303/syn11541569) studies, as well as from our intramural Neurobehavioral Clinical Research study. Data from all studies were processed using a uniform 36-parameter+despiking pipeline. The functional connectivity of four subcortical seed regions (caudate, putamen, nucleus accumbens, amygdala) drawn from the Harvard-Oxford atlas was first examined in a sample of 1705 youth with ADHD diagnoses (1134 males, 66.51%, mean age = 10.86, sd = 2.19) and 6737 unaffected controls (3170 males, 47.05%, mean age = 10.33 years, sd = 1.3) using voxelwise mega-analytic models. Additionally, associations between functional connectivity and ADHD-traits were investigated in a larger cohort (total N = 9,890; n = 4975 males, 50.3%, mean age = 10.77 years, sd = 1.96). In follow-up analyses we aimed to examine whether findings held when examining functional connectivity using ventral and dorsal subdivisions for our regions of interest, based on alternative seed definitions. We also conducted follow-up analyses to examine specificity relative to commonly comorbid internalizing and non-ADHD externalizing problems. To minimize potential confounding factors, matching algorithms were employed to address between group differences in in-scanner motion. Results were assessed following adjustment for estimated general intelligence.

Results: The results from voxelwise mega-analytic models indicated increased connectivity between striatal seeds and temporal, fronto-insular, and supplementary motor regions, as well as between the amygdala and dorsal anterior cingulate cortex in youth with ADHD compared with controls (all voxel-level $p < 0.0001$ uncorrected, cluster $p < 0.05$ fwe corrected). Similar findings were observed when analyzing ADHD-traits and when using alternative seed definitions. The most widespread associations centered on the caudate seed, and in post-hoc partial correlation tests only associations involving this seed remained significant for the group comparison. These findings were independent of in-scanner motion and were distinct from regions associated with commonly comorbid internalizing and externalizing problems. They also held after matching according to in-scanner motion, and after considering estimated general intelligence. However, the effect sizes were consistently small (largest peak d = 0.15).

Conclusions: Our mega-analytic study provides broad support for established links with subcortico-cortical circuits in ADHD, demonstrating robustness to numerous potential confounders and specificity relative to other common childhood behavioral/emotional problems. Nonetheless, the small effect sizes suggest that resting-state subcortico-cortical connectivity may only capture a fraction of the complex pathophysiology of ADHD.

Keywords: ADHD, Resting State Connectivity, Caudate, Mega-Analysis

Disclosure: Nothing to disclose.

P124. Highlighting the Role of Multidimensional Childhood Environments in Functional Brain Network Organization and Cognitive Development

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Background: Cognitive impairments are among the most debilitating and least well understood symptoms of trans-diagnostic psychiatric illness. A growing number of research findings point to developmental origins for such cognitive impairments, with strong associations between early childhood experiences and cognitive functioning in adulthood. Despite the long-recognized importance of childhood environments in shaping individual differences in cognitive neurodevelopment, it is difficult to comprehensively measure and characterize the many inter-connected features of an individual child's environment and experience, and even more difficult to do so at scale in large samples. Moreover, despite clear evidence that psychiatric symptoms and brain organization are both highly heterogeneous among individuals, most neuroimaging studies still rely on group atlases to define functional networks, smearing away inter-individual variation in the spatial layout of functional networks on the cortex ("functional topography"). Here, we overcome these challenges by leveraging supervised and unsupervised computational approaches in a large-scale longitudinal dataset of youth with deep phenotyping of each child's complex, multidimensional environment.

Methods: We conducted pre-registered analyses in matched discovery (n = 5,139, 48.5% female) and replication (n = 5,137, 47.1% female) samples from the Adolescent Brain Cognitive Development Study. To characterize children's multidimensional environments ("exposome"), we applied multilevel (clustered) exploratory factor analysis with a bifactor rotation across 354 variables capturing a variety of child-report, parent-report and geocoded features. In addition to a general exposome factor score for each participant, this bifactor model provided six orthogonal sub-factors capturing specific dimensions of a child's environment: School, Family Values, Family Turmoil, Dense Urban Poverty, Extracurriculars and Screen Time. To define individual-specific maps of functional brain networks we used non-negative matrix factorization to identify seventeen personalized functional brain networks, as in our prior work. We then applied linear mixed effects models and cross-validated ridge regressions to relate individual differences in the exposome to personalized functional brain network topography and individual differences in cognitive functioning across diverse cognitive domains. All models accounted for age, biological sex, family (siblings), scanning site, and head motion.

Results: Our results highlight the important role of a child's exposome in shaping both functional brain network organization and cognitive abilities. Specifically, exposome scores were associated with both current cognition (discovery: βs = 0.12-0.50, all pbonf < .001; replication: βs = 0.15-0.48, all pbonf < .001) and cognitive abilities measured two years later over and above the effect of baseline cognition (discovery: βs = 0.08-0.24, all pbonf < .001; replication: βs = 0.11-0.22, all pbonf < .001). These results were robust to sensitivity tests for the effects of psychiatric diagnoses, medication use, and socio-economic status and held across stratifications by race and biological sex. Moreover, we found that the exposome was reflected in children's' unique patterns of personalized functional brain network topography (association between actual and predicted exposome scores from models trained on multivariate patterns of functional topography: discovery: $r = 0.440$, $p < 0.001$, 95% CI: [0.41, 0.47]; replication: $r = 0.462$, $p < 0.001$, 95% CI: [0.44, 0.49]), suggesting that a child's environment may leave a lasting mark on their cognitive neurodevelopment. We also uncovered both shared and unique contributions of the exposome and functional topography in predicting cognitive abilities. Remarkably, however, we found that models trained on a single variable capturing a child's exposome could more accurately and parsimoniously predict cognitive performance (rs = 0.42-0.46, ps < .001, AICs -454.28--248.82) than models trained on a wealth of personalized neuroimaging data

(rs = 0.41-0.45, ps < .001, AICs = 2.02 x 106), both at baseline and two years later.

Conclusions: Understanding how individual differences in cognitive functioning emerge during childhood is a critical prerequisite for efforts that seek to promote healthy neurocognitive development. By leveraging cutting-edge computational methodologies in a large dataset, our results demonstrate replicable associations between a child's exposome, their functional brain organization, and their future cognitive abilities. Together, these results advance our understanding of how childhood environments may shape unique patterns of functional brain network organization and cognitive development.

Keywords: Cognition, Development, Exposome, Functional Brain Networks, Adolescent Brain Cognitive Development Study

Disclosure: Nothing to disclose.

P125. Shared and Unique Patterns of Connectivity Encode for Impulsivity, Neuroticism and Alcohol-Use Risk in Youth

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Background: Increases in both impulsivity and neuroticism (i.e., the tendency to experience negative affect) are common during adolescence and are both associated with risk for multiple mental health disorders, as well as with increased risk for substance-use initiation. Data-driven, machine learning approaches—when conducted with appropriate cross-validation—enable identification of complex neural networks subserving individual differences in dimensional traits (hereafter referred to as 'neural signatures'), such as impulsivity and neuroticism.

Methods: We used functional connectivity data acquired at age-19 from individuals enrolled in the IMAGEN study, a large, multisite European study of adolescent development (N~1,200). Data were analyzed using connectome-based predictive modeling (CPM) with 10-fold cross-validation. Separate models were built using measures of impulsivity and of neuroticism, respectively, and network anatomies were compared.

Results: CPM accurately predicted both impulsivity and neuroticism, as confirmed via 10-fold cross-validation (r's~.17-.19, p's < .05). The impulsivity network was predominantly characterized by within-network motor/sensory connections. In contrast, the functional connectivity signature of neuroticism was relatively more distributed across multiple canonical networks. Specifically, while neuroticism was also positively predicted by connections within the motor/sensory network, it was also positively predicted by connections between the motor/sensory network and other canonical networks, including the mediofrontal, visual association, salience, subcortical and cerebellum networks. Moreover, neuroticism was also positively predicted by the connections between subcortical and frontoparietal networks, and between subcortical and cerebellum networks. Virtual lesioning analyses confirmed the centrality of the motor/sensory network in predicting impulsivity, whereas virtual lesioning of the neuroticism network indicated relatively comparable contributions of each canonical network alone. Only a single connection was identified as common to both impulsivity and neuroticism networks. Comparison with a previously identified alcohol-use risk network (also identified in the IMAGEN Consortium) indicated that approximately 10% of the connections from each trait overlapped with the alcohol-use risk network; however, these shared connections were distinct between the two traits.

Conclusions: Our findings reveal dissociable functional connectomes between neuroticism and impulsivity in adolescence and elucidate the potential neural basis of interactions between these traits and alcohol-use risk in youth.

Keywords: Neuroticism, Impulsivity, fMRI Functional Connectivity

Disclosure: Nothing to disclose.

P126. Alterations to Stimulus Discriminability in Youth With Misophonia

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Background: Misophonia is a complex condition characterized by extreme emotional distress in response to specific sounds or associated visual stimuli. Despite a growing body of literature concerning the psychological profile there is a paucity of research delving into the biological mechanisms of development and symptomatology. Early work has identified a possible role of circuitry related to the regulation of arousal within the central and autonomic nervous systems. Symptoms such as heightened galvanic skin response increased heart rate, and muscular tension in response to certain auditory stimuli correlate with neuroimaging studies that show heightened vigilance of the auditory system in patients with misophonia compared to controls. The objective of our research was to test the scalability of these elements using neurocognitive testing, and to identify signatures of attentional dysfunction in affected youth that could be readily translated to a clinical setting. To distinguish processes specific for misophonia we recruited a clinical comparison group of youth with anxiety disorders, another condition characterized by alterations in attentional processing, making it possible to distinguish mechanisms specific for misophonia.

Methods: Our study was conducted as part of a larger project conducting deep phenotyping of misophonia in children and adolescents (Guzick et al, 2023). 102 children and adolescents (13.69 ± 2.51 years) (67.6% female) who met the proposed diagnostic criteria for Misophonia and 94 children and adolescents with anxiety disorders (12.41 ± 2.55 years) (57.4% female) were recruited. 72 misophonia (13.74 ± 2.44 years) (64% female) and 89 anxiety patients (12.35 ± 2.57 years) (58.4% female) completed all study measures and were included in our investigation. We used an online implementation the Immediate Memory Task (IMT), created using Gorilla Experiment Builder (Anwyl-Irvine et al., 2021, 2018), to study neurocognitive properties of misophonia including stimulus discriminability, response bias, and response time. In the IMT, the goal is to select if a cue and target stimulus (5 number sequence) match or not, where non-matching sequences can be completely different (random errors) or contain only subtle differences (commission errors). This design distinguishes random and/or impulsive incorrect responses from incorrect anticipatory responses (commission errors), and allows for estimation of response sensitivity and bias. To evaluate IMT performance we estimated signal detection theory measures of response sensitivity (d', standardized difference between hit rate and false-alarm rate) and response bias (c', product of -0.5* the sum of the normalized hit rate and false-alarm rate), as well as reaction times and overall response accuracy by trial type. IMT response accuracy by trial type were analyzed using repeated measures analysis of variance (ANOVA). Signal detection measures and ex-gaussian parameters of the reaction time distribution per subject were compared between groups using independent samples t-tests. To explore

potential relationships between neurocognitive performance and clinical ratings we estimated bootstrapped Pearson's R correlations for misophonia and anxiety groups separately. Correlations were Bonferonni corrected to adjust for multiple comparisons within measurement families.

Results: d' was statistically significantly greater in Misophonia patients compared to anxiety patients ($t = -2.12$, $df = 159$, $p = .036$, $d = -0.336$, CI = -0.65 to -0.02), indicating a higher rate of "hits" to "false alarms." c' was not significantly different between groups ($t = 0.47$, $df = 159$, $p = .64$, $d = 0.07$, CI = -0.24 to 0.38). In Misophonia patients, d' was significantly correlated with the Misophonia Inventory of Sound Triggers (MIST-C) ($r = .33$, $p = .005$) and the Cognitive Emotion Regulation Questionnaire (CERQ) total score ($r = .31$, $p = .008$). No other clinical variables were significantly associated with d' or c scores. Analysis of behavioral performance showed significant main effect of condition ($F = 124.64$, $df = 3$, $p < .001$, $\eta 2 = 0.44$) but not group ($F = 2.06$, $df = 1$, $p < .15$, $\eta 2 = 0.01$) or group*condition ($F = 0.74$, $df = 3$, $p < .53$, $\eta 2 = 0.005$). IMT task performance was associated with reduced accuracy during catch trials relative to other conditions, but performance was not affected by group membership. Reaction times were significantly different between groups, or associated with clinical ratings.

Conclusions: Our findings are in line with previous cognitive and neuroimaging studies, and lend support to an arousal-based model of misophonia, where individuals with misophonia experience a state of heightened vigilance, being more aware of stimuli in the environment. Approaches based on signal detection theory provide a complimentary perspective to existing functional imaging studies by summarizing the computational properties of targeted systems. In this study, we observed a dimensional relationship between misophonic trigger load and response sensitivity. Alterations to neurocognition that promote trigger avoidance might be an avenue for future work aiming to refine therapeutic and clinical targets. Finally, our findings provide a neurocognitive basis for future studies of neurochemical imaging that might further progress towards clinical targets for drug development.

Keywords: Misophonia, Hyperarousal, Attention, Vigilance, Anxiety

Disclosure: Nothing to disclose.

P127. Pubertal Maturation and Hormones Influence Mesocorticolimbic Development in Youth: Implications for Sensitivity to Rewards and Punishments

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Background: Puberty initiates neuroendocrinological changes in adolescence to shape affective and cognitive development. Some have shown that such changes could contribute to heightened psychopathological risk through shifts in sensitivity to positive and negative stimuli. What remains unclear is how various aspects of puberty (e.g., adrenarche and gonadarche) influence the maturation of neural circuitry supporting reward/punishment sensitivity. We address this gap in knowledge by testing longitudinally to what extent pubertal maturation, including changes in levels of testosterone, influence fronto-amygdala and fronto-striatal resting-state functional connectivity (rsFC) and whether such effects impact sensitivity to rewards and punishment in youth.

Methods: We collected self-report measures of puberty, levels of testosterone (3 salivary samples over 4 weeks), and resting-state fMRI data from 126 healthy adolescents (69 girls; mean age = 12.66 ± 1.41) assessed longitudinally up to two times with 2 years (216 total visits). We additionally collected parent report of adolescents' sensitivity to reward and punishment using the Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ). Finally, we used generalized additive mixed models (GAMMs) to test the relationship between pubertal maturation and fronto-amygdala and fronto-striatal rsFC beyond age effects (modeled as a covariate) separately in boys and girls. We used linear mixed effects models to test whether shared or distinct connections supported reward/punishment sensitivity. All statistical tests were Bonferroni-corrected.

Results: Across boys and girls, adrenal axis maturation was associated with strengthening of mesocorticolimbic rsFC whereas gonadal axis maturation was associated with weakening of rsFC. In girls, adrenal axis maturation was associated with fronto-striatal rsFC strengthening between the nucleus accumbens (NAcc) and the following cortical regions: anterior ventromedial prefrontal cortex (vmPFC) ($F = 8.53$, $p = .002$), subgenual anterior cingulate cortex (sgACC) ($F = 9.28$, $p = .001$), rostral ACC (rACC) ($F = 8.30$, $p = .003$), and ventrolateral PFC (vlPFC) ($F = 7.21$, $p = .008$). Adrenal axis maturation was also associated with amygdala rsFC strengthening with the sgACC ($F = 7.51$, $p = .006$), rACC ($F = 8.11$, $p = .003$), and vlPFC ($F = 8.29$, $p = .003$). Gonadal axis maturation in girls was associated with weakening of rsFC in the sgACC – NAcc ($F = 8.96$, $p = .0001$), vlPFC – NAcc ($F = 8.00$, $p = .0004$), as well as amygdala rsFC with the ventral ACC (vACC) ($F = 3.94$, $p = .020$), sgACC ($F = 7.90$, $p = .0004$), and rACC ($F = 5.72$, $p = .003$). In boys, adrenal axis maturation was associated with anterior vmPFC – NAcc rsFC strengthening ($F = 10.97$, $p = .0002$) and with amygdala connectivity strengthening with the anterior vmPFC ($F = 18.15$, $p < .000001$), vACC ($F = 6.26$, $p = .020$), sgACC ($F = 11.10$, $p = .0002$), and rACC ($F = 6.81$, $p = .010$). Gonadal axis maturation was associated with weakening rsFC in the anterior vmPFC – NAcc ($F = 8.32$, $p = .003$) and rACC – amygdala ($F = 5.63$, $p = .040$). Distinct connections supported sensitivity to rewards and punishments in girls and boys. After controlling for age, rACC – NAcc rsFC was positively associated with punishment sensitivity in girls ($\beta = 0.05$, $p = .009$). In boys, anterior vmPFC – NAcc rsFC was negatively associated with reward sensitivity ($\beta = -0.05$, $p = .013$), whereas punishment sensitivity was positively associated with rsFC in the following connections: vlPFC – NAcc ($\beta = 0.06$, $p = .004$) and amygdala rsFC with the anterior vmPFC ($\beta = 0.06$, $p = .001$), sgACC ($\beta = 0.05$, $p = .009$), rACC ($\beta = 0.05$, $p = .007$), and vlPFC ($\beta = 0.06$, $p = .0009$). Finally, in boys, testosterone moderated the relationship between anterior vmPFC and NAcc rsFC and reward sensitivity ($\beta = 0.07$, $p = .0006$). Specifically, the association between stronger rsFC and reduced reward sensitivity was evident only in boys lower in testosterone than their peers. No effects were noted in girls.

Conclusions: The various dimensions of puberty—namely, adrenal and gonadal axis maturation—make unique contributions to the functional neurodevelopment of mesocorticolimbic circuitry. Distinct connections within this circuitry support sensitivity to rewards and punishments differently in boys and girls. Our findings further identify a neuroendocrinological mechanism (i.e., testosterone) that modulates one such relationship between anterior vmPFC – NAcc functional connectivity and reward sensitivity. Taken together, these findings underscore puberty as a multifaceted process with shared and distinct contributions to neurodevelopment in boys and girls, which may explain some sex-related differences in rates of psychopathology that emerge during the adolescent period.

Keywords: Puberty, Resting State Functional Connectivity, Reward Sensitivity, Mesocorticolimbic System, testosterone

Disclosure: Nothing to disclose.

P128. Multivariate Assessment of Inhibitory Control in Youth: Links With Psychopathology and Brain Function

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Background: Inhibitory control refers to the capacity to inhibit a reactive response in order to engage in goal-driven behavior (Miyake et al., 2000). Inhibitory control features prominently in theories of cognitive and brain development (Diamond, 2013; Luna et al., 2015), mediated by the fronto-parietal control network (Cai et al., 2021; Menon, 2011). Further, impairments in inhibitory control are posited to underlie domains of developmental psychopathology (Barkley, 1997; Nigg, 2001). In this study, we used structural equation modeling (SEM) to test the possibility of shared associations between behaviorally-assessed inhibitory control and three common symptom dimensions in youth psychopathology – ADHD, anxiety, and irritability – in a large, clinically-referred sample. We also examined associations between inhibitory control and global efficiency in a fronto-parietal control network delineated during resting-state fMRI.

Methods: 246 youth ages 8-18 (M age = 12.8, SD = 2.6; 48% female) were recruited across several diagnostic groups (ADHD, anxiety disorders, disruptive mood dysregulation disorder, no diagnosis). Participants completed up to four laboratory-based inhibitory control tasks (Anti-Saccade, AX-CPT, Flanker, and Stop-Signal Delay) on which behavioral performance was quantified (eye-tracking, motor behavior), as well as questionnaires assessing ADHD, anxiety, and irritability symptoms. 173 of the participants also completed a resting-state fMRI scan. fMRI data were preprocessed using FMRIPREP (Esteban et al., 2019). We extracted functional connectivity as Pearson correlations across 200 cortical parcels (Schaefer et al., 2018) and 16 subcortical regions (FreeSurfer). Graph theory metrics were derived to quantify global efficiency in a fronto-parietal control network (Brain Connectivity toolbox). All SEM analyses were conducted using Mplus software.

Results: The confirmatory SEM with good model fit (CFI = 0.99, RMSEA = 0.04) indicated that behavior on all four tasks loaded significantly on a latent inhibitory control factor (ps < .001), and symptom levels fit well in a bifactor model including general psychopathology (ps < .01), anxiety-specific (ps < .001), irritability-specific (ps < .01), and ADHD-specific (ps < .001) latent variables. As hypothesized, path analyses within the confirmatory SEM indicated that poorer inhibitory control was a significant predictor of greater general psychopathology (b=-1.55, p=.03), but not a predictor of ADHD-specific (p=.11), anxiety-specific (p=.22), or irritability-specific (p=.11) symptoms. In a second confirmatory SEM including resting-state fMRI (CFI=0.99, RMSEA=0.04), poorer inhibitory control was associated with lower global efficiency in the fronto-parietal control network (b=0.20, p < .05). Exploratory analyses demonstrated that this association was specific to the control network versus the whole-brain network and other specific networks.

Conclusions: These results support performance-based inhibitory control linked to resting-state brain function as an important predictor of comorbidity in youth psychopathology. Specifically, findings provide evidence for inhibitory control as a mechanism underlying the general propensity for childhood psychopathology across mood, anxiety, and disruptive behavior symptoms. Behaviorally-defined inhibitory control also linked with brain connectivity specific to the fronto-parietal control network. Symptom comorbidity is a robust predictor of negative outcomes; as such, treatment and prevention efforts targeting inhibitory control may be valuable to help youth who are highest risk for long-term negative outcomes.

Keywords: Inhibitory Control, Resting State fMRI, Developmental Psychopathology, Structure of Psychopathology

Disclosure: Nothing to disclose.

P129. Evaluating a Novel Digital Neuroplasticity-Based Social Cognitive Intervention for Autistic Teens: Efficacy as Stand-Alone Treatment or as Enhancement to Social Skills Training

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Background: Autism Spectrum Disorder (ASD), a complex neurodevelopmental condition, involves challenges with social communication and interaction, restricted interests, repetitive behaviors, and atypical responses to sensory stimuli (APA, 2013). During the critical period of adolescence, changes in brain networks subserving socioemotional and cognitive processing occur with and adapt to increasingly complex social networks and demands (Andrews et al., 2021). For autistic youth who struggle with novel situations and interpersonal interactions, avoidance of troubling contexts and expectations may hinder development of key experience-dependent social and cognitive skills (Tseng et al., 2020). While evidence-based interventions designed to improve functional impairments in autistic individuals are still lacking, computerized and adaptive brain-based cognitive training programs have been applied effectively to address general cognitive deficits in neuropsychiatry (e.g, schizophrenia, ADHD) (Vinogradov et al., 2012). Building on our preliminary neuroplasticity-based cognitive training findings from autistic adolescents and young adults (Tseng et al., 2023) and prior results from studies in psychosis (Loewy et al., 2016), we developed CICADAS (Care Improving Cognition for ADolescents on the Autism Spectrum), a digital application designed to prime the brain to engage in flexible, adaptive long-term learning about socioemotional events through closed-loop technology. In the present study, we aimed to evaluate the acceptability and preliminary efficacy of the CICADAS app as a stand-alone treatment or in conjunction with remotely delivered PEERS (Program for the Education and Enrichment of Relationship Skills), one of the few evidence-based social skills programs available for autistic teens (Laugeson et al., 2011).

Methods: We recruited adolescents with confirmed diagnoses of ASD from partner clinics delivering PEERS. Participants were enrolled in a three-arm, active-controlled, randomized crossover trial with pre/post-intervention measures to examine the potential of CICADAS in improving social cognition and real-world functioning, and in enhancing response to PEERS. After study eligibility was determined by diagnostic interview with each family (Parent + Teen), informed consent/assent procedures were completed via Zoom. Participants were randomized to the CICADAS only group (N = 19; Age = 14.05 ± 2.04; Sex = 11M, 6F, 2NB), the PEERS + CICADAS group (N = 21; Age = 13.66 ± 2.05; Sex = 14M, 5F, 2 NB), or the PEERS + Active Control group (N = 22; Age = 14.27 ± 1.63; 16M, 6F). Each group spent up to 16 weeks engaged in their assigned intervention; the PEERS program was implemented independently by clinicians blinded to study participation status.

- CICADAS app: Participants completed baseline assessments and neuroplasticity-based social cognitive training (NB-SCT) exercises on a digital device for up to 40 hours through BrainHQ (Posit Science Corporation, San Francisco). The CICADAS app captures user-specific sensory processing abnormalities (SPA)

through 10 brief computerized assessments; these data are used to guide and personalize the delivery of 13 NB-SCT exercises. The NB-SCT exercises are adaptive with the task difficulty adjusting on a trial-to-trial and session-by-session basis to the abilities of each individual. A second set of computerized assessments was administered following the intervention period. Participants had to log in to access these assessments and exercises using a study provided username that contained no personally identifiable information.

- Active Control app: Participants played previously-vetted and popular casual video games for an overall duration time-matched to the CICADAS program. These games were designed to not actively engage the neural systems that underlie ASD. However, the CICADAS and active control programs were matched carefully for level of intensity, modality, reward delivery, and overall engagement. Active control participants also completed the set of brief computerized assessments before and after the intervention period.

Results: Preliminary findings suggest beneficial effects of NB-SCT on cognitive performance. Across all CICADAS app users (CICADAS only and PEERS + CICADAS), we found significant pre-/post-interventions improvements on assessments targeting memory for auditory details ($p = 0.0007$), sound discrimination and processing speed ($p = 0.01$), cognitive flexibility ($p = 0.05$), and executive function and attention ($p = 0.05$). Further, participants in the PEERS + CICADAS group showed significant performance improvements in auditory processing speed ($p = 0.05$) and auditory memory ($p = 0.02$) while participants in the PEERS + Active Control group did not. The abilities to detect subtle differences in auditory stimuli and attend to increasingly complex conversational elements are critical to successful interpersonal interactions. These gains associated with PEERS + CICADAS suggest an enhancement afforded by the NB-SCT app.

Conclusions: While data analyses are ongoing, our initial findings show support for the efficacy and feasibility of our CICADAS app as an augmentation for a group-based social skills intervention and, potentially, as a stand-alone treatment. Further investigation will examine clinical and sensory changes associated with treatment and these data will be used to refine our training programs.

Keywords: Neuroplasticity, Digital Therapeutic, Social Cognitive Skills Training, Autism Spectrum Disorder and Related Syndromes, Adolescent Development

Disclosure: Roche: Contracted Research (Self).

P130. A Machine Learning Approach to Integrate Clinical Studies for the Identification of Multimodal Predictors of Child Internalizing Psychopathology

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Background: Childhood internalizing psychopathology — such as anxiety and depression — significantly impacts lifelong mental health. Aiming to identify the most influential predictors of these symptoms in preschoolers, this study used a data-driven approach to integrate different studies with multiple data modalities within the Research Domain Criteria (RDoC) framework.

Methods: This study integrated data from three small clinical studies to form a combined sample of 181 children (57% female; mean age 5.6 years) and 82 features. The data consisted of electroencephalogram (EEG) measures, behavioral assessments, mother report on child (temperament and psychiatric symptoms) and self (maternal psychopathology), as well as sociodemographic factors. A machine learning-based approach was used for handling missing data (missForest) and Random Forest models to test multimodal predictors of internalizing, anxiety, and depression T-scores from the Child Behavior Checklist (CBCL). Permutation-based importance was used to identify the most influential predictors and post hoc procedures to test for interactions.

Results: For CBCL-Internalizing, the out-of-bag (OOB) performance metrics of the random forest model – MSE and pseudo-R^2 — were 77.25 and 0.48, respectively. The most important predictors of internalizing symptoms were Soothability, a temperament trait measured with the Children's Behavior Questionnaire (CBQ); maternal psychopathology (PTSD symptoms, depression, and trauma history); the Error-Related Positivity (Pe), an EEG index of neural substrate for error awareness in the cognitive control domain. Additionally, greater CBQ Sadness and less error-related alpha suppression — another EEG-derived index of cognitive control — interacted to predict more internalizing symptoms. For the model predicting CBCL-Anxiety (OOB MSE = 51.28; pseudo-$R^2 = 0.43$), Fear was the most influential temperament trait, and the Pe emerged one more time as the most important EEG measure. The interaction of Fear with the Pe was also notably relevant such that medium fear (in contrast to high or low) interacted with larger Pe to predict higher CBCL-Anxiety. Other significant factors in the prediction of CBCL-Anxiety included maternal trauma and maternal PTSD symptoms. Finally, for the model predicting CBCL-Depression (OOB MSE = 32.98; pseudo-$R^2 = 0.33$), Soothability and maternal psychopathology emerged again as highly important, followed by the EEG cognitive control measure stimulus-locked theta power for NoGo trials (channel Cz). The most crucial interaction identified with this model was between Soothability and the distraction time in the Snack Delay Task, a behavioral measure of cognitive control (i.e., lower Soothability associated with more distraction).

Conclusions: In accordance with the RDoC framework, this study offers a multidimensional view of the influential factors in childhood internalizing psychopathology. Notably, this data-driven approach showed that specific temperament traits, maternal psychopathology, and neurophysiological measures differentially predict anxiety, depression, and broad-band internalizing scores. These findings highlight the utility of advanced statistical techniques for implementing a multifaceted, integrative approach for identifying predictors of childhood internalizing psychopathology across brain and behavioral levels of analysis.

Keywords: Machine Learning, Internalizing Disorders, Anxiety and Depression, EEG Biomarkers, Electroencephalography (EEG)

Disclosure: Nothing to disclose.

P131. DHA Supplementation Mitigates the Absolute and Relative Brain Volume Changes Induced by a Prenatal Stress-Associated ASD Murine Model

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Background: Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by social communication difficulties, restricted interests, and repetitive behaviors. Gene-environment interactions appear to contribute to the pathogenesis. One exemplar involves prenatal stress exposed pregnancies in mothers with at least one copy of the short allele in the promotor region of the serotonin transporter gene (SERT), modeled using dams with the heterozygous serotonin transporter knockout (SERT +/-) in mice, whose offspring produce ASD-

associated phenotypical behaviors of low social ability and increased repetitive grooming. Our previous work showed that in the maternal SERT +/- prenatal stress-associated ASD mouse model, DHA supplementation mitigates dopaminergic excess found in the striatum of this model, as well as the behavioral effects. The present study aimed to quantify the absolute and relative brain volume variability across offspring of SERT +/- dams exposed to prenatal stress vs C57BL/6J controls with and without DHA supplementation initiated before pregnancy in the dams and continuing through weaning.

Methods: Offspring of SERT +/- dams exposed to prenatal stress vs C57BL/6J controls with and without DHA supplementation initiated before pregnancy in the dams and continuing through weaning were examined. After weaning, offspring of those given supplementation also received DHA until behavioral testing began, focusing on quantification of repetitive behaviors in an open chamber. Brains were collected from offspring around post-natal day 70. Using deformation-based morphometry at 7T MRI, we assessed neuroanatomical differences across our four conditions (SERT +/- prenatally stressed with DHA supplementation (HS/D), SERT +/- prenatally stressed with no supplementation (HS/ND), C57BL/6J not stressed with DHA supplementation (WT/D), and C57BL/6J not stressed with no supplementation (WT/ND)).

Results: Without DHA supplementation, SERT +/- prenatally stressed offspring were seen to have significantly larger absolute and relative brain volumes in the striatum, nucleus accumbens, olfactory peduncle, flocculus, claustrum and cingulate cortex areas 24a and 24b compared to wildtype controls. DHA appeared to reverse many of these findings, as several areas related to the striatum and dopaminergic pathways were significantly decreased in the HS/D compared to HS/ND offspring including the amygdala, thalamus, olfactory peduncle, flocculus, claustrum, and the cingulate cortex areas 24a, 24b, 24b.1, 29a, 29b, 29c, 30, and 32. Additionally, grooming frequency and grooming duration in the open field correlated with these changes in volume for the cingulate cortex area 24b and the claustrum, and grooming frequency also correlated with changes in volume for the cingulate cortex area 24a, flocculus, and olfactory peduncle.

Conclusions: DHA supplementation in the prenatal stress-associated ASD mouse model seems to influence a wide variety of brain regions including those associated with the striatum and its related dopaminergic pathways. These changes are also associated with changes in repetitive behaviors. These aspects will need to be evaluated in the clinical setting, and the mechanism deserves further exploration.

Keywords: Autism Spectrum Disorder and Related Syndromes, Prenatal Stress Model, Omega-3 Fatty Acids, Repetitive Behavior, Structural MRI

Disclosures: Yamo Pharmaceuticals, Stalicla Biosciences, Impel Pharmaceuticals, Scioto Pharmaceuticals, and Human Biosciences: Consultant (Self).

P132. Neighborhood Resources Shape Neural Circuitry Relevant to the Regulation of Behavior in Youth

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Background: Neighborhoods provide essential resources that influence neurodevelopment and mental health, but mechanisms mediating these relationships are poorly understood. Here, we investigated the relationship between neighborhood resources and the regulation of behavior on a behavioral and neurobiological level.

Methods: In the context of an ongoing study examining a culturally sensitive preventive intervention for risky behavior in African American youth, 92 children (~ 12.7 years, 53% female, no medical or psychiatric diagnosis) completed the Behavior Rating Inventory of Executive Function (BRIEF) and underwent functional magnetic resonance imaging while resting or working on a Go/NoGo task during the baseline assessment. Accounting for the nested nature of the data, we investigated whether neighborhood resources operationalized via the Childhood Opportunity Index (COI) relate to a specific neural signature and explored whether this neural signature mediates the relationship between access to neighborhood resources and problems regulating behavior (indexed via BRIEF).

Results: Youth growing up in neighborhoods with lower COI scores reported more problems regulating their behavior ($\beta = .03$, $\chi2 = 4.58$, $p = .03$). Across the rest and task condition, COI scores were positively related to the functional connectivity of 48 edges interconnecting well-known resting-state networks, and negatively associated with functional connectivity of a small network (13 edges) involving the amygdala, hippocampus, and regions of the default mode, dorsal attention, somatomotor and visual networks (all $pFWE < .05$). This altered functional connectivity pattern mediated the relationship between the COI and problems regulating behavior.

Conclusions: This study adds to a growing literature documenting how inequity may affect the brain, behavior, and youth mental health. Future work should test whether findings generalize to other samples and explore effects on neurodevelopmental trajectories and emerging behavioral problems during adolescence.

Keywords: Neurodevelopmental and Behavioral Deficits, fMRI, Neighborhood Resources, Marginalized Youth

Disclosure: Nothing to disclose.

P133. Peripuberty is a Sensitive Period for Activity-Dependent Maturation of PFC PV Interneurons

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Background: Developmental windows in which experiences can elicit long-lasting effects on brain circuitry and behavior are called 'sensitive periods' and reflect a state of heightened plasticity. The classic example of a sensitive period comes from studies of sensory systems, like the visual system, where early visual experience is required for normal wiring of primary visual cortex and proper visual functioning. At a mechanistic level, loss of incoming visual input results in a decrease in activity in thalamocortical neurons representing the affected eye, resulting in an activity-dependent reduction in the representation of those inputs in the visual cortex and loss of visual perception in that eye. While associative cortical regions like the prefrontal cortex (PFC) do not receive direct sensory input, recent findings demonstrate that changes in activity levels experienced by this region during defined windows in early development may also result in long-lasting changes in prefrontal cortical circuitry, network function and behavior. For example, we recently demonstrated that decreasing the activity of prefrontal parvalbumin-expressing (PV) interneurons during a period of time encompassing peripuberty (postnatal day P14) to adolescence (P50) led to a long-lasting decrease in their functional inhibition of pyramidal cells, as well as impairments in cognitive flexibility. While the effects of manipulating PFC PV interneuron activity were selective to development, and not adulthood, the exact timing of the sensitive period for this manipulation remains unknown.

Methods: To refine the sensitive period in which inhibiting PFC PV cell activity can lead to persistent effects on prefrontal functioning we used a chemogenetic approach to restrict our manipulation of PFC PV activity to two distinct windows: 1) peripuberty (P14-P32) and 2) early adolescence (P33-P50). To do this we used a virus to express the DREADD receptor, hM4D, or the control, mCherry, in PFC PV cells in the developing animal and then administered the ligand, clozapine-N-oxide, during the appropriate developmental window. We then investigated adult behavior after P90. In parallel, we performed histological analysis of molecular markers associated with sensitive period onset (PV cell number and intensity) and offset (intensity of perineuronal nets encapsulating PV cells) in visual cortex (V1), to define the onset and offset of peak sensitive period plasticity in the prefrontal cortex. Both males and females were included, and the statistical analysis is denoted in the results.

Results: We found that inhibition of PV interneurons in peripuberty (P14-P32), but not adolescence (P33-P50), led to an impairment in set shifting behavior in adulthood manifest as an increase in trials to reach criterion performance (1-way ANOVA, $p = 0.0055$, Tukey's post-hoc: control v. peripubertal inhibition, $p = 0.004$; control v. adolescent inhibition, $p = 0.4622$, $n = 12$ control, 4 peripubertal inhibition, 5 adolescent inhibition) and errors (1-way ANOVA, $p = 0.026$, Tukey's post-hoc: control v. peripubertal inhibition, $p = 0.02$; control v. adolescent inhibition, $p = 0.7694$, $n = 12$ control, 4 peripubertal inhibition, 5 adolescent inhibition). Consistent with a pubertal onset of sensitive period plasticity in the PFC, the rate of change of PV cell number and PV intensity was greatest between P14 and P21 and leveled off from that point afterwards into adulthood ($n = 5$ animals per timepoint of analysis). Levels of the perineuronal net marker, WFA, encapsulating PV cells reached adult intensity by P35, indicative of slowing of peak plasticity by this time (1-way ANOVA followed by Bonferroni's post-hoc, P14 versus P90 (adult), $p = 0.0002$; p21 v. P90 (adult), $p = 0.0226$; P35 v. P90 (adult), $p = 0.1433$, $n = 4$-5 animals per timepoint). The time course of expression of these markers was similar in V1.

Conclusions: Both lines of research converge on the peripubertal period (P14-32) as one in which sensitive period plasticity is greatest in the PFC. Further, our direct comparison of markers of sensitive period plasticity across the prefrontal and visual cortex suggests a similar time course of expression, challenging the notion that sensitive periods occur hierarchically. Together, these findings extend our knowledge about the nature and timing of sensitive period plasticity in the developing prefrontal cortex.

Keywords: Medial Prefrontal Cortex, Cognitive Flexibility, Brain Development, Parvalbumin Interneurons, Developmental Sensitive Period

Disclosure: Nothing to disclose.

P134. Inhaled Cannabis Delivery During Pregnancy: Effects on Structural Brain Development, Endocannabinoid and Immune System Functioning, Social and Emotional Behaviour, and Metabolism in Males and Female Rats

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Background: Growing evidence suggests that up to 20% of people report use of cannabis during pregnancy, with many individuals and health professionals considering cannabis natural and safe. However, both clinical studies and animal models of prenatal cannabis exposure (PCE) have shown growth retardation, increased incidence of autism or social behaviour deficits, immune changes, cognitive dysfunction, increased body mass index and adiposity, and signs of disrupted feeding behaviours and glucose metabolism. Nevertheless, little is actually known about the mechanism through which PCE may alter neurodevelopment and subsequent behaviour. Further, while clinical studies are confounded by unknown timing and exposure level, animal models can control for these variables while examining mechanism. Therefore, our studies aim to characterize the effects of inhaled cannabis exposure during pregnancy on a wide range of domains including: maternal outcomes, structural brain development, endocrine and immune system functioning, social behaviour, stress-reactive behaviour, and glucose metabolism and feeding. Moreover, as cannabis exerts its effects through acting on the endocannabinoid (eCB) system, which is involved in many processes of brain development, our studies also aim to determine if PCE acts through direct modulation of the eCB system and/or indirect modulation of other systems, such as the immune or stress-response systems.

Methods: Utilizing a validated vapour chamber system, pregnant rats were exposed to THC-heavy cannabis vapour or vehicle vapour for 15-min a day beginning on gestational day (GD) 1. Our experiments had three aims: 1) fetal development, 2) postnatal development, and 3) long-term effects in adolescence and adulthood. For any given measurement, one male and one female pup from a litter were utilized to avoid litter effects. In total, 8-10 males and 8-10 females were utilized per outcome. In aim 1, dams and their fetuses were euthanized on GD15, 17, or 19 to examine fetal brain development. In aim 2, dams gave birth and a cohort of their offspring were euthanized on postnatal day (PD) 0 and 5 to examine postnatal brain development. Maternal blood and spleen, placenta, and fetal brains were collected via caesarean section surgery from aim 1 and postnatal brains were collected from aim 2 – tissues were analyzed for levels of THC and metabolites, eCB levels, eCB and immune-related gene expression, and baseline cytokine levels. In aim 3, separate cohorts of offspring were tested across the lifespan for the following measurements: 1) cytokine levels after an acute immune challenge on PD14; 2) structural brain development via MRI imaging on PD25-30; 3) social play behaviours on PD33-PD37; 4) anxiety-like behaviour assessed in the elevated plus maze and acute stress response following 30-minute restraint stress in adulthood; and 5) glucose metabolism and feeding patterns following 4-months of high or low fat diet exposure in adulthood.

Results: Preliminary analysis show no impact of PCE on brain eCB levels or cannabinoid receptor gene expression. Analysis of eCB and immune-related gene expression and baseline cytokine levels are ongoing. We have also found no impact of PCE on maternal behaviour, structural brain development, or anxiety-like behaviour. Following acute restraint stress, PCE males did exhibit heightened corticosterone (the main stress hormone in rodents) levels 60-min after the initiation of stress (PCE males had greater levels than control males, main effect of group at $p = 0.028$). Analysis of social play behaviour and cytokine levels following immune challenge are ongoing. Interestingly, prior to special diet exposure, PCE had no effect on adiposity but did increase total daily energy intake (PCE animals had increased light phase and 24-hour intake compared to control animals, main effect of group at $p = 0.04$ and $p = 0.046$ respectively). Further, prior to special diet exposure, PCE improved glucose metabolism (PCE animals had lower blood glucose at 15-min following dextrose injection than control animals, interaction effect of time and group at $p = 0.021$). Following special diet access, PCE had no effect on adiposity or daily energy intake. However, PCE had sex-specific effects on glucose metabolism such that PCE females showed improved glucose metabolism, but PCE males showed impaired glucose metabolism (PCE females had lower blood glucose 120-min after dextrose injection, whereas PCE males had higher blood

glucose at 15- and 60-min after injection (interaction effect of sex, time and group at $p = 0.029$).

Conclusions: Taken together, our findings have illustrated subtle effects of PCE on the stress-response in males and altered energy intake and glucose metabolism in both sexes. However, we have no effects of PCE on maternal behaviour, structural brain development, endocannabinoid functioning, or anxiety-like behaviour. In conjunction with ongoing research examining the functioning of the endocannabinoid and immune systems, our research may help determine the developmental mechanisms through which PCE impacts later-life functioning and behaviour. Our research has and will continue to contribute to the growing body of literature on the effects of cannabis exposure during pregnancy, and may help formulate a more complete picture of the safety of maternal cannabis consumption.

Keywords: Prenatal Cannabis Exposure, Structural MRI, Behavior, Neuroendocrine Responses, Metabolism

Disclosure: Nothing to disclose.

P135. Neuromodulation of the Nucleus Accumbens Through Real-Time fMRI Neurofeedback: A Novel Approach for Examining Age-Related Deficits in Self-Control Over Reward Responding

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Background: The protracted maturation of brain regions involved in cognitive control compared to earlier maturing reward-related regions is believed to underlie heightened risk-taking behaviors during adolescence. Neuroimaging studies that assess cognitive control and reward responsivity separately are limited, however, in their ability to test the dynamic interaction between these constructs simultaneously. Real-time fMRI neurofeedback (rtfMRI-nf), an imaging modality in which participants learn to exert volitional control over targeted brain activity, offers a promising and potentially ecologically valid test of developmental differences posited by dual systems models of adolescent brain development. Work to date supports the feasibility of using rtfMRI-nf to modulate activity in the nucleus accumbens (NAcc), a key region within the mesolimbic dopaminergic system. An important next step is determining the extent to which this probe can detect developmental differences in self-control over reward responding. Toward this aim, the present study examined the ability to modulate activation in the NAcc through rtfMRI-nf in a sample of adolescents and adults.

Methods: Participants were 119 adolescents aged 14 to 16 years old ($n = 52$; 55.8% female; M age = 15.13, SD = .79) and adults aged 25 to 27 years old ($n = 67$; 52.2% female; M age = 25.9, SD = .84) from the NeuroMod Study, a community-recruited study funded by the National Institute on Alcohol Abuse and Alcoholism. During a single study visit, participants completed a rtfMRI-nf task and non-imaging assessments measuring self-regulation, including a 36-item self-reported self-regulation inventory (SRI). Prior to the scan, participants were instructed to up-regulate NAcc activity by thinking of exciting, rewarding stimuli and down-regulate NAcc activity by thinking of boring, dull stimuli. The rtfMRI-nf task was comprised of four runs (run 1: feedback off, run 2: feedback on, run 3: feedback on, run 4: feedback off), with each run consisting of 12 pseudorandomly ordered increase and decrease trials. NAcc activity was collected across run types. Feedback off runs showed a static thermometer image, while feedback on runs showed a thermometer image corresponding to the participant's NAcc activity. The thermometer began at 0% signal change with a range of ±1% signal change. To create measures of peak NAcc activity, trial-wise percent signal change estimates from the first two 2-second volumes immediately following the initial 4-second hemodynamic lag were averaged.

Results: Results from a general linear model showed significant main effects for cued instruction (i.e., increase versus decrease; $F = 45.10$, $p < .001$), run number ($F = 8.06$, $p < .001$), and age group ($F = 7.48$, $p < .01$) on NAcc activity. There was a significant interaction between cued instruction and age group ($F = 7.73$, $p < .01$). Estimated marginal means showed a significant difference in peak signal change in the NAcc for increase ($M = .31$, $SD = .22$)) compared to decrease ($M = .18$, $SD = .18$, $p < 0.001$) trials across both age groups. In both age groups, feedback significantly improved NAcc up-regulation (feedback runs: $M = .33$, $SD = .27$; no feedback runs: $M = .28$, $SD = .24$, $p < .05$) but not NAcc down-regulation. Compared to adolescents, adults had significantly lower peak NAcc activity during decrease trials (adolescents: $M = .23$, $SD = .19$; adults: $M = .14$, $SD = .14$, $p < 01$) but not during increase trials (adolescents: $M = .31$, $SD = .24$; adults: $M = .31$, $SD = .21$, $p = .97$). In terms of self-report data, adults had greater mean scores on the SRI compared to adolescents, indicating higher levels of self-regulation (adolescents: $M = 128.78$, $SD = 15.66$; adults: $M = 135.25$, $SD = 13.24$). In logistic regression analyses, lower peak NAcc activity during decrease trials across runs significantly predicted being in the adult versus adolescent group (OR = 0.02, CI = .00, .28, $p < .01$) over and above sum scores on the SRI (OR = 1.05, CI = 1.02, 1.09, $p < .01$).

Conclusions: Both adolescents and young adults were able to regulate NAcc activity through a rtfMRI-nf task, with feedback particularly aiding in the ability to increase NAcc activity. In line with imbalance models of adolescent brain development, adults were better able to modulate NAcc activity. This difference was especially pronounced during decrease trials, suggesting that adolescents may be less efficient at dampening brain function associated with reward responsivity compared to adults. A potential advantage of rtfMRI-nf over commonly used measures of self-control is its ability to tap more directly into the regulation of reward function by focusing on activation in specific reward-related structures, such as in the NAcc. Examining neural correlates of reward-related self-regulation through rtfMRI-nf has important translational applications. For example, boosting self-regulation in adolescents has been cited as an effective component of substance use prevention and intervention programs. In sum, findings from the present study support rtfMRI-nf as a tool for quantifying individuals' capacity for volitional control over reward responding, allowing us to test hypotheses about the relevance of developmental processes in reward responding more directly.

Keywords: Adolescence, Real-Time fMRI Neurofeedback, Reward Functioning

Disclosure: Nothing to disclose.

P136. Comparing Irritability Across Childhood Psychiatric Disorders Using a Large-Scale Longitudinal Dataset

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Background: Irritability is defined as an increased tendency to anger relative to peers at the same developmental level. Clinically, it manifests as temper outbursts and a sullen, grouchy mood. Irritability is a transdiagnostic construct observed across multiple

psychiatric disorders and one of the most common reasons for child's psychiatric evaluation and care. Utilizing the Adolescent Brain Cognitive Development (ABCD) study database (which tracks over 11,000 children aged 9-10 to early adulthood), we aim to elucidate further the links between irritability and different psychiatric disorders and the association between demographic parameters to irritability.

Methods: An irritability composite score was calculated using a standardized sum of five parent CBCL items and three ODD symptom items from the KSADS-COMP. Irritability scores for those with psychiatric diagnoses were compared to those without the given psychiatric diagnoses using analysis of covariance controlling for age and sex across two-time points (baseline, age 9-10, and 2-year follow-up, age 11-12). All p values were Bonferroni corrected. We used Cohen's D effect size to estimate clinical significance of our findings.

Results: Consistently increased irritability was observed across all evaluated psychiatric disorders at baseline and 2-year follow-up. Children with any mental disorder displayed higher irritability compared to children without mental disorders, with a significant difference (Bassline irritability 2.44 ± 2.08 vs. 0.71 ± 0.98; follow-up irritability 2.22 ± 2.07 vs. 0.68 ± 0.98; p's < 0.0001, corrected) and a large effect size ($d > 1.38$) at both time points. As expected, externalizing disorders, including oppositional defiance disorder (ODD), conduct disorder, and attention deficit hyperactivity disorder (ADHD), exhibited the highest levels of irritability. Surprisingly, internalizing disorders, such as separation anxiety disorder and generalized anxiety disorder, also demonstrated the highest levels of irritability (p-values < 0.0001), with effect sizes falling within the large range ($d > 1.34$) for these specific diagnoses. Notably, while certain disorders, namely non-suicidal self-injury (NSSI) and suicidal ideation, were characterized by the lowest levels of irritability (p-values < 0.0001), their effect sizes ranged from medium to large, depending on the time point. Finally, the subpopulation of irritable children showed significant enrichment for males, individuals of white race, and low income.

Conclusions: Our study reveals that increased irritability extends across various childhood psychiatric disorders, challenging its conventional association mainly with externalizing conditions. Additionally, we identified significant correlations between irritability and demographic parameters. These findings underscore the broad association of irritability with childhood mental health.

Keywords: ABCD Study, Pediatric Irritability, Transdiagnostic, Child and Adolescent Psychiatry

Disclosure: Nothing to disclose.

P137. Concordant Structural Brain Signatures of Immune-Related Blood Biomarkers and Subclinical Psychiatric Symptoms in Youth

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Background: Youth seeking treatment for mental health concerns are at increased risk of developing severe mental illness later in life. In search of early risk biomarkers, tests of peripheral blood offer a cheap and minimally-invasive option. Studies in individuals with schizophrenia and bipolar disorder have found differences in stress- and immune-related peripheral proteins, though it is not known if or how these differences relate to early risk. Here we integrate peripheral blood measures, MRI, and behavioral assessments from two youth cohorts - one treatment-seeking for psychiatric concerns, and the other a non-clinical population sample - to test if patterns of brain structure associated with levels of peripheral biomarkers mirror those found in younger children with emergent mental health symptoms.

Methods: We first analyzed 73 participants from the Toronto Adolescent and Youth Cohort Study (TAY), an ongoing longitudinal clinical cohort study of psychiatric treatment-seeking youth aged 11-24. Participants had 26 proteins quantified in peripheral plasma and underwent MRI at baseline. Linear models tested for cross-sectional associations between each protein with cortical thickness (CT) across 68 regions, covarying for age, sex, ethnicity, and intracranial volume. We also tested associations of proteins with psychosis spectrum symptoms (PSS). Independently, we analyzed data for 9,851 youth aged 9-10 from the Adolescent Brain and Cognitive Development (ABCD) general population-based study who had baseline MRI and Child Behavior Checklist (CBCL) data. Only participants with clinically insignificant CBCL scores were included. We modeled associations between 11 CBCL syndrome scales and CT across the same regions tested in TAY, including the same covariates. Association t-statistics for biomarker effects on CT (TAY) and CBCL effects on CT (ABCD) were then correlated, considering brain regions as observations, for each pair of biomarkers and CBCL scales. P-values were corrected for false discovery rate (pFDR).

Results: In TAY, blood-to-brain associations were strongest between peripheral Resistin, growth differentiation factor 15, interleukin-6 (IL-6) and CT of inferior frontal, inferior temporal, and superior frontal regions, respectively. IL-6, CX3CL1, CCL2, BDNF, and Adiponectin were also nominally associated with greater probability of PSS. In ABCD, several CBCL scales were associated with CT; e.g. social problems with thinner left temporal pole ($pFDR = 1.8x10\text{-}3$) and aggressive behaviors with left midfrontal gyrus ($pFDR = 1.7x10\text{-}3$). Correlating effects of biomarkers (TAY) and CBCL scales (ABCD) between datasets revealed the most significant concordance between neural signatures of peripheral adipsin (Component Factor D) and attention and thought domains of the CBCL. The strongest concordance was observed for thought problems (adipsin correlation $r = 0.56$, $pFDR = 1.6x10\text{-}4$). Selecting one region as an illustrative example; lower adipsin was associated with thinner left precentral gyrus in TAY, which in turn was significantly associated with fewer attention problems in ABCD.

Conclusions: We found associations between several peripheral stress- and immune-related markers and both brain structure and PSS in treatment-seeking youth. Importantly, we also show that the neural signatures of these markers are highly concordant with those associated with subclinical mental health symptoms in younger children in the general population. These findings 1) demonstrate potential for monitoring risk of psychopathology in youth via peripheral markers, 2) propose a map for evaluating the neurostructural basis of this risk, and 3) identify brain regions covarying with peripheral markers that may be useful for optimizing non-invasive interventions. Validation with repeated measures in a larger clinical sample is needed.

Keywords: Psychosis Spectrum Symptoms, Peripheral Blood Marker, Structural MRI

Disclosure: Nothing to disclose.

P138. Neonatal Neural Organization and the Development of Internalizing Problems in Children Born Very Premature

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Background: Premature births in the United States (10.5% of live births) have been increasing in the past few years, representing a significant public health concern (Centers for Disease Control and Prevention [CDC], 2022). Infants born very preterm (VPT; < 30 weeks gestation) are 2-4 times more likely than Term Control (TC) children to report psychiatric problems as adolescents (Rogers et al., 2012). VPT infants also show high rates of internalizing problems during early childhood, which may capture early risk for later emerging psychiatric disorders. Thus, mapping trajectories of internalizing symptoms during childhood may help us improve classification of low- and high-risk children within VPT populations, and chart sensitive developmental windows when clinical interventions may be most effective for high-risk children. Previous work from our lab also indicates that roughly 25% of VPT children show typical socioemotional outcomes through age 5 (Lean et al., 2020), suggesting substantial heterogeneity in VPT children's internalizing symptoms and potential resilience mechanisms that may buffer risk for early psychiatric disorders. Current evidence indicates that high rates of childhood internalizing symptoms may be explained by disruptions in resting-state functional connectivity (Rogers et al., 2017). However, prior studies have only focused on age-specific developmental outcomes and isolated brain regions, rather than investigating how global brain organization may predict change over time in VPT internalizing trajectories. In the present study, we model developmental trajectories of internalizing symptoms between age 2 and 10 and use graph theory and network analysis to examine neonatal whole-brain topology.

Methods: Participants were members of a large prospective cohort of 124 VPT (born at < 30 weeks gestation) and 103 TC children matched for age and sex and followed from birth to age 9-10). Child internalizing symptoms were measured via parent report on the Infant-Toddler Social and Emotional Assessment (ITSEA) at age 2 and the Child Behavioral Checklist (CBCL) at ages 5 and 10. Resting state functional (rsfMRI) data were collected at term (or equivalent in VPT infants). We used functional connectivity matrices (based on correlations between all brain ROIs) to generate graph objects for each individual in the sample using the iGraph package in R. We then extracted topological metrics of interest. These metrics included the clustering coefficient (an index of network segregation), modularity index (a measure of the network's regional organization), and average path length (an index of network integration).

Results: A multi-group linear growth model indicated that both TC and VPT groups showed moderate levels of internalizing symptoms at age two, evident by significant mean intercepts (ITC = 47.39, p = .009; IVPT = 41.88, p = .001), and their symptoms remained stable over time through age 10, as evident by non-significant mean slopes (STC = -1.363, p = .581; SVPT = .716, p = .442). TC and VPT groups differed in the variance of growth parameters, such that the slope variance was significant for the VPT group ($\sigma^2 = 1.952$, p = .050) but not for the TC group. Graph analysis indicated that VPT and TC infants differed in network size and topological metrics. Specifically, VPT infants had on average, 238 more nodes than their counterparts (p = .004). Additionally, VPT infants exhibited a lower cluster coefficient (β = -0.01, p = .006), modularity index (β = -0.02, p = .001), and path length (β = -0.01, p = .008) compared to TC children.

Conclusions: Preliminary analyses suggest that TC and VPT children do not differ in their average trajectories of internalizing symptoms. However, while variance in trajectories of TC children was fully explained by general age changes, VPT children exhibited substantial heterogeneity in their development of internalizing symptoms. Finally, differences in network topology based on resting state functional connectivity suggest that compared to TC infants, VPT infants exhibit more segregated networks (lower clustering and modularity), which may hinder efficiency of information processing. However, they also exhibit higher network integration (shorter path length). Graph theory indicates that shorter path length facilitates information flow between network communities or hubs. Thus, it is possible that a shorter path length in VPT networks may compensate for potential inefficiencies associated with higher network segregation. Analyses are ongoing. We plan to model internalizing trajectories as a function of these topological metrics and expect that higher segregation will predict at-risk internalizing trajectories in VPT children.

Keywords: Prematurity, Internalizing Disorders, Graph Theory

Disclosure: Nothing to disclose.

P139. The Relations Between Chronotype, Academic Performance, and Social Jetlag in the Adolescent Brain Cognitive Development (ABCD) Study

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Background: Academic performance plays a crucial role in long-term educational attainment and job eligibility. Chronotype refers to an individual's daily preferences in times for waking, activity, and sleep, and is influenced by an individual's circadian rhythm. Social jetlag is the mismatch between an individual's chronotype and their social schedule, causing stress across domains of physical, behavioral, and mental health. Perhaps for this reason, late chronotype is associated with substance use and depression and psychosis. Previous studies have explored the relationship between academic performance, chronotype, and social jetlag in different student populations. Because school often start early in the morning, later chronotype is often associated with daytime sleepiness, insufficient sleep, and poor academic performance. However, these relationships have not been extensively examined in large, diverse samples like the Adolescent Brain Cognitive Development (ABCD) study. We hypothesized that in the ABCD cohort, that previously validated predictors of academic performance, including cognitive function, substance abuse and symptoms of mental illness would predict academic performance, and that later chronotypes and social jetlag would further explain some of the variance.

Methods: Year 2 (ages 11-14) cross-sectional data from the ABCD cohort (n = 903 adolescents) was used to evaluate academic performance, NIH Toolbox cognitive performance measures, chronotype, social jetlag, psychosis symptoms, and toxicology results. Self-reported past year grades served as the outcome variable, providing a measure of academic performance. The Munich Chronotype Questionnaire was used to assess chronotype, capturing individuals' preference for wake times, sleep times, and times of activity. The Prodromal Questionnaire Brief Version was utilized to measure psychosis symptoms. Additionally, toxicology data collection provided information on substance use. Hierarchical regression models were employed to examine the association between the NIH Toolbox cognitive performance measures (e.g., NIH Oral Reading Recognition Test of the Language Construct) and academic performance, while controlling for relevant covariates (age, household income, sex, ethnicity). Pearson's correlation coefficients evaluated links between these variables.

Results: Using cross-sectional data from Year 2, we found significant positive associations between poorer academic performance, later chronotype, and increased social jetlag. We found cognitive performance measures such as higher scores for verbal language recognition were linked to higher academic

performance. Psychosis was correlated with later chronotype and increased social jetlag, and associated with poorer academic performance and NIH Toolbox cognitive performance measures. Toxicology results did not correlate with any measure, possibly because our sample primarily consisted of younger students who may engage in substance use less frequently than older age groups.

Conclusions: Our study sheds light on the complex interplay between academic performance, NIH Toolbox cognitive measures, chronotype, social jetlag, and behavioral and mental health outcomes in a sample of 9-10 year old subjects. Our findings emphasize the importance of individual differences in chronotype when designing class schedules, as aligning school activities with student optimal wake times may contribute to improved academic performance, particularly for those with later chronotypes. Understanding of optimal sleep hygiene practices tailored to different chronotypes could further enhance school performance.

Keywords: Chronotype, Academic Performance, Adolescence, ABCD Study, Social Jet Lag

Disclosure: Nothing to disclose.

P140. Neural Markers of Social Responsiveness in the Infant's Developing Brain

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Background: An estimated 1 in 6 American children show social deficits in the first year of life that develop into mental health or neurodevelopmental disorders between ages 2 to 8 years. In addition, half of American children live with at least one risk factor (e.g., neglect, domestic violence, parental psychiatric disorder) known to undermine social development. The fundamental architecture of the social brain is developed in infancy, and early deficits in social development are difficult to compensate for later in life. However, no techniques currently exist that can assess early differences and deficits in the infant's social brain. This has limited our fundamental knowledge of the developing social brain in human infants and has delayed the development of new diagnostics and therapeutics targeting these crucial earliest years. The goal of the present study was to demonstrate the feasibility of using a novel fMRI paradigm to measure infants' developing social responsiveness to the first social partner—the mother—at 6 months of age.

Methods: Our novel fMRI paradigm uses an established MRI protocol to scan infants during natural sleep and relies on the infant's fully developed auditory responsiveness which is present by 6 months and well preserved under sleep. Twenty-four (15 males) typically developing 6-month-old (± 1.0 month) infants underwent scanning during natural sleep, listening to maternal voice, control female voice, and speech-shaped noise (15-sec-long blocks; 7.5-sec inter-block intervals). We measured maternal cue responsiveness, defined as the infant's fMRI response to maternal voice, compared to control voice and speech-shaped noise. Control voices were identified to be distinct from maternal voice on 512 features extracted by the Pyannote machine learning model. Speech-shaped noise consisted of white noise that was edited to match maternal voice on frequency and loudness. FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) version 6.00, part of FSL. A total of 54 runs from 18 infants (11 males) passed quality assurance, showing distinct auditory activation and no excessive movement, and were included in the analysis. Voxel-wise whole-brain analyses examined the infant's fMRI response to: (a) human (maternal and control) voice compared to speech-shaped noise (Human Voice > Noise contrast), and (b) maternal voice compared to control voice (Maternal Voice > Control Voice contrast), adjusting for infant sex, infant age (in weeks), and maternal age. Z-statistic images were thresholded using clusters determined by $Z > 3.1$ and a corrected cluster threshold of $p = .05$. Exploratory correlational analysis examined Pearson's r between the infant's fMRI responses to Maternal Voice > Control Voice contrast and concurrently administered behavioral measures of maternal anxiety (State-Trait Anxiety Inventory), maternal stress (Perceived Stress Scale), maternal depression (Edinburgh Postnatal Depression Scale), and infant negative affectivity (Infant Behavior Questionnaire-Revised, Very Short Form).

Results: Compared to speech-shaped noise, human voice elicited increased activations in multiple cortical regions (all corrected $p < .05$) of the infant's social brain, including the superior temporal gyrus ($Z = 12.75$), anterior cingulate gyrus ($Z = 3.20$), and temporoparietal junction ($Z = 8.17$). Compared to control voice, maternal voice elicited increased activations in all aforementioned cortical regions, and additionally in key dopamine- and oxytocin-rich subcortical regions (all corrected $p < .05$), including the striatum ($Z = 4.40$), amygdala ($Z = 3.79$), and ventral diencephalon (encompassing the hypothalamus, ventral tegmental area/substantia nigra; $Z = 3.58$). Compared to maternal voice, control voice did not elicit any additional activations in the infant's brain. Exploratory correlational analyses suggested that maternal anxiety, maternal stress, maternal depression, and infant negative affectivity were negatively correlated with the infant's preferential brain responses to maternal voice in several important social brain regions, including the striatum, amygdala, ventral diencephalon, and anterior cingulate gyrus (ps < .05).

Conclusions: Six-month-old infants show preferential brain responses to human voice (compared to speech-shaped noise) and voice of their first social partner (compared to control voice). Our findings provide support for the feasibility of using fMRI to measure the developing brain's responsiveness to socially salient cues at 6 months of age. Our findings also provide preliminary evidence that the infant's preferential response to socially salient cues is negatively associated with maternal anxiety, stress, and depression, and infant's negative affectivity. When extended to at-risk infants, this work has the potential to yield breakthroughs in identifying novel neural markers that can detect early differences and deficits in an infant's developing social brain.

Keywords: Infant Neuroimaging, Early Brain Development, Social Responsiveness, Maternal Mental Health

Disclosure: Nothing to disclose.

P141. Screen Media Use Associations With Response Inhibition and Reward Processing in Young Children: Results From the ABCD Study

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Background: Screen media use among children has increased dramatically over the past several decades, with fewer than 1 in 3 children now meeting recommended screen time limits (≤ 2 hours/day). Rapid development of mobile technology has also diversified the types of screen media available. Accordingly, there is widespread interest in understanding the psychological

consequences of screen media use. Studies to date have yielded highly variable findings. Examining behavioral and neurobiological mechanisms that could subserve any potential consequences may explain variability in findings. Greater clarity could enable scientists, clinicians and policymakers to develop more effective recommendations, policies, and interventions to mitigate any negative effects of screen media use. Two potential mechanisms driving adverse consequences of screen media use are: (1) Deficits in response inhibition; and (2) Alterations in reward processing. This project examined associations of self-reported screen media use with these constructs among 9-10 year old children.

Methods: Participants were drawn from the baseline visit of the ABCD Study, which utilized a school-based recruitment approach to obtain a diverse sample that closely mirrors demographics of the broader US population. The present analyses only include participants who provided complete data for analyzed variables that passed quality assurance checks, yielding modestly different sample sizes across topics (Response Inhibition N = 7,461; Reward Processing N = 8,120). Screen media use was assessed through youth self-report interviews. Typical daily use was assessed separately for weekdays and weekends across 6 specific types of screen media watching television, watching videos, playing video games, texting, using social media, video chatting. Response inhibition outcomes included three Stop Signal Task (SST) performance indices (Go Response Time; Stop Trial Correct Rate, Mean Stop Signal Reaction Time) and neural activation for the Stop-Go contrast across four regions of interest (Left and Right Short Insular Gyrus, Right Triangular and Opercular Inferior Frontal Gyrus). Reward processing outcomes included two Monetary Incentive Delay (MID) Task performance indices (Difference in Response Time for Large versus Small Gains and Losses) and neural activation for four different MID contrasts (Anticipation: Gain versus Neutral and Loss versus Neutral; Feedback: Gain Positive versus Negative and Loss Positive versus Negative) across 6 regions of interest (Left and Right Nucleus Accumbens, Caudate and Putamen). Mixed effect models examined the effects of screen time on each outcome. These models included random effects for family and for site or scanner (performance or neural indices), and were adjusted for age, race and ethnicity, parental education, poverty status and handedness.

Results: Participants reported an average of 24.7 hours (SD = 19.8) of weekly screen media use. As expected, typical weekend use (M = 4.3 hours/day, SD = 3.4) exceeded typical weekday use (M = 3.2, SD = 2.8; p < .001). The overwhelming majority of weekly screen media use was accounted for by watching television (~8.4 hours/week), playing video games (~6.6 hours), and watching videos (~6.3 hours). Larger amounts of screen media use were associated with a faster go trial response time (p = .023) and a lower stop trial correct rate (p = .004) on the SST. Effects were driven primarily by watching videos and playing video games and were similar for weekday and weekend screen media use. No neural effects for Stop > Go contrast were observed. Screen media use was not associated with performance task, but higher screen media was associated with reduced neural activation in dorsal striatal regions to anticipatory losses (L Putamen p = .063; R Putamen p = .018; L Caudate p = .017; R Caudate p = .025) and gains (L Putamen p = .002; R Putamen p = .005; L Caudate p = .003; R Caudate p = .005). In contrast, higher screen media use was associated with increased neural activation to gain feedback in the ventral striatum (L Accumbens p = .024) and increased neural activation to loss avoidance across all striatal regions (L Putamen p = .005; R Putamen p = .031; L Caudate p = .002; R Caudate p = .010; L Accumbens p = .004; R Accumbens p < .001). Similar to the SST, effects were not divergent for weekday and weekend use. However, effects were driven by a wider array of screen media types (television and social media use, in addition to watching videos and playing video games).

Conclusions: Screen media use was relatively high overall, with substantial variance. Screen media use associations with performance indices on the SST were generally consistent with deficits in response inhibition (i.e., faster responses, but increased errors), but no significant effects on Stop Signal Reaction Time or neural activations were observed. Effects on reward processing suggest greater screen media use is associated with reduced neural activation to potential future gains and losses (i.e., anticipation), but heightened activation to actual gains and losses (i.e., feedback), particularly the avoidance of negative outcomes. Causal direction cannot be inferred; results could reflect either the effects of screen media use or pre-existing risk factors for greater use. Effect sizes were generally modest, but if caused by screen media, could escalate with continued exposure through adolescence and adulthood.

Keywords: ABCD, Response Inhibition, Reward Processing

Disclosure: Nothing to disclose.

P142. Impact of Unpredictable Maternal Sensory Signals During Early Development on Adolescent Functional Connectivity of the Paraventricular Nucleus of the Thalamus

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Background: Emotional circuit maturation is shaped by sensory signals in the environment during early life. For example, unpredictable parental and environmental sensory signals (high entropy) during early life result in increased hippocampal synaptic pruning and enduring changes in emotional circuitry in rodents. In humans, exposure to such high entropy during infancy is associated with deficits in memory and executive control during childhood and adolescence. Maturation of emotional circuitry is dependent on the integration of numerous processes in several circuits, all involving the paraventricular nucleus of the thalamus (PVT). Indeed, there is growing evidence that the PVT contributes to storing memories of salient experiences over long durations, such as memories of early life adversity. However, PVT connectivity in humans has not yet been evaluated prior to adulthood, and the impact of early life entropy on the development of PVT connectivity to key nodes of emotional circuitry during childhood and adolescence is largely unknown.

Methods: Maternal sensory signals during mother-child interaction were video-recorded and coded at 6 and 12 months age. The predictability of these signals during the interaction was characterized by calculating state transition probabilities (likelihood the mother would change from one sensory signal to another), computing the entropy associated with the transition probabilities, and averaging between the two sessions (as previously described). Two fMRI imaging sessions were conducted during childhood and early adolescence (n = 37, 16 females, 21 males, 9-13.7 years). Six bilateral regions of interest were selected a priori based on the PVT circuitry in rodent literature, including: the anterior cingulate cortex (ACC), amygdala, bed nucleus of the stria terminalis (BNST), hippocampus, locus coeruleus, lateral hypothalamus (LH), and nucleus accumbens (NAc). Measures of functional connectivity between these regions and the PVT were considered in six independent mixed effects models testing for a main effect of entropy adjusted for age at scan and sex.

Results: There was a negative association of entropy with the functional connectivity between the PVT and bilateral LH (T-stat = -2.71, p-uncorrected = 0.0089) and BNST (T-stat = -2.22, p-uncorrected = 0.0308). Functional connectivity between the PVT

and NAc (T-stat = 2.65, p-uncorrected = 0.0105) and between the PVT and ACC (T-stat = 2.62, p-uncorrected = 0.0114) was higher in females compared to males of the same age.

Conclusions: Unpredictable maternal signals during infancy, reflected here as high entropy, may contribute to the development of PVT functional connectivity to the LH and BNST. In rodents, the lateral hypothalamus to PVT projection plays a role in arousal and reward learning, while the BNST to PVT connections may contribute to anxiety behaviors. The sex effects observed here may be due to sex-differences in pubertal timing during adolescence. Future work will employ large, publicly available datasets to further characterize the development of PVT circuitry and its putative role in mediating the effects of diverse early-life factors in the development of mental illness.

Keywords: Paraventricular Nucleus of the Thalamus, Entropy, Unpredictability, Resting State Functional Connectivity, Early-Life Experience

Disclosure: Nothing to disclose.

P143. Reliability and Age-Related Effects on Neural Activation of a Novel Threat Neuroimaging Paradigm

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Background: Leveraging functional magnetic resonance imaging (fMRI), developmental psychiatry has made great strides identifying circuits associated with psychopathology. In these studies, normative brain development is used as a benchmark to assess risk for and severity of psychopathology. However, few studies have examined test-retest reliability of fMRI metrics, which is critical to improve the reproducibility of fMRI findings. In the present study, we adapted the Human Intruder Paradigm (HIP), an extensively validated behavioral task that assesses defensive responses in nonhuman primates (NHP), to a functional neuroimaging paradigm to administer to youth and adults. The NHP HIP consists of three ethologically relevant conditions: 1) the 'alone' (AL) condition, in which the NHP is separated from conspecific(s) and placed in a test environment by itself, inducing separation-related responses, such as increases in cooing and locomotion; 2) the 'no-eye-contact' (NEC) condition, in which an experimenter unfamiliar to the NHP presents their profile to the NHP while avoiding direct eye contact, representing an indirect, potential threat that elicits threat-induced behaviors, such as increased freezing and reduced cooing; and 3) the 'stare' (ST) condition, in which a human intruder stares directly at the NHP making eye contact, representing a direct, proximal threat that elicits hostile behaviors towards the human intruder. We adapted the HIP behavioral NHP task to administer via fMRI. We have two aims: (1) to examine developmental changes in the neural circuitry mediating threat processing; (2) to probe test-retest reliability in a sample of healthy adults who completed the task twice.

Methods: In two ongoing studies, we developed and administered a novel fMRI version of the HIP with three conditions: 1) direct condition (i.e., neutral human face, frontal view); akin to the ST condition of the HIP; 2) indirect condition (i.e., neutral human face, profile view presented); akin to the NEC condition of HIP; 3) control condition (i.e., pixels of each face image scrambled to create a control condition matched on visual properties but with face properties removed); akin to the AL condition of HIP.

In study one, n = 48 participants [21 healthy youth (Mage = 14.37, 8-17 years, 13F) and n = 27 healthy adults (Mage = 27.27, 21-49 years, 12F)] were scanned to evaluate the effects of age on patterns of neural activation associated threat processing. For study two, n = 22 healthy adults completed the task twice (Mtime in between scans = 27.05 days) to examine reliability of neural activation associated with threat processing over time.

Whole brain analyses in AFNI examined neural activation by condition to examine task effects. Voxel-wise analyses were whole brain corrected at p50. In study one, an analysis of covariance (ANCOVA) model was run in AFNI using 3dMVM. Participant age and sex were entered as between-subjects variables and condition was specified as the within-subject variable. In study two, replication of methods from Haller et al., 2018 used a Bayesian adaptation of the intraclass correlation (ICC) to assess reliability of task-evoked BOLD activation for the faces relative to the control contrast. ICC threshold was set to 0.50, which corresponds to moderate reliability (Shrout and Fleiss, 1979).

Results: Across healthy adults and youth (total n = 48) in study one, a significant increase in BOLD signal change for the faces (direct + profile) relative to control (scramble) condition was observed in the bilateral fusiform gyri and occipital gyri, p50. Comparing face conditions, greater activation in the bilateral occipital gyri and fusiform was observed for the profile relative to the direct condition. Additionally, a significant increase in BOLD signal change for the faces (direct + profile) relative to control (scramble) condition was observed in the right amygdala, p50; amygdala activation did not differ significantly between direct vs. profile conditions. No significant interaction effects of participant age or sex were observed in any analysis.

In study two, n = 22 adults from study one completed the task twice, on two separate dates approximately one to two months apart. Stable patterns of BOLD signal were observed across multiple brain regions, including the bilateral occipital gyri, fusiform gyri, and middle cingulate cortex with moderate (ICC between 0.5 and 0.75) to excellent (ICC > 0.9) reliability estimates. Similarly, for the direct versus profile condition, stable patterns of BOLD signal were observed across multiple brain regions, including occipital gyri.

Conclusions: Preliminary evidence from two ongoing studies suggests that the fMRI version of the HIP evokes anticipated visual and threat-related activation to stimuli across adults and youth. Preliminary data suggest stability across two time points in adults. Future analyses with larger samples will: (1) utilize network-based approaches to examine test-retest reliability of functional connectivity; (2) explore functional connectivity as a function of age, and (3) explore patterns of activation and connectivity associated with psychopathology and clinical diagnoses.

Keywords: fMRI, Test-Retest Reliability, Threat Reactivity, Children and Adolescents

Disclosure: Nothing to disclose.

P144. Early Life Adversity Induces Behavioral and Peripheral Biomarker Alterations During Early Development in Mice

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Background: Early life adversity (ELA) such as violence, neglect, and lack of resources increases the likelihood of developing myriad health problems, including mental illness, in later life. There is currently a lack viable predictors of later-life mental illness onset after ELA. Social behavioral deficits often arise during adolescence and have been known to correlate with psychiatric disease progression such as depression and anxiety.

Understanding the developmental trajectory of ELA effects on social behavioral deficits can lead to earlier interventions to mitigate or prevent the symptoms of these conditions. Here, we sought to determine the effects of ELA on development of social behavior and peripheral biomarkers in mice.

Methods: Male and female mice (C57BL/6J; n's = 13-15 mice/group) were subjected to limited bedding and nesting (LBN), a well-characterized procedure used in ELA studies, from postnatal days 2-9 (P2-9). Pup ultrasonic vocalizations (USVs) were recorded at P3, P6, and P9. At P30, mice were then assessed on social interaction (SI). Blood, adrenal glands, and thymus were collected to determine changes in stress and inflammatory endpoints, as well as the production of T-cell receptor excision circles (TRECs), a translationally relevant blood-borne biomarker that can be used as a proxy for thymic function as well as an index of previous stress exposure in both mice and humans. Brains were also flash-frozen and punches of the prefrontal cortex (PFC), nucleus accumbens (NAcc), medial preoptic area (mPOA), and ventral hippocampus (vHPC) were collected to conduct bulk RNA sequencing and assess alterations in inflammatory gene expression. Data were analyzed using analysis of variance (ANOVAs) followed by post-hoc t-tests.

Results: Consistent with previous reports, LBN resulted in fragmented dam behavior as compared to control rearing conditions during P2-P9. Pups did not demonstrate altered USV frequency, number, or call length on P3 or P6, but LBN-exposed pups exhibited significantly increased number of calls and average call length on P9 ($p = 0.0120$ and $p = 0.0002$, respectively). Preliminary data reveal that at P30, male but not female LBN-exposed mice trended towards a lower SI ratio ($p = 0.07$), suggesting social avoidance. While adrenal weights did not differ across groups, thymus weights of P30 LBN male and female mice were significantly increased compared to control mice, and females had higher thymic weights as compared to males (ANOVA; Treatment x Sex, $p = 0.6375$; Treatment, $p = 0.0010$; Sex, $p = 0.0032$). However, despite changes in thymic size, there were no differences in TREC production in either sex (ANOVA; Treatment x Sex $p = 0.8481$; Treatment $p = 0.5409$; Sex $p = 0.2497$).

Conclusions: Our data demonstrate that: 1) LBN induces social deficits that emerge as early as P9; 2) these behavioral alterations persist to P30 only in males; and 3) the thymi of LBN-exposed mice are enlarged during adolescence without altering production of TRECs. Overall, these results reveal ELA-induced behavioral and peripheral biomarkers that are evident during adolescence and provide the basis for future studies that more thoroughly examine these endpoints as translationally relevant predictors of later psychiatric disease onset and/or targets for novel therapeutics.

Keywords: Early Life Adversity, Social Deficits, Ultrasonic Vocalizations, Thymus, Peripheral Biomarkers

Disclosure: Nothing to disclose.

P145. Synergistic Effects of Prenatal Cannabis and Childhood Trauma Exposure on Functional Connectivity of Large-Scale Neurocognitive Networks in Youth: A Double Hit to the Developing Endocannabinoid System?

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Background: Recent years have witnessed an uptick in the use of cannabis during pregnancy as well as a rapid rise in cannabis potency. Together, these trends have amplified concerns regarding the potential harms to brain development for individuals exposed in utero. Indeed, prenatal cannabis exposure (PCE) may disrupt the endocannabinoid system, which plays a key role in shaping pre- and post-natal brain development, including the fine-tuning of neuronal networks. Childhood trauma exposure (CTE) is thought to further disturb endocannabinoid signaling, and has been shown to contribute to differences in brain structure and function in youth and increased risk of psychiatric disorders. Here, we examine the interactive effects of PCE and CTE on organization of large-scale functional brain networks in youth.

Methods: This study included neuroimaging and developmental history data collected from 9,862 children ($M \pm SD = 10.93 \pm 0.64$ years, 48% female; 66% White) from the Adolescent Brain Cognitive Development (ABCD®) study. We focused on resting-state functional connectivity of the salience network (SN), in particular, which plays a key role in supporting higher-order cognitive and emotion-related processing. The SN is shown to be sensitive to developmental insults, including PCE and CTE, in separate studies. We also examined connectivity of the SN with two other large-scale neurocognitive networks, the default mode network (DMN) and the frontoparietal network (FPN), as interactions between these networks and the SN change dynamically across development and may be sensitive to early exposures. Linear mixed-effect models examined effects of the PCE x CTE interaction term in predicting resting-state connectivity, while adjusting for site, family, and other covariates (e.g., socioeconomic status, prenatal exposure to other substances).

Results: Thirty-five percent of youth in the sample reported CTE and 3.8% of caregivers reported using cannabis during pregnancy. We found a significant interaction between PCE and CTE in predicting SN-FPN resting-state functional connectivity in youth ($\beta = -0.03$, CI = -0.06 to -0.001, $p = 0.044$), such that PCE was associated with lower connectivity only within those also with CTE. A sex x PCE x CTE interaction was also significant, such that lower SN-FPN connectivity was observed for females (but not males) with both PCE and CTE ($p < 0.05$). PCE exposure was not associated with SN-FPN connectivity in those without CTE. There were no interactive effects of PCE and CTE on SN-SN or SN-DMN connectivity.

Conclusions: These results suggest that PCE may contribute to a later susceptibility to effects of CTE on functional organization of large-scale neural networks associated with higher-order cognitive and emotion-related functioning in children. Functional alterations of these networks—particularly during development—are linked to cognitive, behavioral, and psychiatric problems during childhood and later in life. More granular assessments of the potential sensitizing and/or synergistic effects of PCE and CTE on the endocannabinoid system is needed for a thorough understanding of their impact on the developing brain. Together, the present findings provide support for the so-called "double hit" hypothesis, and may provide insight in the link between potent early exposures and later psychiatric risk.

Keywords: Cannabis, Childhood Trauma, Prenatal Drug Exposure, Human Neuroimaging, Resting State Functional Connectivity

Disclosure: Nothing to disclose.

P146. Clarifying the Influence of Measures Included in the Area-level Deprivation Index in Relation to Hippocampal Volume: The Moderating Role of Positive School Environments

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Background: Adverse neighborhood characteristics, including the area-level deprivation index (ADI), have been shown to be inversely related to hippocampal volume (HV) in youth. The ADI is operationalized as the average z-score of 9 items: percentage of the adult population with at least a high school diploma, education, average household income, income disparity, percentage of home ownership, percentage unemployed, percentage of residents living below the poverty line, percentage of residents living below 138% below the poverty line, percentage of single-parent households, and percentage of residents who do not own a car. Items included in the ADI have been used to index neighborhood-level social fragmentation, which has been consistently associated with prognostic outcomes in early-onset psychosis spectrum disorders. Recent findings suggest that the inverse relations between ADI and regional brain morphometry may be better accounted for by differences in the social environment rather than the socioeconomic aspects of one's neighborhood (i.e., poverty), especially among youth with early-onset psychosis. Still, it is unclear which aspects of ADI are relevant for hippocampal development. It is unknown whether the school environment may buffer against the deleterious effects of ADI. This study sought to determine which aspects of the ADI are relevant to bilateral HV and whether aspects of the school environment may moderate relations between ADI and HV.

Methods: This study sample included 10,114 participants between the ages of 9 and 10 from the Adolescent Brain and Cognitive Development Study (52.4% male, 62.5% White non-Hispanic). Generalized linear mixed models tested associations between the 9 measures of ADI and right and left HV with family groups and sites as random intercepts. First, all 9 measures of ADI were entered into the model, and backward stepwise selection with a cut-off of $p < 0.05$ was used to select individual items of ADI that were significantly related to HV. Second, retained items from ADI were adjusted for 6 individual-level covariates including age, biological sex, ethnoracial minority status, parental education, household income, and estimated intracranial volume. Third, interaction terms between the ADI measures that were included in the optimized model for bilateral HV (i.e., were significant at the stepwise selection step) and school environment were added.

Results: For right HV, after backward stepwise selection, only 3 neighborhood-level characteristics remained inversely associated with right HV: lower income, unemployment, and single-parent household. After adjusting for 6 individual-level covariates, neighborhood-level single-parent households remained significantly inversely associated with right HV (adjusted B: -1.09; SE: 0.46; $p = 0.02$). None of the indices of ADI were significantly associated with left HV after adjusting for individual-level covariates. Neighborhood-level single-parent households significantly interacted with the school environment in predicting right HV (adjusted B: -0.24; SE: 0.08; $p < 0.01$). The slope of the association between neighborhood-level single-parent households and right HV became flatter with a more positive school environment.

Conclusions: This study sought to clarify the role of measures included in the ADI in relation to HV and found that a greater percentage of neighborhood-level single-parent households are associated with reduced right HV and that more positive school environments may buffer the impact of single-parent households on the right HV. Future research into the impact of neighborhood-level characteristics and school environments on trajectories of HV development would be necessary to better understand the complex interactions between various social environments on mental health among adolescents.

Keywords: Hippocampal Volume, Social Determinants of Health, Neighborhood Socioeconomic Deprivation, Children and Adolescents, School Environment

Disclosure: Nothing to disclose.

P147. The Gut Microbiome is Associated With Insula Structure in Neonates

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Background: The gut microbiome is a complex ecosystem associated with brain development across species. The insula, a brain region located within the lateral sulcus, is crucial for sensorimotor and socioemotional processing. Of interest, the anterior insula is involved in a neural network in charge of detecting salient signals from the body, including those from the gastrointestinal system. In infants, resting functional connectivity (rFC) between the anterior insula and the anterior cingulate is negatively associated with alpha diversity. Similarly, in adults, the microbiome has been associated with rFC between the insula and the frontal pole. Moreover, gastrointestinal disease and microbiome composition have been shown to influence insula structure. This literature suggests that across the lifespan, the microbiome is associated with both structure and connectivity of the insula. However, because a child's gut microbiome experiences rapid changes across the first three years of life, it is important to further understand this association. Additionally, because its structure and connectivity are involved in several psychiatric phenotypes, the insula might be a particularly important region to study in terms of the gut microbiome.

Methods: The present study aims to explore associations between the gut microbiome and insula volume. Neonates ($n = 88$) completed a 3T MRI scan and provided fecal samples at 2 weeks of age. Insula bilateral volumes were estimated using an infant-specific multi-atlas segmentation workflow at the UNC NIRAL. Infant fecal samples were sequenced using Whole Genome Sequencing. We built Random Forest (RF) regressions to explore the predictive capacity of bacteria genera on total insula volume. We specified the model using 501 trees and determined significance after permutation ($n = 1000$).

Results: The microbiome predictive capacity showed adequate fit and was determined significant on the insula ($r2 = 9.97$, $p = .004$). We used percent increase in Mean Square Error (MSE) to calculate the importance score of each genus and identified the top 10 genera driving the significance: Veillonella, Enterococcus, Finegoldia, Pseudomonas, Peptacetobacter, Flavobacterium, Acinetobacter, Massilistercora, Lacticaseibacillus, Shewanella. To reduce dimensionality and address multicollinearity we conducted a Principal Component Analysis (PCA) using the top 10 identified features. The first principal component (PC) explained 32% of the variance, and representative genus (loading >.30) included: Pseudomonas, Flavobacterium, Massilistercora, Lacticaseibacillus, and Shewanella. The second PC explained 15% of the variance, and representative genus included: Veillonella, Enterococcus, and Acinetobacter. The PCs were extracted and used as predictors of insula volume in an ordinary least squares multiple regression. An ordination analysis was conducted to identify salient covaries associated with the insula-related genera using 999 imputations; only child's sex showed a significant association. A multiple regression was conducted with insula total volume as the dependent variable of the two PCs. Covariates included child's sex, total intracranial volume, maternal education, child's age at day of scanner, maternal age at birth, child's weight at birth, and gestational age at birth. The second PC was significantly associated with insula volume before ($B = -133.74$, $p = .01$), and after ($B = -7.802$, $p = .03$) controlling for covariates.

Conclusions: In summary, these results highlight the contributions of both the community-level gut microbiome and specific genera on the structure of the insula in neonates. Moreover, it shows similar associations found in other neuroimaging studies with both infant and adults, where the insula has been linked to both the gut microbiome and gastrointestinal disease. Additionally, this is the first study to show these associations as early as 2 weeks of age. The insula receives afferent visceral input and is involved in complex socioemotional processes such as emotion reactivity and regulation. Likewise, because of its role in interoception and other sensorimotor processes, the insula might be one the first brain regions to be influenced by the microbiome early in development. This makes it a key region in understanding the gut-brain axis, and the development of future nutrition-based interventions to treat behavioral conditions that involve insula-related differences such as anxiety disorders.

Keywords: Gut Microbiome, Insula, Structural MRI

Disclosure: Nothing to disclose.

P148. Exploring Risk and Resilience Following 11β-Hydroxylase Antagonism in a Rodent Model of Maternal Immune Activation

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Background: Severe illnesses during pregnancy can elevate the risk for neurodevelopmental disorders such as autism and schizophrenia in the offspring. These mental health disorders are associated with disruptions in social processes such as social recognition and motivation. The mechanistic underpinnings of these social disruptions remain undefined but are a likely path for identifying factors contributing to resilience vs susceptibility against challenges to mental health functioning. While it is known that immune signaling is critical to these phenotypical changes in offspring behavior, it is unclear how the stress response is involved and if it can be manipulated as a potential intervention at the time of maternal immune activation (MIA).

Methods: We used a rodent model of MIA to recapitulate disruptions in social experiences by treating pregnant rat dams ($N = 70$) with the clinically available 11β-hydroxylase inhibitor metyrapone (MET, 100 mg/kg, i.p; or saline control), known to attenuate the hormonal stress response, followed by administration of either the bacterial mimetic lipopolysaccharide (LPS;100 µg/kg, i.p), or saline vehicle on gestational day (G)15. Three hours later, using standard ELISA techniques, concentrations of corticosterone were evaluated in maternal plasma, placenta, and fetal brain while11ßHSD enzymes type 1 and 2 were evaluated in placenta ($n = 7$). Additionally, levels of interleukin (IL)-17A and IL-6 were analyzed in maternal plasma via ELISA and IL-6 receptor levels assessed in male and female fetal brains utilizing western blots. In a separate cohort of animals, male and female offspring were evaluated across development on a series of social measures including isolation and play ultrasonic vocalizations (USVs), in addition to social preference and discrimination tasks ($n = 10$-11). RNA sequencing was used to evaluate transcriptional changes in adult offspring ventral hippocampus ($n = 3$), a brain region implicated in social behavior. Fetal tissues were adequately powered to detect sex-differences so three-way ANOVAs (Sex by G15 MET/Saline by G15 LPS/Saline) were utilized with litter used as a covariate. CLC Genomics Workbench v.23.0.2 software (QIAGEN Digital Insights, Aarhus, Denmark) was used to perform differential expression analysis on RNA sequencing data. Two-way ANOVAs were used as appropriate for all other measures (G15 MET/Saline by G15 LPS/Saline).

Results: Three hours post LPS, concentrations of corticosterone were elevated in maternal plasma (MET by LPS interaction: $F(1, 24) = 10.401$, $p = 0.004$), and male and female placenta (MET by LPS interaction: $F(1, 47) = 160.380$, $p = 0.001$) and fetal brains (MET by LPS interaction: $F(1, 47) = 14.736$, $p = 0.001$) which were prevented by MET treatment (saline-LPS vs MET-LPS: $p = 0.001$each for maternal plasma, placenta and fetal brains). While placental 11ßHSD1 increased in both sexes following LPS challenge, irrespective of metyrapone treatment (saline-saline vs saline-LPS: $p = 0.001$; saline-LPS vs MET-LPS: $p > 0.05$), decreased placental 11ßHSD2 following LPS + metyrapone was only observed in female placentas (saline-LPS vs MET-LPS: $p = 0.001$; female: $p > 0.05$); this suggests less corticosterone was inactivated in female offspring. MET protected males against LPS-induced disruptions in USVs during juvenile play (saline-LPS vs MET -LPS: $p = 0.048$) and social discrimination ability across development (saline-LPS vs MET-LPS juvenile and adult: $p = 0.001$). In contrast, LPS-induced disruptions in female offspring were unaffected by metyrapone (USVs; $p > 0.05$) or led to worsened performance on social tasks (saline-LPS vs MET-LPS juvenile and adult social discrimination: $p = 0.001$; adult social preference: $p = 0.001$). Notably, MET had no effect on either elevated maternal plasma IL-17A (main effect of LPS: $p = 0.003$; main effect of MET: $p > 0.05$) or the increased levels of IL-6 receptor observed in male fetal brain following MIA (main effect of LPS: $p = 0.007$; main effect of MET: $p > 0.05$). Furthermore, MET had only a partially protective effect on elevations in maternal plasma IL-6 following MIA (MET by LPS interaction: $F(1, 24) = 10.401$, $p = 0.004$), indicating that mechanisms outside of the immune system were likely involved in the mitigative effects of metyrapone. Indeed, transcriptomic analyses of the ventral hippocampus revealed that metyrapone-exposure reversed many of the MIA-induced alterations in dopamine-, serotonin-, GABA-, and glutamate-related genes in males, but not females.

Conclusions: Overall, MIA-induced hormonal stress responses act alongside the immune system to produce behavioral susceptibility to social deficits in male and female offspring. These immune and endocrine interactions work together within maternal, placental, and fetal compartments and appear to affect neurotransmitter functioning within the ventral hippocampus which can be offset by 11β-hydroxylase inhibition, at least in male offspring. As a clinical drug currently available to pregnant people and neonates for the treatment of hypercortisolism, the sex-specific benefits and drawbacks of metyrapone should be investigated further as a potential means of reducing neurodevelopmental risks due to gestational MIA.

Keywords: Maternal Immune Activation, Sex Difference, 11β-hydroxylase Inhibitor, Fetal Programming

Disclosure: Novartis: Employee (Spouse/Partner)

P149. Amphetamine in Adolescence Induces a Sex-Specific Mesolimbic Dopamine Trait in the Prefrontal Cortex in Adulthood

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Background: During adolescence, the mesocorticolimbic dopamine system undergoes significant maturational changes, most notably the protracted growth of dopamine axons to the

prefrontal cortex, which is controlled by the Netrin-1 guidance cue receptor, DCC. Environmental insults can dysregulate this guidance pathway, significantly altering prefrontal cortex dopamine development and related cognitive processing and behavior. In male mice, recreational-like doses of amphetamine (AMPH) in early adolescence induce ectopic growth of mesolimbic dopamine axons to the prefrontal cortex. This effect is mediated by drug-induced downregulation of DCC receptors in mesolimbic dopamine axons and leads to impaired inhibitory control in adulthood (Reynolds et a.,l Nat Comm 2023.) Here we investigated if the ectopic input of nucleus accumbens dopamine axons to the prefrontal cortex alters adult (i) local expression of the dopamine transporter (DAT), (ii) baseline dopamine kinetics, and (iii) dopamine signaling in response to the dopamine releaser and DAT blocker, methylphenidate. We examined if these effects can be prevented by upregulating DCC receptor expression in adolescence, using CRISPRa.

Methods: Adolescent male and female mice received saline (SAL) or AMPH (4 mg/kg, i.p.) injections from PND 22 to PND 31. Mice were placed in distinctive compartments of a place-preference box and preference was assessed at PND 32 and again at PND 80. One day later, mice were euthanized and DAT expression in dopamine axons was quantified in the prefrontal cortex using stereology. In a separate cohort, the dopamine sensor, GrabDA2h, was unilaterally microinjected into the prefrontal cortex and an optical fiber was implanted. Dopamine dynamics were measured with fiber photometry at baseline, following an acute i.p. injections of SAL, and an acute i.p. injection of methylphenidate (10 mg/kg). In a separate cohort of adolescent male mice, CRISPRa, with sgRNAs targeting Dcc, or LacZ as a control, was microinjected into the ventral tegmental area at PND 21, followed by the AMPH or SAL treatment and dopamine dynamics were assessed in adulthood.

Results: Male and female AMPH-treated mice exhibit short- and long-lasting place preference. However, in adulthood AMPH-treated males, but not females, show increased density of DAT-positive varicosities in the prefrontal cortex, compared to SAL controls. This male-specific increase in DAT expression is associated with (i) a decrease in baseline dopamine transients and an increase in their amplitude, (ii) faster dopamine release and a trend towards faster absorption in response to acute SAL, and (iii) exaggerated prefrontal cortex dopamine signal in response to methylphenidate. DCC upregulation prevents both the development of place preference to AMPH and alterations in adult dopamine dynamics. Notably, DAT expression in the prefrontal cortex is significantly higher in females than males in the SAL groups.

Conclusions: The male-specific increase in DAT expression and in dopamine dynamics observed in the prefrontal cortex in adulthood is likely to result from ectopic innervation of mesolimbic DA axons, which seem to retain the anatomical and functional properties of their intended target.

Keywords: Amphetamine, Mice, Dopamine, Medial Prefrontal Cortex, Fiber Photometry

Disclosure: Nothing to disclose.

P150. Sex Differences in Cortical Gene Expression Patterns During Development in a Model System of Autism Spectrum Disorders

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Background: Autism Spectrum Disorders (ASD) have increasing prevalence in North America, with no known pharmacological interventions to alleviate core symptoms of the disorder. Symptom presentation upon the autism spectrum is heterogeneous and relies on the diagnosis solely on behavioral phenotypes. Sex differences in the disorder exist in age of onset, prevalence, etiology, and presentation of symptoms, yet underlying mechanisms for these differences are unknown. Animal models are paramount to study aspects of ASD, yet females are still underutilized, resulting in a dearth of information on the sex-dependent molecular underpinnings. Therefore, employing the valproic acid (VPA) model, widely used to study aspects of idiopathic ASD, sex differences in cortical gene transcription at postnatal day (PND) 0 and 35 were evaluated.

Methods: Pregnant Sprague Dawley rats were injected with VPA (500 mg/kg, i.p.) or saline on gestational day 12.5. Total RNA was isolated from prefrontal cortical (PFC) tissue of male and female animals and purified at PND 0 (birth) or 35 (adolescence) using the RNeasy Mini Kit (Qiagen, Hilden, Germany) ($N = 5$/group). RNA quality was assessed by NanoDrop (ThermoScientific). Samples were then sent to the TCAG Microarray Facility at Sick Kids Hospital (Toronto) for microarray processing. Analysis of genes that were transcriptionally altered was performed on GeneSpring GX 14.9.1 (Agilent). A fold change of 1.3 was deemed the threshold for significant change to assess differences between VPA and control groups, for each sex, at each time point. Gene ontology (GO) analysis for enriched genes was performed using the NIH DAVID Bioinformatics Database, Functional Annotation Tool.

Results: At PND 0, a total of 176 and 149 genes showed a 1.3 or greater fold change in expression in the VPA female and male PFC respectively, 24 of these genes were shared. In the female VPA cortical tissue, most of these genes were downregulated (72%) whereas in the male tissue they were upregulated (77%). The expression of genes involved in growth factor signaling, such as egfr, fgf15 and hgf, were elevated in female VPA PFC. Neuropeptide genes such as tac1, cartpt, pdyn, and penk were downregulated as were many receptor RNAs including drd1a, drd2, adora2a, mc4r, and htr3a. In contrast to the females, the tissue from VPA male PFC showed elevations in the neuropeptide RNAs tac1, cartpt, pdyn, and penk as well as in receptor RNAs such as drd1a, adora2a, and mc4r. At PND 35, a total of 395 and 154 genes were altered in VPA female and male PFC respectively, the majority of which were downregulated. Only 11 genes were shared. For the females, gene transcripts involved in vascular permeability, such as gpr116, cldn5, flt1, rgs5, angptl4, esam, and decorin showed the greatest fold reduction in expression. In male rats, genes that showed the greatest changes were those involved in neurotransmitter/ neuropeptide signaling and included an upregulation of drd2, cartpt, adora2a, and pdyn, and a downregulation of nxph4 and several solute carrier genes. In both sexes, genes associated with myelination, rxrg, opalin and mog, were downregulated.

Conclusions: These findings indicate that in the VPA model cortical gene expression changes between male and female animals were for the most part distinct. Whereas gene expression associated with myelination appears to be altered in both sexes, processes involving vascular permeability may warrant attention in the female animals.

Keywords: Autism Spectrum Disorders, Gene Expression, Cortical Development, Sex Differences

Disclosure: Nothing to disclose.

P151. Neocortical Excitation/Inhibition Imbalance During Early Development Leads to Permanent Socio-Behavioral, Transcriptomic and Connectivity Alterations

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Background: Autism and related developmental disorders encompass a wide range of heterogeneous conditions with an overall prevalence of 1 % in the human population. Although autism varies in symptomatology and severity, its two main core symptom domains (i.e. social and communicative impairment, and restricted and repetitive behaviors) appear to be consistently affected across the spectrum. However, how diverse etiological mechanisms converge to produce the relatively narrow set of behavioral manifestations that characterize autism remains unclear.

A popular theory posits that an imbalance between excitatory and inhibitory (E/I) activity might contribute to the etiology of autism (Rubenstein and Merzenich, 2003). However, controversy exists about whether markers of E/I imbalance measured in autistic individuals and animal models are indicative of a direct, causal contribution to the underlying pathology, or if they instead represent a compensatory, epiphenomenal phenotype of limited etiological relevance.

Here, we test the hypothesis that alterations in E/I ratio (a possible consequence of a genetic or developmental insult) occurring during early infancy are sufficient to produce lasting autism-relevant behavioral, transcriptomic and connectivity alterations via interference with ongoing developmental processes. To probe this notion, we used chemogenetics in mice to transiently increase the neocortical E/I balance during early postnatal phase and measured the ensuing behavioral and neuroimaging phenotypes longitudinally until late adulthood. In keeping with our hypothesis, our results show that this simple manipulation results in permanently impaired social behavior, as well as transcriptional and connectivity alterations of high translational relevance for autism.

Methods: Cell-type specific increase of neuronal excitability (pyramidal neurons) was obtained using intersectional genetics. Specifically, we expressed excitatory DREADD receptors hM3Dq in Vglut1-cre mice (Giorgi et al., 2017) and induced E/I imbalance by chronically treating mice with CNO during the first two postnatal weeks (Control $N = 29$, Vglut1-gq $N = 27$, mixed sexes). Longitudinal behavioral tests, resting state fMRI and RNA- sequencing were performed at multiple developmental stages (late infancy, adolescence and adulthood).

Results: Chemogenetically increasing E/I balance during early development resulted in robustly impaired sociability, and increased repetitive behavior, that lasted throughout adulthood ($p < 0.002$, liner Mixed model, age x sex x genotype). Importantly, socio-behavioral alterations were not associated with anxiety-like phenotypes, impairments in olfactory abilities, tactile sensitivity, motor coordination, working or long-term memory. Corroborating the developmental specificity of these results, control studies in which the same manipulation was applied to adolescent mice did not result in any behavioral alteration ($p > 0.23$, all behaviours).

Transcriptomic analyses showed that transient hyperexcitability during early development results in permanent alterations in the expression level of a large set of ribosomal and synaptic-related transcripts. Importantly, the list of differentially expressed genes was significantly enriched for known autism– associated genes (SFARI, $OR = 2.11$ $p = 0.0093$), thus putatively implicating activity-dependent transcriptional mechanisms in the generation of the observed phenotypes.

We next interrogated circuit organization of the manipulated mice using resting state fMRI in adult animals. We found that chemogenetically–manipulated animals exhibited profoundly disrupted fronto-hippocampal functional connectivity, but preserved coupling in motor–sensory regions ($Z > 4$, cluster corrected at $p < 0.001$). Corroborating the behavioral relevance of these findings, multivariate modelling revealed that functional hypo–connectivity was highly predictive of behavioral disruption in manipulated animals (PLS, $p < 0.001$, 1st latent variable).

Conclusions: We document that E/I imbalance during early development is sufficient to produce a wide array of autism–related phenotypes in rodents. Transcriptional analyses suggest that these alterations may be underpinned by activity-dependent epigenetic mechanisms triggered by developmental hyperexcitability of pyramidal neurons, resulting in long-term dysconnectivity of socially-relevant brain circuits. Taken together, our results also support a causal (as opposed to compensatory) contribution of E/I imbalance to the pathogenesis of autism and related developmental conditions.

Keywords: Chemogenetics, Resting-State fMRI, Autism, Excitation-Inhibition Balance, RNAseq

Disclosure: Nothing to disclose.

P152. Associations Between Early Life Housing Code Violations and Parent and Child Psychological and Cognitive Functioning

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Background: In low-income communities, housing code violations such as rodent infestation, cockroach infestation, structural defects, and mold are common. Property owners have a legal responsibility to prevent and remediate these issues; yet, the New York City Department of Housing Preservation and Development received over 500,000 housing code complaints in 2022 alone. Mice, rats, cockroaches, and mold are well-established infectious and immunologic health threats that contribute to further infrastructural decay and further infestation. These contaminants are stressful, unpredictable, unsightly, physically threatening, and dehumanizing. Furthermore, a hazardous living space is particularly impactful on children given the amount of time they spend at home. However, little to no research has examined the cognitive and psychological toll that housing violations take on families.

Methods: Here, we use data from a longitudinal birth cohort in New York City to examine associations between housing code violations and maternal and child psychological and cognitive wellbeing. The Mothers and Newborns longitudinal birth cohort (recruitment: 1998-2006) included almost exclusively low-income, pregnant Black and/or Latinx women in northern Manhattan. Mothers who smoked during pregnancy or had gestational diabetes, hypertension, or HIV were not eligible. Beginning in the third trimester of pregnancy, mothers completed questionnaires assessing the characteristics of their housing, finances, mood, environmental exposures, and health behaviors every two years. Housing code violations included in the present analysis included the presence of rodents, cockroaches, mold, peeling paint, holes in the ceiling or walls, and leaky pipes. Maternal psychological distress was assessed at each timepoint by the Psychiatric Epidemiology Research Instrument Demoralization Scale (PERI-D) survey. Child behavior was assessed by the Child Behavior Checklist at age five and seven, and cognition was assessed by the Wechsler Intelligence Scale for Children-III (WISC-III) at age seven. Associations between total number of housing code violations and psychological/cognitive outcomes were

tested using multiple linear regression with the following covariates: household income, inability to pay for housing, inability to pay for utilities, maternal education, and ethnicity as a crude proxy for the cumulative, unique experiences of discrimination faced by the ethnoracial groups involved in the study.

Results: Mothers who resided in homes with more housing code violations had greater psychological distress during the third trimester and at child ages 1 year ($p = .034$), 2 ($p = .046$), 3 ($p = .001$), and 5 ($p = .0009$) p's < 0.05), though not 7 ($p = .13$). Their children had more externalizing symptoms ($p = .02$) and more total problems ($p = .02$) at age 5. They also had lower scores on assessments of verbal comprehension ($p = .05$) and perceptual reasoning ($p = .02$), but not working memory ($p = .99$) or processing speed ($p = .68$).

Conclusions: Housing code violations are extremely common and understudied. The current exploratory analyses find evidence of associations between these environmental threats and child and parent psychological and cognitive functioning. Remediation of these modifiable and illegal conditions could improve family wellbeing on a population level.

Keywords: Epidemiology, Health Disparities, Mental Health Disorders

Disclosure: Nothing to disclose.

P153. Patterns of Alternative Splicing in Layer 3 Pyramidal Neuron Subtypes of the Dorsolateral Prefrontal Cortex Between Pre-Pubertal and Adult Macaques

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Background: Schizophrenia (SZ) is a neurodevelopmental disorder that is characterized, in part, by dysfunction of the dorsolateral prefrontal cortex (DLPFC) and working memory (WM) deficits. The emergence of these deficits during adolescence coincides with developmental changes in the DLPFC including pruning of dendritic spines on layer 3 pyramidal neurons (L3PNs), a critical cell type that supports WM function. Key subtypes of L3PNs that furnish projections to ipsilateral posterior parietal cortex (IP) or contralateral DLPFC (CP) differ in dendritic spine density, maturational course, and role in WM. However, the transcriptomic basis for the maturation of L3PNs over the adolescent period is unclear. Prior studies, predominately conducted in tissue homogenates of the DLPFC, suggest that maturation over the adolescent period is associated with few, if any, isoform-level differences. In contrast, other reports suggest later cortical maturation reflects, in part, a proliferation in transcript isoforms arising from alternative splicing (AS), a process that alters the composition of exons and retained introns in mature RNA transcripts. Therefore, we compared AS in IP and CP L3PNs between pre-pubertal and adult rhesus macaques.

Methods: IP and CP L3PN subtypes in the DLPFC were identified by fluorescent retrograde tracers in pre-pubertal ($N = 9$, females = 5, Mage = 19.7mo) and adult ($N = 8$, females = 4, Mage = 56.6mo) macaques. Pools of 120 IP and CP neurons were collected in replicate by laser microdissection, sequenced to an average depth of 50 million 100nt paired-end reads, filtered to remove low quality mappings, and aligned to the MMul10 genome using STAR in two-pass mode to improve annotation of novel splice junctions. Junction saturation curves approached asymptotes in each L3PN subtype and in each developmental group, suggesting data were suitable for AS analyses. LeafCutter were used to extract and cluster junction-spanning reads for AS. Analyses were adjusted for sex and performed on samples run in a single sequencing batch. AS differences with FDR-corrected p-values10% difference in splicing were considered significant. Pathway enrichment analyses were performed for biological processes and cellular components using PANTHER, reported pathways contained >=10 genes. All procedures were approved by the University of Pittsburgh IACUC.

Results: Developmental shifts in AS were more extensive in IP than in CP L3PNs, with 257 events in 231 unique genes alternatively spliced between pre-pubertal and adult IP L3PNs compared to 172 events in 157 unique genes in CP L3PNs. Genes ($n = 43$) shared between pre-pubertal and adult macaques in both IP and CP L3PNs included specific AS events previously shown to alter dendritic spine number, synaptic plasticity, and synaptic signaling (e.g., GRIN1 exon 5, GABRG2 long/short). Alternative splicing effect sizes were highly correlated for genes with developmental AS differences in both L3PN subtypes ($r = 0.89$, $p < 0.001$). Developmental AS differences in IP L3PNs (and not CP) L3PNs included events shown to regulate spine density, morphology, and/or stability in experimental studies (e.g., usage of CDC42 exon 6 or 7; skipping of CAMK2G F-actin binding domain) and events that modulate neural transmission and trans-synaptic signaling (e.g., SNAP25 exons 5a/5b, NRXN1-gamma). Developmental AS differences in CP (and not IP) L3PNs included events shown to alter trans-synaptic composition and signaling (e.g., NRXN1 SS6, CACNA1E exon 19). Genes with developmental AS shared between IP and CP L3PN subtypes were enriched for 6 processes and 13 components. Top processes included cell-to-cell and synaptic signaling; top components included the synapse and cell junction. Genes with developmental AS differences in only IP L3PNs were enriched for 19 processes and 13 components. Top processes included modulation of chemical synaptic transmission, regulation of trans-synaptic signaling, and regulation of morphogenesis; top components included the postsynapse, postsynaptic density, and cell junction. Finally, genes with developmental AS differences in only CP L3PNs were enriched for 2 processes (regulation of trans-synaptic signaling and modulation of chemical synaptic transmission) and 0 components.

Conclusions: IP and CP L3PN subtypes share some AS differences between pre-pubertal and adult macaques, which may contribute to functional and structural maturation of L3PNs (e.g., synaptic pruning). In support of this interpretation, specific AS events shown to alter dendritic spine density in experimental studies (e.g., GRIN1 exon 5) demonstrated patterns of AS consistent with findings of lower spine density in adult than pre-pubertal L3PNs. The presence of more extensive AS differences in IP than CP L3PNs suggests a particular role for AS in the maturation of IP L3PNs. Genes with developmental AS differences in IP L3PNs or in both L3PN subtypes were enriched for synaptic structures/processes, suggesting a role for AS in fine-tuning synaptic signaling in L3PNs across adolescence. Consistent with this interpretation, functional AS isoforms of GABRG2 and GRIN1, which encode constitutive GABA-A and NMDA receptor subunits, showed comparable changes in AS over development in both IP and CP L3PNs. The findings of AS differences in these genes in SZ suggests that AS may play a role in altered L3PN development in the illness.

Keywords: Alternative Splicing, Pyramidal Neuron, Developmental Transcriptome, Dorsolateral Prefrontal Cortex (DLPFC), Schizophrenia (SCZ)

Disclosure: Nothing to disclose.

P154. Polygenic Risk for Alzheimer's Disease and Heavy Alcohol Use Influence Trajectories of Neural Connectivity and Related Neuropsychiatric Outcomes Across the Lifespan

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Background: Known genetic risk factors for Alzheimer's disease (including Apolipoprotein (APOε4) and associated variants) have been associated with poorer cognitive outcomes and other adverse health conditions later in life among individuals without Alzheimer's. Recent studies have also demonstrated early developmental changes in brain structures and function in healthy young APOε4 carriers (< age 20). Studying genetic risk factors for Alzheimer's beginning in childhood and adolescence may provide the earliest indicators for individuals who might benefit from interventions or preventive measures for cognitive impairments and other complex health conditions that manifest across the lifespan. In a separate literature, heavy alcohol use has been shown to negatively impact brain development and cognitive functioning throughout the lifespan, with new data suggesting that alcohol abuse is associated with a 4-fold increase in risk for Alzheimer's. The primary aim of this study is to investigate the association of polygenic risk for Alzheimer's disease (including and excluding APOEε4, given its large effect size) with measures of brain functioning (i.e., neural connectivity, memory problems) across the lifespan (ages 7-96), and the moderating influence of alcohol use behavior on these associations.

Methods: Data was drawn from the Collaborative Study on the Genetics of Alcoholism (COGA), a large and diverse family sample of individuals ranging from ages 7-96, enriched for alcohol use problems (N: 17,854, 53% female, 28% Black; 8% Hispanic). Polygenic risk scores (PRS) were derived on 12,751 participants for whom genome-wide association data was available. PRS were computed based on summary statistics from a meta-analysis of 111,326 Alzheimer's cases and 677,663 unaffected individuals (Bellenguez et al., 2022, Nature Genetics) and calculated using PRS-CSx. Neural connectivity was measured with inter- and intra-hemispheric EEG coherence (eyes closed resting state in the theta (3-7 Hz), alpha (7-12 Hz) and beta (12-28 Hz) frequency bands), which captures the degree of synchrony in brain oscillatory activity between neural network ensembles in two brain regions and provides temporal resolution on the order of milliseconds—a scale at which most relevant sensory, motor, and cognitive processes take place at the neural level. Data on alcohol use behaviors, including age of initiation of regular drinking, alcohol use frequency in a typical week, and DSM-5 alcohol use disorder symptoms, were obtained from structural clinical interviews (SSAGA; Semi-Structured Assessment for the Genetics of Alcoholism). Data on memory problems and other medical outcomes were available on a subset of these individuals, selected for being over the age of 50 with a history of AUD (N = 690).

Results: The PRS was associated with increased EEG coherence in the alpha frequency among COGA participants, and significant differences based on age, sex and ancestry were observed. Among males, the most robust associations ($p < 5x10\text{-}8$) were observed after age 24 between the PRS and alpha inter-hemispheric fronto-central coherence pairs. Associations between the PRS and alpha coherence were less robustly associated ($p < 5x10\text{-}4$) among females, with associations between PRS and fronto-central coherence pairs only observed at ages 12-15. Further, interactions were observed with two alcohol-related behaviors: age of initiation of regular drinking and DSM-5 alcohol use disorder symptoms ($p < 5x10\text{-}6$). Among males, those with higher PRS displayed increased alpha fronto-central coherence beginning at age 24, and effects become more prominent as participants aged (24 through 96). This distinction between trajectories of neural connectivity between those with lower and higher PRS was more extreme among those who started drinking regularly before age 14 and among those with a greater number of alcohol use disorder symptoms. The PRS was also associated with an increased odds of having memory related impairments (OR: 3.5, $p < 0.01$), alcohol and other substance use problems (ORs:1.5-2.5, $p < 0.01$), and pulmonary conditions (OR: 1.6, $p < 0.01$) in the subset of older participants with a history of AUD.

Conclusions: These findings suggest that polygenic risk for Alzheimer's Disease shapes developmental changes in neural connectivity across the lifespan among individuals unaffected by Alzheimer's as early as age 24. Our findings also point to additional risk conferred by heavy alcohol use and related problems, but not light or moderate alcohol use, for disturbances in neural development. Polygenic risk for Alzheimer's Disease also increases risk for a range of adverse health outcomes, including substance use problems, pulmonary conditions, and memory related impairments. Targeting APOε4 carriers beginning early in development for interventions or preventive measures for neural impairments may help prevent downstream risk for a wide range of health conditions that manifest across the lifespan. Additionally, efforts to reduce heavy alcohol use among high-risk individuals should be prioritized.

Keywords: Alzheimer's Disease, Polygenic Risk Score, Alcohol Use Disorders, Developmental Trajectories, EEG Connectivity

Disclosure: Nothing to disclose.

P155. Polygenic Risk Score for C-Reactive Protein is Associated With Accelerated Cortical Thinning and Increased Psychopathology in Adolescents: A Population-Based Longitudinal Cohort Study

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Background: This study investigates the potential impact of a genetic predisposition to elevated C-reactive protein (CRP) on neurodevelopment and susceptibility to psychopathology among adolescents. CRP is a marker of systemic inflammation used in clinical practice, and chronic inflammation is believed to contribute to the development of a distinct subtype of psychiatric disorders. Previous research has associated CRP with structural brain alterations and psychiatric disorders such as depression. In add-on, disruptions in cortical thickness, which decreases during adolescence due to synaptic pruning, can influence the onset of psychiatric disorders. Here, we use the polygenic risk score for CRP (PGS_CRP) as an indicator of a genetic predisposition to elevated CRP to examine its relation to cortical thickness and psychopathology.

Methods: Cortical thickness maps were extracted from T1-weighted structural magnetic resonance images obtained from a large, diverse sample from the Adolescent Brain Cognitive Development (ABCD) study. We analyzed baseline data (9 and 10-year-olds, N = 11283) and two-year follow-up data (N = 7578) from 21 US sites. The PGS_CRP was computed based on the meta-analysis results from the CHARGE Consortium and divided into four groups based on the quantiles. Psychopathology was measured using the syndrome scales for externalizing, internalizing, and depression t-scores from the Child Behavior Checklist. Linear mixed-effects models were utilized to explore the impact of PGS_CRP on cortical thickness and psychopathology. Specifically, we examined genetic and environmental interactions on these outcomes using a parents-reported binary variable of early-life infection. Fixed effects included time, age, sex, BMI, race, parents'

education level, household income, and 10 principal components of genetic ancestry, while participants' ID, nested within study sites and family IDs, was considered a random effect.

Results: Each standard deviation (SD) increase of PRS_CRP correlated with a 0.027 SD reduction in mean cortical thickness (beta = -0.027, p = 0.029). Post hoc analyses indicated that the bilateral temporal lobe primarily drove this effect. Early-life infection was significantly associated with increased depression, internalizing, and externalizing psychopathology (Cohen's d ranged from 0.23 ~ 0.24, p < 0.001). A significant interaction effect was found between PGS_CRP levels and early-life infection on cortical thickness and psychopathology. Compared to individuals with the lowest PGS_CRP level (bottom 25% quantile) and without early-life infection, those with high PGS_CRP level (Top 25% quantile) and early-life infection exhibited reduced mean cortical thickness (d = -0.20, p = 0.003) and increased scores for depression (d = 0.38, p < 0.001) and internalizing (d = 0.37, p < 0.001). Higher PGS_CRP levels also independently impacted the externalizing score (d = 0.13, p < 0.001), irrespective of early-life infection.

Conclusions: The findings suggest a complex interplay between genetic predisposition to elevated C-reactive protein (CRP), early-life infection, and neurodevelopmental and psychopathological outcomes. There are several mechanistic processes that could contribute to these findings. First, a genetic predisposition to higher inflammation may contribute to reduced cortical thickness, which may be due to the deleterious effects of chronic inflammation on neuronal health and synaptic pruning processes. Second, the temporal lobe may be particularly vulnerable to the effects of inflammation, possibly due to its late maturation during neurodevelopment. Third, early-life infection has been associated with increased depression, internalizing, and externalizing psychopathology, which could be due to the acute and chronic effects of infection on the developing brain. Fourth, the interaction effect between PGS_CRP and early-life infection on cortical thickness and psychopathology suggests that these factors may have a synergistic effect, which provides further support for the "double hit" hypothesis, where the combination of chronic inflammation (indicated by high PGS_CRP) and acute inflammation from early-life infection leads to more severe disruption of neurodevelopment and higher risk of psychopathology. Finally, higher PGS_CRP levels could independently impact the externalizing score, irrespective of early-life infection.

Keywords: Polygenic Risk for Inflammation, Cortical Thickness, Early Life Infection, Psychopathology

Disclosure: Nothing to disclose.

P156. Genetic Contributions to Insula Structure and Function With a Focus on the 7q11.23 Williams Syndrome Critical Region

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Background: Williams syndrome (WS) is a rare neurodevelopmental disorder caused by hemideletion of ~26 genes at the 7q11.23 chromosomal locus. Because both the genetics and neurobehavioral phenotype associated with WS are well-circumscribed and well-defined, studying WS offers a valuable opportunity to understand how genetic changes affect brain development and ultimately translate into complex behaviors. Nearly all individuals with WS have the same 25-27 genes hemideleted and are behaviorally typified by increased social drive and problems with visuospatial processing. Previous studies in adults with WS have associated the hypersocial phenotype with structural and functional changes in the anterior insular cortex (Jabbi 2012). Moreover, one of the affected genes in the 7q11.23 WS Critical Region (WSCR), GTF2I, has been linked to the WS social phenotype (Jarvinen 2013; Crespi 2014). Here, we first sought to determine whether the structural and functional changes previously identified in the anterior insula region of adults with WS are also present earlier in development. Additionally, in a data-driven manner, we sought to identify which, if any, of the genes in the WSCR might be associated with insula structure by conducting a region-wide association study ("mini-GWAS") of just the WSCR, testing whether variation in the quantitative phenotype of anterior insular volume significantly related to 7q11.23 WSCR SNP variation in healthy adult populations.

Methods: First, to determine whether structural insular brain changes previously found in adults with WS are present earlier in development, we compared longitudinally-collected gray matter volume (GMV) derived from T1-weighted MEMPRAGE scans of 30 children with WS (79 cumulative visits, age = 12.6 + /- 4.7 years, 10 males) to that of 74 typically developing children ([TD]; 197 cumulative visits, age = 12.4 + /- 3.3 years, 27 male). We tested for group differences using linear mixed-effects modeling, controlling for age and sex. Next, to translate our structural findings into insular function, we tested whether regional cerebral blood flow (rCBF) collected with ASL MRI in 13 children with WS (age = 15.1 + /- 5.5 years, 1 male) differed from that in 24 TD children (age = 15.3 + /- 5.5 years, 2 males) using general linear modeling, controlling for age and sex. Finally, to investigate the genetic underpinnings of insular structure, we tested whether variation in GMV of the anterior insula related to genetic variation at any loci in the WSCR in 296 healthy adults of European descent using a mini-GWAS of just this genetic region. To further replicate these findings, we performed an identical mini-GWAS in a second, independent sample of 276 healthy adults of European descent from the Human Connectome Project (HCP) Young Adult Sample, again using anterior insula GMV as a quantitative phenotype. Imaging analyses were FDR-corrected for multiple comparisons, and mini-GWAS analyses were Bonferroni-corrected for the number of LD-independent signals in the WSCR.

Results: GMV was significantly altered in children with WS in two distinct portions of the anterior insula bilaterally: a superior portion of the anterior insula showed greater GMV in WS compared to TDs (both p < 0.001, FDR-corrected), whereas an inferior subregion of the frontoinsular cortex showed less GMV in WS (both p < 0.001, FDR-corrected). Additionally, rCBF of the bilateral anterior insula was significantly increased in children with WS (both p = 0.012, FDR-corrected) in regions that overlapped the area of increased GMV in children with WS. Further, in the discovery group of healthy adults, insula GMV significantly related to a block of SNPs spanning the initiation site of the GTF2I gene (peak SNP p = 9.54x10-4). This finding was replicated in the HCP sample, with a similarly significant block of SNPs spanning from the termination site of the GTF2IRD1 gene through the initiation site of GTF2I (peak SNP p = 1.0x10-3).

Conclusions: These results in children with WS, together with structural MRI in the general population, provide evidence for a GTF2I-based neurogenetic mechanism that is important in the structural and functional organization of the insular cortex. The GTF2I gene is highly expressed in the developing brain and is implicated in the process of myelination, both in mouse models and in postmortem human brains (Barak 2019). Akin to the increased social drive in WS, both the domestication of wolves to dogs and the friendliness of different dog breeds have been linked to variation in GTF2I and GTF2IRD1 genes (vonHoldt 2017). Moreover, in a mouse model of GTF2I CNVs that included hemideletions, diploid (wild type), duplications, and triplications,

a separation anxiety phenotype varied with GTF2I copy number such that mice with hemideletions had the least, and triplications the most separation anxiety (Mervis 2012). Additionally, a whole-brain neuroanatomical measure of the WS phenotype that included insula has been related to variation in the GTF2IRD1 gene (Fan 2018). Given the known associations of GTF2I with the social phenotype in WS and with neural myelination, our striking results linking insula structure with GTF2I SNP variation underscore a critical role for GTF2I in the behavioral and brain alterations in WS. Future work will extend the current findings to explore whether specific white matter tracts emanating from the insula, such as the uncinate fasciculus, are also related to GTF2I CNV and/or GTF2I common SNP variation.

Keywords: Williams Syndrome, Anterior Insula, Cerebral Blood Flow, Genetic Association Study, Gray Matter Volumes

Disclosure: Nothing to disclose.

P157. Neurobiological Underpinnings of Altered Social and Visuospatial Processing Accompanying Williams Syndrome in Children and Adolescents: Convergent Results From Four Independent fMRI Tasks

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Background: Efforts toward understanding genetic mechanisms underlying complex neuropsychiatric disorders, such as autism and schizophrenia, are complicated by the inherent clinical and neurobiological heterogeneity of many of these diseases. Studying genetically-driven neurodevelopmental disorders, wherein the specific genetic alterations are known and the characteristic behavioral phenotypes can be delineated, offers a pathway toward investigation of the impacts of particular genes on neural structure and function, and ultimately behavior, and has the potential to provide important mechanistic information. One such condition, Williams syndrome (WS), results from hemideletion of approximately 26 genes at chromosomal locus 7q11.23, and individuals with this rare disorder are typified by increased social drive (sometimes termed "hypersociability") along with deficits in visuospatial abilities. Previous fMRI studies have aimed to establish neural correlates of these characteristic behavioral features and have demonstrated that the neural response to social faces is greater in the fusiform gyri of adults with WS than in healthy controls (Mobbs 2004, O'Hearn 2011). In contrast, neural activation of the intraparietal sulcus is decreased in response to processing visuospatial stimuli (Meyer-Lindenberg 2004). In the present study, by integrating information from four separate fMRI tasks, we sought to understand the developmental nature of these findings —specifically, whether the patterns of neural activation previously observed in adults with WS are also present earlier in development, in children and adolescents with WS.

Methods: Children and adolescents with WS, together with age- and sex-similar typically developing (TD) controls, participated in the National Institute of Mental Health Intramural Research Program Study of Williams syndrome. Participants underwent structural and functional MRI scanning, including four separate task-based fMRI sessions. Two of these fMRI tasks targeted face processing and two targeted visuospatial processing. One of the two face processing tasks involved matching faces to a concurrently-displayed sample face, while in the other task, participants matched faces in a one-back task design. Of the two visuospatial fMRI probes, one was a Tetris-like task, while the other involved matching the spatial location of two sequentially-displayed images. Participants for the two face processing tasks were 20-21 individuals with WS and 40-42 TDs; mean ages: 12.5 ± 3 (WS) and 12.3-12.5 ± 3.5 (TD); approximately 30% male. Participants for the visuospatial processing tasks were 16-18 individuals with WS and 32-36 TDs; mean ages = 12.8-13.8 ± 3 (WS) and 12.9 ± 3 (TD); approximately 25% male. Functional scans were processed for slice-time correction with AFNI's 3dTshift, motion-corrected with SPM5, and coregistered to each individual's structural T1-weighted scan. Data were then aligned to a study-specific template that equally represented both TD and WS participants using ANTs software. Task effects within the TD group were modeled for each task separately using AFNI's 3dttest++, controlling for age and sex effects, to ensure each task activated the expected network. Group differences between WS and TD participants were also examined for each task with 3dttest++, also controlling for age and sex effects. The resulting between-group statistical maps were combined in a meta-analytic fashion using Stouffer's Z-method to represent the combined task activation from each of the two similar tasks (e.g., face processing or visuospatial processing). The resulting meta-analytic maps were thresholded at an FWE-corrected $p < 0.05$, using an uncorrected voxelwise significance of $p < 0.001$ and a cluster size threshold determined by AFNI's 3dClustSim.

Results: Activation patterns in the TD group were as expected for each task, with the two visuospatial tasks preferentially activating dorsal visual processing stream regions, including the bilateral intraparietal sulci, and the two face processing tasks activating ventral visual processing stream regions, including bilateral fusiform gyri. The between-group analyses for the two meta-analytically combined face processing tasks demonstrated bilateral hyperactivation of the fusiform gyri in people with WS compared to TDs when viewing faces. Between-group analyses for the two combined visuospatial tasks demonstrated hypoactivation of the intraparietal sulcus in participants with WS relative to TDs.

Conclusions: Results from this study establish that altered neural processes in people with WS, particularly in the fusiform gyri and intraparietal sulci, are present during childhood. Future work will investigate how these processes may change longitudinally through development and will examine whether specific 7q11.23 genes that are hemideleted in WS may be linked to these neurobiologic phenotypes. In addition to providing key insights regarding this fascinating copy number variant, the present data may offer clues to inform investigations of developmental and neurogenetic mechanisms underlying more genetically and behaviorally complex neuropsychiatric disorders.

Keywords: Williams Syndrome, Functional Neuroimaging, Neurodevelopmental Disorders

Disclosure: Nothing to disclose.

P158. Polygenic Risk for ADHD and its Contributions to Brain and Behavioral Phenotypes in Noonan Syndrome

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Background: ADHD occurs in approximately 48% of children with Noonan syndrome (NS), a single gene disorder caused by pathogenic variants disrupting the Ras/mitogen-activated protein kinase pathway. Subthreshold ADHD symptoms occur even more commonly in approximately 64% of children with NS. Recent studies characterizing NS phenotypes, including those from our group, have detected deficits in executive function and attentional problems, and lower volumes in subcortical structures linked to attentional processing and ADHD in children with NS relative to

controls. Although NS results from single gene pathogenic variants (most commonly in the PTPN11 and SOS1 genes), variability in neuropsychiatric phenotypes of NS implicate the role of other common genetic factors. Polygenic risk scores (PRS), summarizing effects of common genetic variants, present a unique opportunity to better understand the contribution of "background" genetic factors associated with ADHD to NS phenotypes. In this preliminary study, we examined how ADHD PRS associates with (1) ADHD psychopathology, (2) cognitive and behavioral measures, and (3) brain structure.

Methods: We collected cognitive-behavioral data and T1-weighted structural brain MRI from 42 children with NS (age range 4.43-12.3, 27 female) caused by PTPN11 or SOS1 variants. Saliva samples were collected and genotyped from a subset of 18 children using the Illumina Infinium PsychArray-24v1.3 BeadChip at Stanford Genomics, containing approximately 50,000 markers implicated in common psychiatric conditions. Following standard pre-processing and quality control procedures with PLINKv1.9, we generated ADHD PRS from ADHD GWAS data (Psychiatric Genetics Consortium) using the classic clumping and thresholding approach at seven p-value thresholds (0.001, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5).

Attention and executive function were evaluated with NEPSY-II, while hyperactivity and inattention symptoms were assessed with BASC-2. Participants were assessed for psychiatric diagnoses with K-SADS-PL. For structural MRI, cortical and subcortical reconstruction and volumetric segmentation were performed with FreeSurfer v5.3.

First, we tested associations between each calculated ADHD PRS and ADHD diagnostic status (from K-SADS-PL) with logistic regression, using age, sex, and the first two principal components as covariates. Then, we conducted linear regression with the same covariates to explore the relationship between ADHD PRSs and NEPSY-II attention and executive function measures, BASC-2 hyperactivity and inattention measures, whole brain measures (total brain volume, total surface area, and weighted mean thickness), and subcortical gray matter volumes. For each association, we used Nagelkerke's pseudo R2 to select the best-fit model.

Results: We detected significant associations between ADHD PRS and two NEPSY-II subscales related to executive function. Higher best-fit ADHD PRS (pthreshold = 0.1) associated with higher NEPSY-II Inhibition-Switching scores ($R2 = 0.443$, $p = 0.015$). We observed a similar association between ADHD PRS (pthreshold = 0.05) and NEPSY-II Switching scores ($R2 = 0.454$, $p = 0.035$). For behavioral measures, ADHD PRS (pthreshold = 0.3) predicted BASC-2 ADHD Composite Score (Attention Problems and Hyperactivity subscales) ($R2 = 0.267$, $p = 0.043$), but did not predict ADHD diagnostic status.

On the whole brain level, higher ADHD PRS (pthreshold = 0.001) was associated with total surface area reduction ($R2 = 0.133$ pnominal = 0.013, pFDR-corrected = 0.038), following FDR correction for multiple comparisons. For subcortical structures, ADHD PRS (pthreshold = 0.2) predicted left accumbens area ($R2 = 0.291$, $p = 0.045$), left thalamus ($R2 = 0.264$, $p = 0.035$), and right caudate volumes ($R2 = 0.201$, $p = 0.031$). Right thalamus ($R2 = 0.313$, $p = 0.009$) and right hippocampus volumes ($R2 = 0.355$ $p = 0.031$) were also associated with best-fit ADHD PRSs, using pthreshold = 0.1 and pthreshold = 0.05, respectively. Associations between ADHD PRSs and cognitive-behavioral or subcortical measures were nominally significant and did not survive FDR correction.

Conclusions: Our cognitive-behavioral findings suggest that polygenic risk for ADHD is associated with inattention and hyperactivity symptoms and dysregulation in executive function, but not with ADHD diagnosis in children with NS. Our preliminary results show that ADHD PRS may predict ADHD symptomatology in NS. In the brain, we observed that higher polygenic burden for ADHD was associated with decreased total surface area and, generally, with lower subcortical volumes. Given that NS has been previously linked to ADHD symptoms and volume reductions in total surface area and subcortical structures, it is possible that ADHD risk conferred by "background" genetic factors functions as a "second hit", further contributing to behavioral and neuroanatomical phenotypes resulting from "first hit" variants of NS. Due to this study's sample size limitation (n = 18), it is important to confirm these results and the "two hits" model in larger cohorts of children with NS. Prior studies in 22q11.2 deletion syndrome, a high-risk copy number variant for schizophrenia, support the utility of PRS in assessing risk for neuropsychiatric disorders in at-risk clinical populations. This proof-of-concept study tests this notion, assessing ADHD risk in a preliminary cohort of children with NS, and illustrates the potential value of genotype-phenotype discovery in clinical prognosis and surveillance.

Keywords: Polygenetic Risk Score, ADHD, Noonan Syndrome, Structural Neuroimaging

Disclosure: Nothing to disclose.

P159. Precise Gene Methylation Analysis as a Predictor of Protein Expression and Drug Target Engagement in Fragile X Syndrome

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Background: Fragile X syndrome (FXS) is the most common single gene cause of autism and the most common inherited form of developmental disability. In recent years we and others have established that deficits in fragile X messenger ribonucleoprotein (FMRP) expression in FXS are not absolute with continuous measure of FMRP deficit measurable in blood in the majority of males and females with FXS. This includes the discovery that about half of males with FXS whose traditional Southern Blot and PCR testing FMR1 gene testing notes a fully methylated full mutation (greater than 200 CGG repeats in the FMR1 gene) make measurable amounts of FMRP in their blood. Given this finding, we have added peripheral FMRP assay to all clinical trials at our Center. In doing this we have determined that FMRP level may be a predictor of human brain electrophysiology findings and a predictor of treatment response in single-dose study of baclofen in humans with FXS. Given all of the above findings, we now need to determine the molecular genetics causes of FMRP variation in FXS as FMRP measurement in blood has become a reliable biological marker in this field.

Methods: We utilized Oxford Nanopore Sequencing (GridIon machine) to sequence the five prime untranslated region of the FMR1 gene in humans with FXS who have previously had peripheral blood FMRP quantitation and participated in a double-blind placebo-controlled single-dose challenge study of the GABA B agonist baclofen. The triple repeat 5-UTR region of FMR1 was isolated using guide RNA and Cas9 techniques as previously published with the Oxford Nanopore Technology. Read outs from these analyses include CGG repeat number and methylation status with CGG methylation counted on a methylation site by methylation site basis.

Results: We were able to successfully sequence the 5-UTR region of the FMR1 gene using a Cas9 approach and Oxford Nanopore long read sequencing technology. This sequencing included exact counting of CGG repeat status as well as most importantly the preservation of repeat methylation status using the this long read technology. As we are completing our analyses, we hypothesize that exact methylation status as determine by a ratio of methylated CGG repeats to total methylation sites

correlates strongly with FMRP level as expressed in blood. We will additionally test the hypothesis that increasing methylation rates will correlate with improvement in baclofen-associated resting EEG gamma band activity. This result would align with prior reporting that lower peripheral FMRP correlated with enhanced reduction (reduction) of excessive resting state high frequency gamma band activity with baclofen treatment.

Conclusions: Long read sequencing using the Oxford Nanopore platform provides a methodology to enhance exact description of FMR1 gene methylation status which is an advance over decades of use of Southern Blot and PCR to characterize the FMR1 gene in fragile X syndrome. We propose to utilize long read sequencing in future clinical trial subject characterization to more precisely quantity subject molecular genetic variation which may impact drug treatment response in this field.

Keywords: Fragile X Syndrome, CpG Methylation, Baclofen

Disclosure: Nothing to disclose.

P160. Environmental Stress and its Impact on Brain Network Reconfiguration Efficiency in Adolescents

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Background: Adolescence, a critical phase in human development, is marked by significant reconfiguration of brain networks. This neurodevelopmental process is susceptible to environmental stressors, which can disrupt typical brain network development and potentially lead to enduring cognitive and emotional consequences. African American adolescents often encounter elevated environmental stressors, including socio-economic hardships and racial discrimination. These experiences can exacerbate the impact of stress on the developing brain, potentially altering brain network organization.

This study aims to explore how adverse environmental stressors affect brain network organization in African American adolescents, specifically examining how stress affects task-related brain network reconfiguration. Reconfiguration efficacy is defined as the efficiency of updates in brain network organization in response to task demands. Previous research has found how smaller changes in functional connectivity (FC) between resting and task-conditions are positively related to general intelligence and cognitive performance. Our hypothesis is that exposure to adverse environmental stressors is associated with lower reconfiguration efficacy in brain network organization, which in turn are related to poorer cognitive control and executive performances.

Methods: The data presented was collected in the context of an ongoing study examining neurocircuitry changes associated with a culturally sensitive preventive intervention for risky behavior in African American youth (clinical trial number: NCT03370393). The data presented are the baseline (pre-intervention) data. Subjects were adolescents of African American heritage between the ages of 11 and 14. Subjects with a preexisting mental health condition were excluded. Sociodemographic, behavioral and neuroimaging data were collected cross-sectionally. Environmental stressors were assessed through the Adolescent Stress Questionnaire (ASQ). Magnetic resonance imaging (MRI) data were collected on a 3T scanner; functional MRI (fMRI) data were acquired during rest, and during the execution of a Go/No-go task. After the preprocessing of fMRI images, BOLD (Blood Oxygenation Level Dependent) time series were extracted from 100 cortical parcels and 16 subcortical regions. Nuisance regressors and task-related events were regressed out from the time series. Both full and partial correlation functional connectivity matrices for resting state and task-based data were then generated. To assess the similarity between the resting-state and task-based matrices, Pearson's correlation was performed between the upper matrices for each subject. Finally, a Fisher transformation was applied to the correlation coefficients. This value represented the reconfiguration efficacy, with higher values indicating higher efficiency, and vice versa. Permutation Analysis of Linear Models (PALM) was used to assess whether reconfiguration efficiency was related to ASQ levels.

Results: After excluding images with high framewise displacement (> 0.5), 85 subjects were considered for the analyses. Mean and standard deviation age was 13.19 ± 2.14, 51% were female. ASQ for the whole sample was 133.186 ± 93.56.

ASQ was negatively correlated with the reconfiguration efficiency index ($r = -.08$, $t = 9.62$, $p < .001$). In other words, higher levels of perceived stress were associated with a lower efficiency in reconfiguring and adapting the resting state networks to the execution of the Go/NoGo task.

This relationship was driven by 54 edges ($pFWE < .05$). The networks most represented were the Central Executive Network (CEN), the Default Mode Network (DMN) and the Dorsal and Ventral Attention Networks (DAN, VAN).

Conclusions: Our study suggests how environmental stress can affect the organization of brain networks during adolescence. Specifically, higher levels of stress seem to impact the magnitude of connectivity updates during high demand cognitive processes in networks involved in cognitive control and executive functioning. Future studies are needed to replicate the findings in other samples and in longitudinal studies, and to explore the behavioral and cognitive correlates of these neuroimaging findings.

Funding: This work was supported in part by grants from the National Institutes of Health (NIH) under Grants R01DA040966, R01DA058794, R01HL164823, R01 MD010757, and R01 MH108155. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Keywords: Resting and Task fMRI, Early Life Stress (ELS), Adolescence, African American

Disclosure: Nothing to disclose.

P161. The Effects of Adverse Life Events on Brain Development in the ABCD Study: A Propensity Weighted Analysis

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Background: Early life stress can have enduring effects on the brain, leading to increased risk for a multitude of psychiatric disorders and other poor health outcomes. However, longitudinal examinations of the effects of stress on human brain development are sparse. Furthermore, human observational studies are confounded by the fact that adverse exposures in childhood often occur within developmental contexts (e.g., lack of socioeconomic security) that increase the propensity to experience such events. As a result, analyses of the effects of discrete adverse events are often confounded by these other variables. Weighting individuals based on their propensity to experience stressors can balance the distribution of covariates across degrees of exposures, thereby reducing the bias in effect estimates. Here we leveraged data from the Adolescent Brain and Cognitive Development (ABCD) Study and employed a propensity score-weighted machine learning analysis to identify brain functional connections from resting-state fMRI for which their developmental change was associated with the experience of adverse events.

Methods: 7185 youth (46% female) with complete resting-state fMRI scans at both baseline (8.9-11.1 years old) and 2-year follow-

up (10.6-12.8 years old) were included. Subjects were divided into a training group (80%; n = 5,750) and a test group (20%, n = 1,435), balanced on site composition and adverse life event exposures. Using only training data, we developed a resting-state functional connectivity predictor of adverse event exposure that was subsequently evaluated in the independent testing group.

Resting-state data at baseline and 2-year follow-up included Fisher z-transformed Pearson correlation coefficients measuring: 1) the functional connectivity within and between 13 large-scale cortical brain networks; and 2) the functional connectivity between the networks and 19 anatomically-defined subcortical regions. In total, 338 connections were considered. Functional connectivity values were cleaned for effects of motion and scanner manufacturer. Next, follow-up functional connectivity values were regressed on the corresponding values at baseline to provide a measure of brain development.

The total number of adverse life events occurring between the baseline and 2-year follow-up was estimated as the number of negatively-rated life events reported in the past year on the Adverse Life Events Scale for the 1-year and 2-year follow-ups combined.

Propensity scores for the training group were calculated with the weightit() function in R, which also calculated the weights to balance the variables across the number of adverse life events. Variables used to construct propensity scores included study site, baseline functional connectivity, in-scanner motion, prior adverse life events, sex, and race.

We used elastic net regularization (lassoglm.m in MATLAB) with a 10-fold cross-validation and inverse propensity weighting of observations to predict adverse life events from functional connectivity data. Prediction accuracy was based on Spearman correlation coefficients between the actual and predicted number of events. The procedure was repeated 5 times with different cross-validation partitions to ensure stability of the results. Functional connections were designated as significant predictors if they correlated with the predicted values with a strength of r≥0.40 across iterations.

We also examined the relationship between functional connectivity predictors of adverse life events and changes in internalizing and externalizing scores on the Child Behavior Checklist over the two-year period using Pearson correlation analyses.

Results: Brain development between baseline and follow-up significantly predicted the number of adverse events during that same time period across the five iterations in both cross-validation (ρ's from 0.078-0.095, p's < 0.001) and the independent test group (ρ's from 0.088-0.091, p's < 0.001). Nineteen functional connections showed negative effects of adverse life events on development based on their moderate-to-strong (r > 0.4) correlation with the predictor, whereas four connections showed positive effects. Overall, adverse life events related to reduced network-to-subcortical functional connectivity development, particularly for the cingulo-opercular network and sensorimotor network. Increased connectivity was detected within sensorimotor networks. Although the number of adverse life events was associated with increased internalizing (ρ = 0.05, p = 0.002) and externalizing (ρ = 0.08, p < 0.001) symptoms, we unexpectedly found inverse associations between brain functional connections associated with adverse life events and development of internalizing symptoms: The correlation between the first principal component of significant functional connectivity predictors and internalizing symptoms (r = 0.05, p < 0.001) indicated that functional connectivity changes in the same direction as observed for adverse event effects was related to lesser psychopathology.

Conclusions: The current data suggest that experiences of adverse events may lead to altered development of functional connectivity of motor networks, as well as between large-scale task-related networks and subcortical brain regions. The identified brain alterations in response to discrete adverse events may reflect adaptive changes with the potential to protect against stress-related development of mood and anxiety disorders.

Keywords: Resting-state fMRI, Machine Learning, Early Life Stress (ELS), Adverse Childhood Events, Adolescent Brain Cognitive Development Study

Disclosure: Nothing to disclose.

P162. When Bigger Isn't Necessarily Better: Uncovering and Mitigating Bias in Large, Automated MRI Analyses of Brain Development

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Background: Large, population-based MRI studies of adolescents promise transformational insights into neurodevelopment and mental illness risk. Such insights could be harnessed in efforts to develop improved early recognition and treatment, outcomes that may help ameliorate the current youth mental health crisis. However, MRI studies of youth are especially susceptible to motion and other artifacts that may introduce non-random noise, and that may predispose to either false positives or negatives. Here, we conducted rigorous quality assessments of >12,000 baseline and year 2 (Y2) scans obtained from 9-12 year-old youths participating in the ongoing Adolescent Brain Cognitive Development (ABCD) study. Our goals were to (1) contrast the sensitivity of manual versus automated quality control for detecting low-quality scans; (2) demonstrate effects of rigorous quality control on risk for error in applied analyses that relate MRI and clinical phenotypes; and (3) provide quality control recommendations that balance efficiency and rigor for large-scale structural MRI analyses.

Methods: Minimally processed T1 volumes for baseline scans (age 9-10, n = 11,263, 50% male) and a semi-random, matched set of Y2 scans (age 11-12, n = 1,000) were downloaded from the NIMH Data Archive (NDA; ABCD release 4.0) and pre-processed in Freesurfer v7.1. Scans were visually inspected by trained raters and evaluated on a 4-point manual quality control (MQC) scale based on overall appearance and presence of segmentation and other errors ("1"=minimal errors; "2"=moderate errors; "3"=substantial errors; "4"=unusable). Scans that failed preprocessing or demonstrated cysts >1cm were removed from analyses. MQC ratings were compared to automated "use/do not use" designations posted at the NDA as well as to surface hole number (SHN), an automated measure of scan quality provided by Freesurfer based on the Euler number. Association of structural MRI indices with MQC and SHN were assessed using linear regression, adjusting for scanner, study site, age, sex, and intracranial volume. Next, associations between baseline cortical measurements (thickness, volume) and clinical measures (age, externalizing symptoms) were examined based on MQC-based thresholds of inclusion (i.e., most liberal = "1" to "4"; most conservative = "1" only). Finally, a randomly selected subset of 180 scans with better MQC ratings (N = 150 "1", N = 30 "2") underwent manual cortical edits by trained technicians, to determine effects of these edits on cortical thickness and volume measurements.

Results: Among baseline scans, 45% received a "1" MQC rating, 40% received a "2", 13% received a "3", and 2% received a "4". We have posted these ratings to the NDA (ID #1944). Of "3" and "4"

scans, 11% and 49% respectively received "do not use" designations. We uncovered bias in structural MRI measurements arising from the 55% of the sample with suboptimal image quality, such that poorer quality scans tended to deflate thickness and inflate surface area (Cohen's d = 0.14-2.84 across 68 regions-of-interest). Further, these biases impacted applied analyses relating structural MRI and clinical measures. Inclusion of lower quality scans tended to increase false negative age-thickness associations (Type II error) and to increase false positive volume-externalizing psychopathology associations (Type I error); for example, of 43 cortical regions with significant associations between volume and psychopathology in the entire sample (n = 10,257), only 24 retained significance when using "1" and "2" scans only (n = 8,674), and only 3 retained significance when using "1" scans only (n = 4,617). SHN correlated strongly with MQC ratings (rho = 0.59), and mean SHN differed between all MQC level pairs (p's≤1.02E-121). Three SHN cutoff levels were optimized based on receiver operating characteristic curves of MQC ratings; these cutoffs reproducibly identified lower-quality scans with good specificity (0.81-0.93 at Baseline, 0.88-1.00 at Year 2). Inclusion of SHN as a covariate partially mitigated quality-related bias in primary measurements (i.e., thickness, volume) but not in applied analyses. Correction of segmentation errors with manual edits reproducibly altered thickness measurements across much of the cortex (d = 0.15-0.92) and strengthened age-thickness associations (d = 0.10, p = .024).

Conclusions: The results demonstrate that inadequate QC of youth MRI scans can undermine advantages of large sample size to detect meaningful associations. However, image quality-related biases can be mitigated through additional automated (SHN) and manual (cortical edits) interventions.

Keywords: Structural MRI, ABCD Study, Neurodevelopment, Quality Control, Big Data Analysis

Disclosure: Nothing to disclose.

P163. Impact of Early Life Adversity and Effects on Metabolism and Energy Balance

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Background: Early life adversity has enduring effects on neuroendocrine function and behavior and increases susceptibility to later-life psychopathology and chronic metabolic disorders. Stress and metabolism are biologically intertwined, and the sensitive periods for effects of early life stress overlap with sensitive periods for metabolic programming. In rodents, neural circuity from the arcuate nucleus to other hypothalamic regions governing appetite and energy regulation are immature at birth and are driven partly by a postnatal leptin surge occurring from approximately postnatal day (P) 6-11. This early postnatal period is also a stress hyporesponsive period characterized by reduced ACTH and corticosterone response to stress. Early life adversity during the development of hypothalamic neural circuitry may not only alter stress physiology, but also affect maturation of metabolic systems by disrupting energy balance.

The limited bedding and nesting material (LBN) paradigm in rodents is a pre-clinical model of early life adversity. LBN changes offspring stress response, social behavior, and cognition in adulthood, but acute effects of LBN on metabolic development are not well explored. LBN induces more fragmented maternal care primarily attributed to maternal stress caused by the low-resource environment. However, early adversity through LBN may also change maternal milk composition and nest temperature which may contribute to LBN effects on pup development. The current study uses LBN to explore the effects of early adversity timing (before or during the postnatal leptin surge) on pup metabolic markers, dam milk composition, pup body temperature, and maternal behavior.

Methods: Long-Evans dams and pups were randomly assigned to LBN or control conditions on postnatal day (P) 1-6. Pups were cross fostered to the same or different condition on P6-13, creating four groups: Control-control (n = 10), Control-LBN (n = 9), LBN-Control (n = 10), and LBN-LBN (n = 10). One male and one female pup from each litter was implanted with a passive integrated transponder chip to monitor core body temperature in the home cage on P3 or P6 and temperature reads were taken eight times per day for the remainder of the experiment. Home cage behavior was monitored with 24-hour side-view video recordings on P3 and P7 and analyzed with an automated analysis pipeline (AMBER). In the AMBER pipeline, pose estimation data from DeepLabCut models trained to track dams and pups is used with Simple Behavior Analysis software behavior classifiers trained to identify seven maternal behaviors with high accuracy (all F1 > .95). Milk was collected from dams on P6 and P12 and concentrations of lactose, total protein, triglycerides, cortisol, and leptin were measured with ELISA. Serum and brain tissue were collected from male and female pups on P13. Serum metabolic hormones levels were determined with a metabolic multiplex assay. Data was analyzed using linear mixed models to test for the effects of P0-6 LBN, P6-13 LBN, pup sex, and pup age with cohort and litter ID included as random factors for pup measures and to test for effects of LBN and litter age with cohort and litter ID included as random factors for dam measures.

Results: Dams in the LBN condition spent more on the nest, licking and grooming pups, and nursing pups, had shorter intervals between bouts, and had more behavioral transitions on P3 and P7 (p < .05). On P7, dams in the control condition that received pups that were previously in LBN on P0-6 had longer nursing bout lengths compared to control dams that received pups that were previously in the control condition on P0-6 (p < .05). Total protein, lactose, leptin, and corticosterone concentrations in dam milk on P6 and P12 was not affected by dam exposure to LBN, although milk lactose concentration was higher on P12 compared to P6 (p < .05). However, dams exposed to LBN had elevated milk triglyceride levels on P6 but not on P12 (p < .05).

On P6, pups exposed to LBN weighed less than pups in the control condition (p < .05) and on P13, pup exposed to LBN P0-6 and LBN P6-13 weighed less than pups in the other three groups (p < .05). Pup core body temperature increased with pup age, was higher during dam nest attendance, was higher during active nursing, and was lower in pups in the LBN condition compared to control pups at time of measurement (p < .05). P13 pup serum levels of brain-derived neurotrophic factor, glucagon, insulin, c-peptide, amylin, pancreatic polypeptide, peptide tyrosine tyrosine, Il-6, glucagon-like peptide -1, and leptin were reduced by LBN exposure on P0-6, but only leptin was also affected by LBN exposure on P6-12 (p < .05). Male pups had lower serum concentrations of insulin, C-peptide, glucagon-like peptide -1, pancreatic polypeptide, peptide YY, leptin, and glycogen and higher serum concentrations of growth hormone compared to female pups (p < .05).

Conclusions: These data suggest that early adversity before the postnatal leptin surge has stronger effects on metabolic programming. Although LBN dams spent more time nursing, had longer nursing bouts, and supplied milk with higher caloric density on P0-6, LBN during that time reduced levels of several metabolic hormones and slowed pup weight gain, perhaps partly due to increased energy demand to maintain body temperature in the LBN condition. These findings demonstrate multifaceted effects of early adversity that may contribute to increased vulnerability to chronic metabolic disorders and disrupted endocrine function later in life.

Keywords: Early-Life Adversity, Maternal Behavior, Energy Metabolism, Temperature Regulation, Macronutrient

Disclosure: Nothing to disclose.

P164. Exposomic and Genomic Contributions to Allostatic Load in Youth

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Background: The concept of allostatic load (AL) captures the biological burden that environmental stress imposes on an individual over time. We aimed to test environmental and genetic contributions to AL in a large sample of genotyped youth who were deeply phenotyped for environmental stress exposures and for physiological and biochemical measures. We hypothesized that exposure to environmental childhood adversity, as well as genetic susceptibility to both metabolic disorders and to stress-related disorders, will be associated with greater AL during adolescence.

Methods: We analyzed data on youth who participated in the Adolescent Brain Cognitive Development (ABCD) Study for whom there was available data on anthropometric and biological measures (N = 4,545, mean age 12, 47.5% female). AL was calculated for participants at age 12 through a factor analysis of measures that were previously related in the literature to AL in adolescence, including body mass index, waist circumference, blood pressure, blood Hemoglobin A1C (as an indicator for blood sugar levels), blood cholesterol (LDL and HDL), and salivary DHEA. Structure of allostatic load variables was first determined by EFA using least-squares extraction and oblimin rotation. The number of factors to extract was determined by interpretability and the minimum sample-size adjusted Bayesian information criterion (BIC). To obtain an overall estimate of allostatic load, we estimated a bifactor model in which all variables load on both a specific factor (e.g., systolic and diastolic on "blood pressure") and a general (overall) AL factor. Poly-environmental adversity was modeled with a single exposomic risk score derived from a bifactor model that captured the totality of environmental exposures collected on participants at a previous ABCD Study assessment wave (mean age 11). Genetic susceptibility was modeled using polygenic risk scores of type 2 diabetes and of major depressive disorder using summary statistics of respective GWAS in independent (non-ABCD) samples. Associations between exposomic and polygenic risks with AL were tested in linear mixed-effects models with the general AL factor as the dependent variable with random intercepts for study site and family-relatedness. Models adjusted for age, sex, race, ethnicity, household income, and parent education. G X E interactions were introduced in separate models as the product of exposomic and polygenic scores. Models that included polygenic scores were limited to include participants of European ancestry (n = 2,745) due to limited availability of diverse ancestry GWAS needed to calculate polygenic scores.

Results: Exposomic risk score was significantly associated with the general AL score a year later in the entire study population (estimate = 0.16, 95%CI 0.10-0.21, P < .001). Polygenic risk score of both type 2 diabetes and of major depressive disorder were independently associated with the general AL score in the European ancestry participants (estimate = 0.11, 95%CI 0.08-0.14, P < .001 and estimate = 0.04, 95%CI 0.00-0.08, P = .03). For both polygenic scores, there was a significant exposomic by polygenic score interaction, whereby participants with greater polygenic scores showed a stronger association between exposomic risk and AL (both P's = .025). Sensitivity analysis using polygenic scores of other metabolic (body mass index PRS) or psychiatric (cross-diagnostic PRS) yielded similar findings as in the main analyses.

Conclusions: We show that AL, a construct that was conceptualized through a theoretical framework of the biological embedding of stress, can be empirically modeled in youth using anthropometric and biological measures. We provide internal validity for AL by showing that it maps to measurements of earlier environmental stress and of genetic susceptibility to both metabolic and psychiatric conditions, as hypothesized based on theory. The significant G X E interaction in association with AL may suggest that polygenic scores and environmental factors should both be considered in models of stress related health conditions.

Keywords: Allostatic Load, Exposome, Polygenic Risk Score, Early Life Stress (ELS), Gene Environment Interaction

Disclosure: Taliaz Health: Advisory Board, Stock / Equity (Self).

P165. Relationship Between the Dysregulation of Steroid Hormone Pathways and the Development of Adolescent Depression

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Background: Major depressive disorder affects approximately 13% of adolescents in the United States. While classical antidepressant treatments effectively reduce depressive symptoms in adolescents, low remission rates and potential adverse effects, such as suicide-related thoughts, highlight the need for the development of novel pharmacotherapies for the treatment of adolescent depression. During adolescence, there is a surge in steroid gonadal hormones that impact behavior and brain functions in both humans and mice. The combination of stress and increased gonadal hormones in adolescents is associated with adolescent depression and suicidality. We hypothesize that the increased risk of depression in adolescents is due to the combined effects of increased stress and heightened production of gonadal hormones in both sexes and that targeting gonadal steroid hormone systems could serve as an intervention for adolescent depression and suicidality.

Methods: Adolescent male (n = 10) and female (n = 10) mice were exposed to acute foot-shock stress and assessed the development of anhedonia, social preference deficits, and anxiety-like behavior using the sucrose preference test, social interaction test, and light/dark box test, respectively. Behavioral data were analyzed with two-way ANOVA followed by Holm-Sidak test when appropriate. To investigate the role of gonadal hormone systems in these effects, we conducted bioinformatic analysis of publicly available RNAseq data (GSE101521) on the prefrontal cortex of both sexes of depressed patients, comparing adults (n = 10) and adolescents (n = 5) as well as non-depressed adolescents (n = 7) with depressed adolescents (n = 5). Gene ontology and Pathway enrichment analysis were performed using Biojupies and p values were corrected using the False Discovery Ratio analysis (adjusted $p < 0.05$ to reach significance).

Results: Stress exposure in adolescent mice resulted in social interaction deficits ($p = 0.009$), but no changes were observed in anxiety behavior or anhedonia. Gene ontology analysis for biological processes of RNAseq data from the prefrontal cortex revealed a significant downregulation in cholesterol metabolic ($p < 0.001$) and biosynthetic processes ($p < 0.001$) in adolescent depressed patients compared to non-depressed adolescent controls. Cholesterol serves as the precursor for most steroid

gonadal hormones. Additionally, pathway enrichment analysis indicated downregulation in steroid biosynthesis in depressed adolescent patients compared with non-depressed adolescents ($p < 0.001$). Similarly, comparison between depressed adults and depressed adolescent patients revealed a downregulation of the estrogen receptor binding in the Gene ontology analysis for molecular function ($p = 0.001$).

Conclusions: In contrast with our hypothesis these findings suggest that downregulation in steroid gonadal hormone synthesis during adolescence elevates the risk of developing depression. Consequently, targeting these systems could represent a novel strategy for treating adolescent depression.

Keywords: Adolescent Depression, Neurosteroid, Prefrontal Cortex

Disclosure: Nothing to disclose.

P166. Acetate Supplementation Rescues Social Deficits and Alters Transcriptional Regulation in Prefrontal Cortex of Shank3 Deficient Mice

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Background: The pathophysiology of autism spectrum disorder (ASD) involves genetic and environmental factors. Mounting evidence demonstrates a role for the gut microbiome in ASD, with signaling via short-chain fatty acids (SCFA) as one mechanism. Here, we utilize mice carrying deletion to exons 4-22 of Shank3 (Shank3KO) to model gene by microbiome interactions in ASD. We identify SCFA acetate as a mediator of gut-brain interactions and show acetate supplementation reverses social deficits concomitant with alterations to prefrontal cortex (mPFC) transcriptional regulation independent of microbiome status.

Methods: Shank3KO and wild-type (Wt) littermates were divided into control, Abx, Acetate and Abx+Acetate groups upon weaning. After six weeks, animals underwent behavioral testing. Molecular analysis including 16S and metagenomic sequencing, metabolomic and transcriptional profiling were conducted. Additionally, targeted serum metabolomic data from Phelan McDermid Syndrome (PMS) patients (who are heterozygous for the Shank3 gene) were leveraged to assess levels of SCFA's relative to ASD clinical measures.

Results: Shank3KO mice were found to display social deficits, dysregulated gut microbiome and decreased cecal levels of acetate – effects exacerbated by Abx treatment. RNA-sequencing of mPFC showed unique gene expression signature induced by microbiome depletion in the Shank3KO mice. Oral treatment with acetate reverses social deficits and results in marked changes in gene expression enriched for synaptic signaling, pathways among others, even in Abx treated mice. Clinical data showed sex specific correlations between levels of acetate and hyperactivity scores.

Conclusions: These results suggest a key role for the gut microbiome and the neuroactive metabolite acetate in regulating ASD-like behaviors.

Keywords: Autism Spectrum Disorder, Microbiome, Metabolomics, Social Deficits, Transcriptomics

Disclosure: Nothing to disclose.

P167. Patient-Device Interactions in a Digital Therapeutic for Inattention Predict ADHD-Related Clinical Outcomes: Replication Across Three Independent Trials of AKL-T01

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Background: An advantage of digital therapeutics (DTx) is the ability to conduct real-time assessment of mental health symptoms via the collection of high dimensional data on patients as they engage with treatment. However, extracting meaningful clinical information from patient-device interactions is complex, requiring theoretical mapping of data from these interactions onto the therapeutic properties of the DTx, as well as validation with clinical outcomes. AKL-T01 is a videogame-like DTx that is FDA-authorized for children ages 8-12 with ADHD. AKL-T01 targets attention and cognitive control by generating cognitive conflict at dynamically updated levels of difficulty during multitasking (navigating a ship while tapping in response to target creatures and ignoring distractors), which is thought to enhance the sustained efficiency of frontoparietal networks. Clinical trials of AKL-T01 support its efficacy in children, adolescents, and adults for improving attentional functioning. However, there is a need to better understand how AKL-T01 patient-device interactions can be used to measure changes in attentional functioning over time. In the present study, we aimed to (1) derive a cognitive metric from AKL-T01 patient-device interactions that is grounded in theoretical models of attention, can be measured in real-time, and follows a predictable temporal pattern across subjects, and (2) validate this metric by evaluating its associations to clinical outcomes across three independent clinical trials.

Methods: Trials of AKL-T01 included: STARS-ADHD-Adult (NCT05183919), a 6-week trial in adults 18 and older ($n = 221$ enrolled; M age = 39.9; 70% female); STARS-ADHD-Adolescent (NCT04897074), a 4-week trial in adolescents ages 13-17 ($n = 162$ enrolled; M age =14.4; 41% female); and STARS-ADHD (NCT02674633), a 4-week trial in children ages 8-12 ($n = 180$ enrolled; M age = 9.7; 31% female). All participants had a diagnosis of ADHD and demonstrated impairment in attentional functioning. The primary clinical outcome was the Test of Variables of Attention - Attention Comparison Score (TOVA-ACS), an objective, computerized measure of attentional functioning. In the adult study, we also measured ADHD-related quality of life on the Adult ADHD Quality of Life (AAQoL) rating scale.

We extracted measures of targeting response speed, targeting accuracy (d-prime), and navigation skill level (80% psychometric threshold). After rescaling, we combined the two targeting variables using their vector norm, then averaged the intermediate targeting variable and navigation threshold variable to form the cognitive metric. At the group-level, the cognitive metric increased smoothly and logarithmically over missions played, suggesting initial rapid improvement followed by more gradual improvement. To capture this pattern at the individual patient level, we fit a Bayesian hierarchical linear-log model to each individual's gameplay data across the 4-6 week treatment. Cognitive metric change scores were computed using subject-level model parameters. To validate the cognitive metric, we analyzed the relationship between cognitive metric change and clinical outcome change. We regressed the change in TOVA-ACS (baseline to study endpoint) on the change in the cognitive metric (initial measurement to final score), controlling for TOVA-ACS baseline, cognitive metric baseline, age, and sex. In the adult data, we conducted a similar model with AAQoL change as the outcome.

Results: The Bayesian hierarchical linear-log model converged properly and fit well to individual subject data, and the vast majority of subjects showed a consistent logarithmic trend in the cognitive metric over the course of treatment. Increases in the cognitive metric significantly predicted increases in TOVA-ACS in the adult ($\beta = .16$, SE = .04, $p < .001$), adolescent ($\beta = .09$, SE = .03, $p = .007$), and pediatric ($\beta = .06$, SE = .02, $p = .014$) trials. The magnitude of the effect was largest in adults ($F2 = .18$), followed by adolescents ($F2 = .08$), with the smallest effect size observed in the pediatric trial ($F2 = .05$). In the adult data, increases in the cognitive metric were also related to statistically significant increases in self-reported quality of life ($\beta = .25$, SE = .11, $p = .020$), with a small effect size ($F2 = .04$).

Conclusions: Findings support the clinical validity of a real-time measure of attention derived from AKL-T01 patient-device interactions, with replication across the lifespan providing increased confidence in effects. We saw the strongest association between the cognitive metric and TOVA-ACS in the adult population, followed by adolescents, and then children; this pattern mirrors the magnitude of TOVA-ACS change reported in each of the clinical trials, with a nearly 7x improvement in adults and a nearly 3x improvement in adolescents relative to the pediatric trial. Given that we sampled patients with high levels of attentional impairment, it will be important for future studies to evaluate the generalizability of the cognitive metric derived here in more heterogeneous samples. Nevertheless, the present findings underscore the potential for DTx to use information from patient-device interactions to provide in-vivo monitoring of cognitive changes during treatment.

Keywords: ADHD, Digital Assessment, Mobile Technology, Attention, Digital Therapeutic

Disclosure: Akili Interactive Labs: Employee, Stock / Equity (Self) Google: Employee, Stock / Equity (Self).

P168. Stress Buffering in Adolescents at Risk for Suicide: An Ecological Momentary Assessment Study

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Background: Adolescent suicide is a public health emergency, as rates of suicide are steadily increasing. Thus, there is an urgent need to better understand factors that potentiate risk acute for suicidal thoughts and behaviors (STB) among adolescents. Interpersonal theories of suicide have consistently posited that stress exposure may contribute to greater STB risk, while social support is a protective factor. Yet, prior research is limited, as most adolescent STB research has not captured how social contexts can affect dynamic changes in stress among high risk samples. In particular, gaps in knowledge exist as to whether time spent with peers and caregivers can serve as a short-term protective factor against heightened stress and negative affect among youth at risk for STB. Further, it is unclear whether such reductions in stress and affect buffer against subsequent STB. As acute levels of stress can change rapidly over days or even hours, identification of factors protecting against stress requires repeated and frequent assessments. Given prior work linking social stress with STB, we hypothesized differences between psychiatric controls and participants with STB history in associations between being alone (versus with others) and stress.

Methods: Adolescents ages 13-18-years-old (N = 185) reporting depressive, anxiety, and/or substance use disorders were recruited from psychiatric outpatient programs, emergency departments, medical center research registries, and social media. 66% were experiencing current suicidal ideation, with 27% reporting a past year attempt. STB and symptom assessment interviews were completed at baseline, 1-, 3-, and 6-month follow-up visits. Additionally, smartphone-based ecological momentary assessment (EMA) was used to probe 1-week bursts at each study visit to assess time spent with peers and caregivers (versus being alone) over the past several hours, as well as momentary stress, positive affect, and negative affect. During EMA bursts, participants received prompts 4X/day on weekdays and 7X/day on weekends. Multilevel linear and logistic regression models were used to test proximal associations between time spent with caregivers/peers and stress, as well as associations with suicidal ideation.

Results: Multilevel regression models indicated that adolescents reporting current suicidal ideation or attempts in the year prior to the baseline visit spent a higher proportion of time alone compared to psychiatric controls ($z = -2.4$, $p = 0.015$), and reported greater stress ($t(180.81) = 2.82$, $p = .005$) during EMA periods. Across the whole cohort, participants reported reduced stress ($t(156.78) = -3.18$, $p = .002$), reduced negative affect ($t(134.05) = -5.32$, $p < .001$), and increased positive affect ($t(170.05) = 11.8$, $p < .001$) when spending time with peers or caregivers (at the same prompt), relative to being alone. Being with others was also associated with reduced stress ($t(49.34) = -2.64$, $p = .011$), reduced negative affect ($t(101.89) = -3.30$, $p = .001$), and increased positive affect ($t(64.12) = 4.11$, $p = < .001$) at the following prompt. Preliminary findings indicated that adolescents with current ideation and past year attempts showed smaller decreases in stress relative to psychiatric controls during time spent with caregivers or peers ($t(90.23) = 1.99$, $p = .049$). However, no differences in stress or the influence of social context were identified between adolescents reporting current suicidal ideation versus past year attempts. While between-participant differences in stress ($t(107.77) = 2.31$, $p = .023$) and proportion of time spent alone ($t(160.99) = 3.08$, $p = .002$) were associated with suicidal ideation over the 6-month study window, within-participant changes in such factors were not associated with concurrent change in suicidal ideation.

Conclusions: Our findings underscore the importance of evaluating caregiver and peer relationships among adolescents at risk for STB. Future work will explore differential buffering by peers versus caregivers on stress and associated STB risk, as well as the short-term prediction of suicidal behaviors.

Keywords: Adolescent, Suicide, Stress, Suicidal Ideation, Ecological Momentary Assessment

Disclosure: Nothing to disclose.

P169. Bringing Research to the People: Initial Feasibility, Acceptability, and Reliability of Cognitive, Molecular, and Neurophysiological Testing Completed in the Home of Individuals With Fragile X Syndrome

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Background: Current research studies conducted at Cincinnati Fragile X Center have been limited to individuals who can

participate in traditional research visits at the hospital. Numerous factors, including those directly related to the Fragile X phenotype (e.g., clinically significant anxiety, irritability, challenging behaviors) and those associated with marginalized backgrounds (e.g., low SES, rurality), prevent many families from participating in traditional research visits. Thus, conducting at-home research visits ("home visits") is necessary to include the most representative sample of FXS. No FXS research study, to date, has conducted at-home blood draws and/or EEG testing.

Methods: We demonstrate high feasibility and acceptability of home visits, with overwhelmingly positive feedback from the parents and participants themselves via informal communication and formal survey data. Test-retest reliability is $r = 0.94$ and $r = 0.91$ for nonverbal and verbal IQ raw scores, respectively. EEG data quality is medium to high for all 10 participants, with 3 participants previously providing unusable data due to excessive movement and behaviors interfering with data collection. Test-retest values for individualized peak alpha frequency and relative power of frequency bands will be provided at time of ACNP. Blood was collected from 8 of 10 participants. One participant declined (as did previously) and we could not collect from one other participant due to scheduling conflict with research nurse. FMRP values with our optimized assay show high test-retest reliability at $r = 0.99$, and mRNA concentrations will be analyzed by time of ACNP.

Results: We demonstrate high feasibility and acceptability of home visits, with overwhelmingly positive feedback from the parents and participants themselves via informal communication and formal survey data. Test-retest reliability is $r = 0.94$ and $r = 0.91$ for nonverbal and verbal IQ raw scores, respectively. EEG data quality is medium to high for all 10 participants, with 3 participants previously providing unusable data due to excessive movement and behaviors interfering with data collection. Test-retest values for individualized peak alpha frequency and relative power of frequency bands will be provided at time of ACNP. Blood was collected from 8 of 10 participants, 1 declined (as did previously) and 1 could not collect due to scheduling conflict with research nurse. FMRP values with our optimized assay show high test-retest reliability at $r = 0.99$.

Conclusions: Thus far, we demonstrate high feasibility, acceptability, and test-retest reliability of home data collection of cognitive, molecular, and neurophysiological data in individuals with FXS. This pilot study provides critical data to support larger scale efforts of collecting a multi-modal battery of testing within the home as well as opens possibility of future home-based or hybrid clinical trials. By reducing barriers to research participation, we will be able to better capture the full spectrum of participants with FXS in our research studies, thus better understanding the molecular and neurophysiological mechanisms underlying this disorder.

Keywords: Fragile X Syndrome, Electroencephalography (EEG), Molecular Profiling, Diversity and Inclusion

Disclosure: Nothing to disclose.

P170. Model Driven Patient Selection Using Eye-Tracking Features for Improved Efficacy in a Phase 2 Trial in Autism Spectrum Disorder With JNJ-42165279, a Fatty Acid Amide Hydrolase (FAAH) Inhibitor

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Background: Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by impairments in social communication, and the presence of restricted and repetitive behavior. There are currently no medications approved for the treatment of core symptoms of ASD.

JNJ-42165279 is a potent, selective, and orally bioavailable inhibitor of FAAH. In a phase 2, placebo-controlled study (NCT03664232) efficacy of JNJ-42165279 was compared with placebo in the improvement on core symptoms of ASD during the 12 weeks of treatment on the Autism Behavior Inventory (ABI) as the primary objective. The secondary objectives include assessing the effect of JNJ-42165279 compared with placebo on the Social Responsiveness Scale (SRS-2) total score.

Eye-tracking (ET) features discriminate between autism spectrum disorder (ASD) and typically developing (TD) groups across paradigms, reliably showing gaze abnormalities in attention to social versus nonsocial stimuli. In this abstract, we show improved efficacy in patients enriched using ET features of JNJ-42165279 as measured with the secondary outcome measures, namely, SRS-2 total score.

Methods: Baseline ET features related to gaze and focus during different tasks such as activity monitoring, visual exploration, and social orienting were obtained from two large-scale studies, namely, ASD002 (ASD ($N = 144$) and TD ($N = 41$)), NCT02668991 and AUT0002 (TD) $N = 55$. The distribution of age and gender across these studies were matched. Extreme Gradient Boosting (XGB) machine learning model was trained to distinguish ASD (positive class) from TD (negative class) using ET features capturing gaze and attention patterns. A repeated cross-validation procedure with stratified split (5 folds, 10 repeats) was used to evaluate the model. The features included in stratification across folds were age, gender, and class distribution (ASD vs TD). trained model predicted a group of patients as ASD (positive class) in the independent test dataset, namely, AUT2001 phase 2 trial, NCT0366423 ($N = 28$) using baseline ET features. A bootstrapping approach was applied by randomly sampling patients (originally included in the study using the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2)) to match the number of patients selected by the model to provide a balanced assessment of efficacy. The effect size (measured by Cohen's d) of change in SRS-2 total score at week 12 compared to baseline was assessed between the ASD patients selected by ADOS-2 and ADOS-2 + model driven patient selection.

Results: The XGB model achieved a mean cross validation balanced accuracy of 0.64 across 5 folds and 10 repeats. The ADOS-2 + model driven patient selection achieved a Cohen's d (baseline-endpoint) of 0.8 compared to a mean of 0.5 from bootstrapping.

Conclusions: These results indicate that the ADOS-2 + model driven patient selection approach employing ET features for enrichment achieved a better efficacy (~0.3 more in Cohen's d) compared to ADOS-2 only patient selection. These findings highlight the advantage of using more objective measure-based patient selection in developing a precision medicine approach in ASD trials. As the model driven approach is based on ET features, it provides a unique opportunity for scalable deployment in future clinical trials to recruit the appropriate patient population for potential success.

Keywords: Predictive Biomarker, Eye Tracking, Autism Spectrum Disorders

Disclosure: Johnson and Johnson: Employee (Self).

P171. Novel Measures of Social Determinants of Health and Associated Child Brain and Body Outcomes

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Background: Health inequities in children are predominantly influenced by social determinants of health (SDoH). Despite their significance, in-depth studies that identify SDoH patterns from high-dimensional factors and analyze their association with child development remain limited.

Methods: A population-based cohort study was conducted involving children aged 9 to 10 years, participating in the Adolescent Brain Cognitive Development study from September 1, 2016, to April 24, 2021, across 21 sites in 17 U.S. states. 84 neighborhood-level variables spanning seven different SDoH domains were included, and hierarchical agglomerative clustering was utilized to detect SDoH patterns. Developmental outcomes such as mental health, suicidal behaviors, cognitive function, and physical health outcomes were measured.

Results: Among 10,504 children, we identified four distinct SDoH patterns: Affluence, Structural Discrimination, Socioeconomic Deprivation, and High Crime/Drug Sales, Low Education, and Populated. Children in the Socioeconomic Deprivation pattern displayed the most adverse health profiles, including higher mental health problems, suicidal behaviors, lower cognitive performance, and adverse physical health. Conversely, the Affluent Communities pattern showed better health conditions, while the Structural Discrimination and High Crime/Drug Sales pattern indicated moderate negative correlations with cognitive abilities and poor physical health outcomes.

Conclusions: Our research identified four unique, multidimensional SDoH patterns and their correlations with child development. Children exposed to socioeconomic deprivation suffered the most severe adverse developmental outcomes across mental, cognitive, and physical health domains. The enhancement of social policies to alleviate socioeconomic deprivation is critical to improving child development.

Keywords: Social Determinants of Health Inequity, Mental Health and Health Disparities, Children and Adolescents, Machine Learning Clustering

Disclosure: Nothing to disclose.

P172. Altered Metabolite and Microbiota Profiles Associated With Behavioral Phenotypes in Autistic Individuals and Their Unaffected Siblings Compared to Typically Developing Controls

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Background: Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder with impaired social communication and interactions and restricted repetitive behaviors/interests. Despite extensive research, the underlying pathogenic mechanisms of ASD remain unclear, resulting in limited information about biomarkers, effective early detection, and treatment for its core symptoms. Recent attention has turned to the role of gut-brain axis in neuropsychiatric disorders and gastrointestinal (GI) symptoms in autistic individuals, suggesting a potential link between ASD and the microbial environment. Various studies (Korteniemi et al., 2023) and our recent investigation in a sample of 82 boys and young men with ASD and 31 typically developing controls (TDC) aged 6-25 (Chen et al., 2022) have highlighted metagenomic dysregulation as associated with behavioral problems in ASD. However, its connection to GI symptoms has not been established (Chen et al., 2022). Research on the gut microbiome's relation to ASD has yielded inconsistent results, highlighting the disorder's complexity and the need for more sophisticated experimental designs (Dan et al., 2020, Korteniemi et al., 2023). Since the composition of microorganisms is significantly influenced by geographical and cultural factors, there is a lack of data from East Asian populations in this context. To address these gaps, we investigated the microbial profiles among individuals with ASD, their biologically healthy unaffected siblings, and TDC within a substantial sample from a single site, National Taiwan University Hospital, Taipei, Taiwan. By investigating the gut microbiota and its correlations with clinical presentation across the three groups, we hope to identify potential markers and protective factors associated with ASD in this specific population.

Methods: We conducted a longitudinal follow-up study involving 239 autistic individuals (M:F = 200: 39, mean age ± SD = 12.1 ± 6.2), 102 unaffected siblings (M:F = 52:50, age = 12.0 ± 5.8), and 81 TDC (M:F = 54:27, age = 13.8 ± 5.9), aged 4 to 25 years, with multi-dimensional assessments including diagnosis (Autism Diagnostic Interview-Revised, Autism Diagnostic Observation Scale, Kiddie SADS-E), behavioral phenotypes (Social Responsiveness Scale, SNAP-IV, Child Behavioral Checklist), neuropsychological tests, brain images, microbiomes (stool samples) and metabolomics (blood samples). We have completed the Time 1 data collection. The stool samples underwent 16S rRNA amplicon library construction and Illumina V3V4 sequencing analysis. We performed taxonomic diversity analysis, microbiota differential abundance analysis, and pathway analyses to identify variations in microbial composition. We used QIIME2 and R software with a linear mixed-effect model to correlate microbiota profiles with gastrointestinal symptoms, autistic symptoms, and emotional/behavioral problems across the three groups. The significance level was set at the FDR-corrected q value < 0.05.

Results: Autistic individuals exhibited more severe gastrointestinal symptoms, social deficits, and a wide range of emotional and behavioral symptoms than unaffected siblings and TDC. Despite no group differences in F/B ratios, unaffected siblings demonstrated higher alpha diversity than ASD and TDC. The highest beta diversity was observed in ASD, followed by unaffected siblings and TDC, the least. The gut microbiome of ASD contained a higher species level of relative abundance of Lactobacillus mucosae, Lactococcus garvieae subsp garvieae, Clostridium baratii and butyricum than unaffected siblings and TDC. The gut microbiome of unaffected siblings showed a higher relative abundance of species, including Methanobavteriaceae, Methanobacterials, Deferribacterales, Deferribacteraceae, and Mucispirillum, than ASD and TDC. Using multiple response permutation procedure and Adnosis, ASD vs. TDC was the only significant comparison in microbiome structural community. The unaffected siblings carried functions primarily associated with metabolism, organismal systems, human diseases, and environmental information processing. Many of these pathways were upregulated in unaffected siblings compared to ASD and TDC. The abundance of microbiota was associated with the concentration of L- asparagine, glucosamine 6- sulfate, and DL- glutamate, with higher expression levels in ASD than unaffected siblings and TDC. Further, the total SRS score significantly positively correlated to the abundance of Lactobacillus mucosae and Lactococcus garvieae subsp garvieae in ASD and unaffected siblings. Finally, L-asparagine was associated with altered microbiota and the severity of social deficits in ASD and their siblings.

Conclusions: Our study revealed distinct microbial and metabolomic compositions in ASD and their unaffected siblings. Importantly, we established a correlation between behavioral symptoms and altered microbiota profiles, mainly involving amino

acid and protein metabolism demonstrated in the peripheral circulation. Moreover, specific microbiota in autistic individuals and unaffected siblings are associated with clinical presentation and social deficits, suggesting that gut microbiota dysbiosis could influence behavioral phenotypes. The microbiome, primarily serving metabolic functions, shows promising potential in developing treatments for ASD.

Keywords: Autism Spectrum Disorder, Intestinal Microbiome, Sibling Design, Gut-Brain Axis

Disclosure: Nothing to disclose.

P173. Possible Sensitization Effects of Amphetamine Treatment in Drug-Naive Youth With ADHD

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Background: Emerging evidence of sensitization following stimulant administration in humans represents a challenge for understanding the relationship between stimulant treatment for attention-deficit/hyperactivity disorder (ADHD) and the subsequent development of substance use disorders (SUD). We have proposed a hybrid model – that the majority of youth with ADHD greatly benefit from treatments with stimulants, which are in turn protective against the development of SUD; however, a much smaller number may have heightened vulnerability, based on altered responsiveness of the brain reward system either at baseline or in response to stimulant treatment. To investigate this model, we used functional neuroimaging paired with ratings of reward sensitivity and sensation-seeking to assess changes in activation of the brain reward system and behavioral indices thought to underlie SUD risk in drug-naïve youth at high and low risk for SUD before and after treatment with mixed amphetamine salts extended release (MAS-XR; i.e., stimulant medication).

Methods: We recruited 14 drug-naive youth ages 7-12 diagnosed with ADHD and defined as Low Risk (LR, ADHD only) vs High Risk (HR, ADHD + ODD/CD) for later SUD. All participants received treatment with MAS-XR and underwent pre and post treatment fMRI scans while performing a reward task. Participants' assessment included KSADS, Family History, ADHD-RS, Kirby delay discounting task, BIS/BAS and UPPS (urgency, perseverance, premeditation sensitivity). Our primary outcomes include imaging indexes from selected regions of interest (ROI), processed on SPM 12; secondary outcomes include behavioral scores from psychometric instruments. Data was analyzed using repeated measures ANOVA with significance set at $p < .05$ in 50 voxels.

Results: The two groups showed no significant differences in demographics; ADHD related measures were no significantly different pre and post treatment. Participants received MAS-XR treatment for mean duration 66 days at mean dose 18mg/day and mean total dose 1220mg; however HR participants received significantly lower total dose of MAS-XR (595,1mg vs 745mg).

Imaging Results: We detected significant differences (all $p < .05$) in brain activation between the LR vs HR groups at baseline (before MAS treatment) in relation to reward outcomes as follows:

i) the HR group showed higher activation than LR participants during Reward outcome (e.g. winning $1) in the right caudate and amygdala and also ii) during Surprising Non-Reward outcome (i.e. violation of expected reward) in the right insula.

After treatment, the two groups showed differences in brain activation in relation to both group status and reward outcome as follows:

i) the LR group showed greater increase in activation during Reward outcome (e.g. winning $1) in the right amygdala and anterior cingulate cortex (ACC);

ii) the HR participants showed greater increase in activation during the Surprising Non-Reward outcome (i.e. violation of expected reward) in the right amygdala, ACC and ventro-latral prefrontal cortex (VLPFC).

Behavioral Results: We also detected significant pre- to post treatment change in the UPPS Sensation Seeking scores, which decreased for the HR group and increased for LR group (group risk X time interaction $p = .039$). The HR group also showed decrease of Kirby Large Win scores - with group Risk x Time interaction approaching significance.

Conclusions: Our preliminary results from this experimental protocol suggest that MAS-XR differentially affects brain activation in HR vs LR youth and that the affected network may be linked to changes in indexes of behavioral control (UPPS and Kirby scores). Further, our results suggest that stimulant treatment in HR youth may reduce impulsivity indexes relevant to SUD onset (e.g. sensation seeking and delay discounting). These preliminary results also suggest that brain regions relevant to SUD in adults (e.g., VLPFC) might be a future target for minimal risk registration and intervention techniques in children at risk, using modalities like near infrared imaging (fNIRS), transcranial magnetic stimulation (TMS), transcranial direct current stimulation (tDCS) or biophotostimulation.

Keywords: ADHD, Stimulants, fMRI

Disclosure: Nothing to disclose.

P174. Pharmacotherapeutic Effects of Cannabidiol in Mouse Models of Autism Spectrum Disorders

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Background: Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders characterized by social deficits and restrictive/repetitive behaviour. ASD is diagnosed in roughly 1-2% of the global population. Despite the prevalence of ASD, very few pharmaceutical interventions have been approved for its treatment. The endocannabinoid system (ECS) has recently gained interest as a potential target for treating ASD. Cannabidiol (CBD), a compound found in the Cannabis (C. Sativa) plant, is non-psychoactive, and it has shown promise in ameliorating ASD-associated symptoms in Fragile X syndrome (FXS) patients. In this present study, we aimed to evaluate the efficacy of CBD in treating social deficits and restrictive/repetitive behaviours in Fmr1 and Shank3 knockout (KO) mice, two well-characterized monogenetic animal models of ASD.

Methods: Male and female mice from each genotype were tested in the social novelty/social preference ($n = 9$-13/group/sex) or open field/self-grooming tests ($n = 4$-7/group/sex) to establish baseline behaviors. Then, they received five daily subcutaneous injections of either vehicle or CBD (5mg/kg for males, 50mg/kg for females). An hour after the last injection, mice were tested again, as described above. Male and female data were analyzed separately with mix-design three-way ANOVAs, with treatment (vehicle vs. CBD) and genotype (wildtype vs. Fmr1 KO vs. Shank3 KO) as independent variables and time-point (pre- vs. post-treatment) as dependent variable. Significant effects or interactions were followed up with Tukey's multiple comparison tests.

Results: At baseline, Fmr1 and Shank3 knockout mice displayed a lack of preference for a novel social stimulus (e.g., social novelty index [SNI] < 0.5 – Male Fmr1 KO: 0.42; Female Fmr1 KO: 0.48; Male

Shank3 KO: 0.45; Female Shank3 KO: 0.48) compared to their wildtype counterparts (SNI Male wildtype: 0.69; Female wildtype: 0.64; $p < 0.05$ for all KO vs. wildtype comparisons). This deficit was restored following the administration of CBD in both strains and sexes (SNI – Male Fmr1 KO + CBD 0.71: 0.42; Female Fmr1 KO + CBD: 0.69; Male Shank3 KO: 0.71; Female Shank3 KO: 0.78; $p < 0.05$ vs. the respective vehicle-treated KO group). Notably, CBD did not affect social preference or novelty in wildtype mice, indicating specificity toward the disorder models. Increased self-grooming in Shank3 KO mice and hyperactivity in Fmr1 KO mice were not changed after CBD treatment.

Conclusions: Repeated treatment with CBD restored the preference for a social stimulus in male and female, Fmr1 and Shank3 KO mice. The dose used for female mice was 10 times greater than that used in males, suggesting that sex is crucial in determining a therapeutic dose in ASD mouse models. With the present treatment regime, increased self-grooming and hyperactivity (characteristic of Shank3 and Frm1 KO mice, respectively) were not affected, suggesting that social behaviour/memory networks are more sensitive to CBD treatment.

Keywords: Autism Spectrum Disorders, Cannabidiol, Social Behavior, Sex Differences

Disclosure: Nothing to disclose.

P175. Unraveling the Mechanism of Action of Viloxazine ER in ADHD

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Background: Many therapeutics for attention-deficit/hyperactivity disorder (ADHD) affect norepinephrine (NE) and dopamine (DA) neurotransmission in regions of the brain important to the disease state, including the prefrontal cortex (PFC). Qelbree® (viloxazine extended release (ER); VLX) is an FDA-approved, nonscheduled treatment for pediatric (≥6 years) and adult ADHD. Historically, VLX has been described as a moderate and selective norepinephrine transporter (NET) inhibitor due to selectivity for NET relative to dopamine (DAT) and serotonin (SERT) transporters. Activity on NET supports preclinical findings showing an increase in NE and DA in the PFC. However, VLX occupancy of NET has never been measured at therapeutically relevant concentrations for ADHD. In addition, until recently, the role of VLX on serotoninergic receptors has also not been explored, despite studies suggesting that VLX may engage these receptors. Therefore, a revision of VLX in vitro and in vivo pharmacology was undertaken to unravel its mechanism of action beyond its action as an NRI. These studies, described below, also aimed to establish whether observed pharmacological effects of VLX translate to therapeutic efficacy for ADHD, based on comparison of plasma concentrations in preclinical models to those seen with VLX-ER use in children and adults with ADHD.

Methods: To better characterize the effect of VLX on NET and serotonin (5-HT) receptors, in vitro binding competition assays were conducted using human isoforms of NET, SERT, 5-HT2A, 5-HT2B, 5-HT2C, and 5-HT7 receptors expressed in different cell lines. IC50 and Ki values were calculated based on inhibition of specific radioligand binding, including [3H]nisoxetine for NET, [3H] imipramine for SERT, [3H]mesulergine for 5-HT2B, [125I]±DOI for 5-HT2C, [3H]ketanserin for 5-HT2A, and [3H]LSD for 5-HT7. Follow-up in vitro cellular uptake and functional assays were performed in HEK cells, expressing human isoforms, using radioligands for the previously mentioned transporters and receptors to determine IC50's and/or EC50's of VLX. To determine the relationship between brain extracellular VLX concentrations and changes in [NE], [DA], [5-HT] and their metabolites in the PFC, an in vivo microdialysis study was conducted in Sprague-Dawley rats ($n = 5$-6/group, vehicle: $n = 7$) administered VLX 1, 3, 10, or 30 mg/kg or vehicle (IP).

Ex vivo dose occupancy of VLX at NET in the rat brain was determined using the NET specific radioligand, [11C]methyl reboxetine ([11C]MRB). Rats ($n = 20$, males) were injected (IP) with VLX (1, 3, 10 and 30 mg/kg) or vehicle ($n = 4$/group), 45 minutes prior to IV administration of [11C]MRB. Plasma samples were collected pre-and 40 minutes post- [11C]MRB administration, after which rats were euthanized, brains resected, and radioligand activity was determined. The % occupancy of VLX at NET was calculated using VLX plasma concentrations as a reference.

The role of VLX in recruiting 5-HT2C receptors was determined via a PET imaging study in anesthetized cynomolgus monkeys (2F/2M, $n = 4$) using the 5-HT2A/2C radioligand agonist, [11C]CIMBI-36. PET scans were performed at baseline and 30 minutes after IV bolus infusion of VLX (3, 6, or 12 mg/kg). Vt values were calculated from dynamic PET data and were used to determine non-displaceable binding potential (BPND) for different brain regions. Occupancy of VLX at 5-HT2 receptors was calculated based on the change in BPND between baseline and post-viloxazine treatment.

Animal research was performed at Charles River Laboratories (South San Francisco, CA, USA), Karolinska Institute Centre (Stockholm, Sweden), and Invicro (London, UK). All protocols were approved by local ethics and animal care committees. Animals were cared for according to international standards.

Results: In vitro binding competition assays indicate VLX directly binds to NET, 5-HT2C, 5-HT2B, and 5-HT7 (Ki = 0.13, 0.66, 0.84, 1.90μM, respectively), with lower affinities at SERT (Ki = 14.4 μM) and 5-HT2A (Ki = 16.3 μM). Functionally, VLX inhibits the human isoform of NET with IC50 = 0.2 μM. Microdialysis studies showed that 30 mg/kg VLX significantly increased NE, DA, and 5-HT in rat PFC, while 10mg/kg VLX significantly increased NE and DA; moreover both these doses were found to be clinically relevant based on comparison of average unbound plasma concentrations in rats to those for children and adults taking approved doses of VLX-ER. Ex vivo occupancy studies in rats also found that VLX 10 or 30mg/kg, yielded NET occupancy of 85% and 95% respectively, with an EC50 = 0.19 μM. Free-VLX plasma concentrations for 10 and 30 mg/kg were 0.9μM ± 0.19μM ($n = 4$) and 3.05 μM ± 0.64μM ($n = 4$) respectively, (within the human therapeutically relevant range). Finally, the in vivo PET study in monkeys showed dose-dependent reductions in [11C]CIMBI-36 binding in most brain regions (ΔBPND), with the greatest reductions in 5-HT2C-rich regions (EC50 = 4.1 μM).

Conclusions: At plasma VLX concentrations that are clinically relevant for ADHD treatment, significant (>85%) NET occupancy and in vivo increases of NE, DA, and 5-HT levels were observed in the PFC of rats. The effect of VLX on 5-HT is bolstered by in vivo results in monkeys showing binding to the 5-HT2C receptor. The therapeutic relevance of VLX activity at 5-HT receptors is still under investigation, but the current data suggest that VLX-ER has a broader mechanism of action in ADHD treatment than simply inhibition of NET.

Keywords: ADHD, Norepinephrine Transporter, Serotonin 5-HT2C Receptor, PET, Viloxazine

Disclosure: Pharmaceutical Company: Employee (Self).

P176. Does Acute Psychedelic "Therapy" Persistently Reverse Reward-Related Anhedonia Induced by Early Life Adversity?

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Background: Depression, addiction, and disordered eating are common mental health disorders that affect millions of people worldwide, and these disorders involve dysregulated reward circuitry leading to abnormally low, or abnormally high reward pursuit. Risk of developing these disorders is increased in those with a history of stress or parental neglect during childhood. However, it is impossible to investigate the causal nature of developmental stress on these disorders in humans, so translationally-relevant animal models are required--such as our limited bedding and nesting model of early-life adversity/poverty in rodents.

In the last few years, psychedelic drugs like LSD, psilocybin, and DMT have re-emerged in psychiatry, with promise for treating depression, addiction, and other disorders. These promising findings inspired us to develop a rat model of "psychedelic therapy" to ameliorate ELA-induced alterations in reward seeking.

Methods: Male rats were subjected to the limited bedding and nesting model of early-life adversity (ELA), which causes a phenotype related to anhedonia and poor spatial memory, replicated here. After baseline testing that confirmed the ELA phenotype (sucrose preference, binge-like eating of palatable food, object location memory), we subject rats to a single dose of the 5-HT2A agonist DOI, administered in a comfortable "set and setting". We then re-tested rats on the same behavioral battery. We also examined potential neural correlates of psychedelic drug effects using anatomical and immunohistochemical methods, focusing initially on astrocytes and microglia.

Results: Our preliminary data provides evidence that a single dose of the psychedelic serotonin 2A (5-HT2A) receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) may reverse ELA-induced anhedonia in male rats.

Conclusions: We hope these studies will contribute to the sorely-needed body of basic research on psychedelic drug effects, and provide new insights that could be capitalized upon when developing maximally effective, but minimally disruptive therapeutic strategies in humans. Our intriguing new findings, and our new model of "psychedelic therapy" in rats will be discussed, as will our future plans for the project.

Keywords: Psychedelic Therapy, Anhedonia, Animal Models, Microglia, Astrocytes

Disclosure: Nothing to disclose.

P177. Associations of Parent-Child Relationship Dynamics With NR3C1 Methylation, Telomere Attrition and Mitochondrial DNA Copy Number in Pre-School Aged Children With and Without Maltreatment

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Background: A growing body of evidence demonstrates effects of early life stress on biological processes, including methylation of the glucocorticoid receptor gene (NR3C1), telomere attrition, and mitochondrial biogenesis, which are shown to be associated with a number of mental and physical health conditions. However, there is little previous research on the role of parent-child relationship dynamics on these biological processes. This study examined associations of parent-child relationship dynamics (relationship quality, cohesion, enmeshment, and disengagement) and NR3C1 methylation, telomere length, and mitochondrial DNA copy number (mtDNAcn) in pre-school aged children with and without maltreatment.

Methods: Participants (N = 254; 53% female) ages 3-5 years old (M = 51.04 months, SD = 8.94 months) were recruited from the state child protection agency, a pediatric medical clinic during a well-child visit, and childcare centers, with 52% of the children with a history of maltreatment. Families completed two home visits with a free play task as well as standardized questionnaires. Parent-child relationship characteristics were assessed from videotaped 15-minute observations of parent-child free play tasks, which were coded using scales for cohesion, disengagement, enmeshment, as well as the Iowa Family Interaction Rating Scale for relationship quality. Saliva samples were collected and analyzed for DNA methylation using bisulfite pyrosequencing within two locations of the promoter region of NR3C1, exons 1D and 1F. Quantitative polymerase chain reactions (qPCR) were performed to assess mtDNAcn and telomere length. Relationships were examined using four separate analytic models wherein each parent-child relationship characteristic was specified as a predictor of the four biological variables.

Results: Path analyses revealed that higher parent-child relationship quality was associated with lower levels of methylation of NR3C1 exon 1D, $\beta = -.15$, $p = .02$, higher parent-child cohesion was associated with lower levels of methylation of NR3C1 exon 1D, $\beta = -.15$, $p = .02$, and longer telomeres, $\beta = .13$, $p = .046$, and higher parent-child disengagement was associated with higher levels of methylation of NR3C1 exon 1D, $\beta = .16$, $p = .02$, and shorter telomeres, $\beta = -.14$, $p = .03$. Child maltreatment status did not significantly moderate any of the associations between parent-child relationship characteristics and the biological variables.

Conclusions: These findings suggest that parent-child relationship dynamics may have distinct biological effects on children, which highlights a potential target for preventative intervention for supporting children and families living in contexts of risk.

Keywords: Glucocorticoid Receptor Gene, Mitochondrial DNA Copy Numbers, Telomere Length, Early life Stress (ELS)

Disclosure: Nothing to disclose.

P178. Sex-Dependent Effect of Peripubertal Stressors on Thalamic Reticular and Reuniens Nucleus Activity in Rats

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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 has a significant impact on the development of neuropathological states with males and females having different vulnerability periods. Male rats are sensitive to stress experienced during peripuberty periods (PeriP; postnatal days, PD31-40) and females to postpuberty (PostP; PD41-50) in inducing adult hyperdopaminergic state and deficits in cognition. Moreover, the apparent male susceptibility is underlined by increased basolateral amygdala activity but not in females. The precise neurobiological mechanisms/circuits that contribute to differential sex responsivity to different periods of susceptibility to stress are understudied. Here we investigate if the nucleus reuniens of thalamus (RE) and thalamic reticular nucleus (TRN) could be involved in the disparity observed in male and female susceptibility to stress, considering that they are important areas related to dopaminergic activity and cognitive regulation. TRN has extensive expression of parvalbumin interneurons and their perineuronal nets (PNN); an extracellular matrix which is implicated in the feedforward inhibition of

thalamic relay neurons, such as RE. Here we investigated the impact of stress exposure during PeriP and PostP periods on adult RE activity, TRN gamma oscillatory activity across development, and stress and PV/PNN staining after PostP stress in males and females.

Methods: Male and female Sprague-Dawley rats were subjected to a combination of footshock/restraint stress during PeriP (PD31-40) and PostP (PD41-50). The rats were tested for single-unit extracellular electrophysiology recording of RE neurons 5-6 weeks after stress (PeriP, PD75-82; PostP, PD85-92). Independent groups of animals were submitted or not to stress and recorded in the TRN for local field potential at PD31, PD41, PD51, and PD75 for PeriP stress and at PD41, PD51, PD61, and PD85 for PostP stress. Low (30-50Hz) and high (60-120Hz) gamma were analyzed. Posterior immunohistochemistry analysis of PV/PNN content was evaluated in the TRN one week after PostP stress (PD61). 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: PostP stress increased the number of active RE neurons only in females after 5-6 weeks of stress ($n = 6$ all groups; Sex and Stress factor, $F1,36 = 16.74$, $p < 0.05$, Two-way ANOVA) with no effect of PeriP stress (Naïve, $n = 8$ male and $n = 9$ female; Stress, $n = 12$ male and $n = 11$ female; $p > 0.05$). Sex differences were observed in both experiments with males having more spontaneously active RE neurons at baseline (PeriP, $F1,20 = 21.56$, $p < 0.05$; PostP, $F1,36 = 5.28$, $p < 0.05$; Two-way ANOVA). Firing rate was not affected by PeriP and PostP stress ($p > 0.05$). Decreased % of spikes in bursts was observed in PeriP-stressed males (Kruskal-Wallis, 11.29, $p < 0.05$). For local field potential, PeriP stress (Females, PD31: $n = 6$ naïve, PD41: $n = 5$ naïve and $n = 6$ stress, PD51: $n = 6$ naïve and stress, PD75: $n = 9$ naïve and stress) increased low gamma power (mV2) only in females at PD51 (one week after stress; $F1,35 = 5.48$, $p < 0.05$, Two-way ANOVA) and PostP stress (Females, PD41: $n = 5$ naïve, PD51: $n = 7$ naïve and $n = 3$ stress, PD61: $n = 6$ naïve and $n = 8$ stress, PD85: $n = 6$ naïve and $n = 5$ stress) increased low gamma at PD51 (one day after stress; stress and age interaction, $F2,29 = 6.10$, $p < 0.05$, Two-way ANOVA). High gamma was not changed significantly in females after PeriP and PostP stress ($p > 0.05$). No effect was observed in males for all time points (PeriP, PD31: $n = 6$ naïve, PD41: $n = 6$ naïve and stress, PD51: $n = 6$ naïve and stress, PD75: $n = 6$ naïve and $n = 7$ stress; PostP, PD41: $n = 6$ naïve, PD51: $n = 7$ naïve and $n = 3$ stress, PD61: $n = 2$ naïve and $n = 4$ stress, PD85: $n = 7$ naïve and stress; $p > 0.05$). For PV/PNN staining, PostP stress decreased the number of PV+ interneurons in the TRN at PD61 only in females (one week after stress; $n = 2$ male and $n = 5$ female naïve, $n = 4$ male and $n = 9$ female stress; sex and stress interaction, $F1,16 = 7.53$, $p < 0.05$, Two-way ANOVA). Females were found to have more PV+ interneurons than males (sex, $F1,16 = 5.94$, $p < 0.05$, Two-way ANOVA). No effect in PNN count and PV/PNN co-localization were observed ($p > 0.05$).

Conclusions: Our findings indicate females are sensitive to the effect of PostP stress which is potentially mediated by RE and TRN activity. PostP stress increased the adult RE activity, increased low and high gamma immediately after stress, and decreased PV+ cells one week after stress. Stress appears to impact PV-TRN transmission during PostP stress with a later impairment in PV content that ultimately leads to disruption in the inhibitory drive to RE, driving increased RE activity. TRN gamma oscillations and RE activity were not affected by either PeriP or PostP stress which suggests that these areas do not underly male vulnerability to stress. Therefore, TRN and RE seem to be involved in the sex-dependent neurobiological disrupted circuit underlying early-life stress vulnerability, especially in females.

Keywords: Adolescence, Early Life Stress, Sex Difference, Thalamus - Reticular Nucleus, Nucleus Reuniens

Disclosure: Nothing to disclose.

P179. Basolateral Amygdala Activity During Social Approach and Interaction

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Background: Almost all organisms exhibit species-specific social behaviors, ranging from social affiliation to mating and parenting behaviors. Deficits in social behavior can be maladaptive and result in decreased quality of life or reduced survival. Social behaviors are highly complex and the neuronal population and circuitry underlying them are not fully understood. Because of their importance in daily life and their vulnerability to disruption in neuropsychiatric and neurodevelopmental disorders such as schizophrenia and Autism Spectrum Disorder, and lack of targeted treatments for social deficits, it is crucial to define the circuit and molecular signatures of social behaviors.

Methods: The basolateral amygdala (BLA) has been shown by us and others to be important for social affiliative behaviors in which sexual and aggressive motivations have been minimized. Here, we used a genetically encoded calcium indicator and fiber photometry system to optically image activity in different BLA neural populations during the three-chamber social approach test and free social interaction.

Results: Our data indicate that glutamatergic BLA neurons are significantly more active during social sniffing than during investigation of a novel object or an empty cylinder, in a time-locked manner, and as a function of distance from the social stimulus, as measured by %ΔF/F. In addition, we find specific patterns of activation in CaMKII+ BLA neurons during discrete events of free social interaction, including preference for anogenital sniffing and no increase in activity during non-social behavior such as rearing and grooming. Interestingly, inhibition of these neurons using DREADDS does not decrease social approach but tends to increase aggressive-like behaviors in free interaction. We do not find similar increases in activity during social events in another population of BLA neurons, those that project to the medial prefrontal cortex (mPFC).

Conclusions: These data show specific BLA neural populations are preferentially active preference during particular social behaviors and may begin to identify novel treatment targets for social deficits.

Keywords: Social Behavior, Autism Spectrum Disorder, Basolateral Amygdala, In Vivo Fiber Photometry

Disclosure: Nothing to disclose.

P180. Sex Differences in Astrocyte Neuronal Metabolic Coupling During Chronic Inflammatory Pain in the Anterior Cingulate Cortex of Mice

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Background: Chronic pain is a major risk factor for anxiety and depression, with over sixty percent of people with chronic pain experiencing severe depression and anxiety. Tragically, the risk of death by suicide is doubled in people with chronic pain. Alarmingly, one in five people suffer from chronic pain, and women make up 67% of this group. Present treatment options are woefully inadequate, with less than half of all patients reporting pain relief with current treatments. Human and rodent

neuroimaging studies indicate that chronic pain corresponds with reorganization of an emotion-pain brain circuit, and evidence indicates that neuroplasticity of the anterior cingulate cortex (ACC) is a critical step in this reorganization. We previously showed chronic pain and fear learning enhance neuronal excitability and induce similar plasticity-related gene expression changes in the anterior cingulate cortex of mice. We recently showed that fear learning requires astrocyte-neuronal lactate shuttling (ANLS) in the dorsal hippocampus, and that ANLS is necessary for learning-induced associated molecular changes, including increases in plasticity-related gene expression. Here, using a murine model of inflammatory pain, we present data showing that the development of chronic inflammatory pain engages ANLS in the ACC in a sex-specific manner.

Methods: We exposed female and male adult mice to a model of chronic inflammatory pain by injecting complete Freund's adjuvant (CFA) into the left hindpaw. Vehicle injections were used as control. We assessed pain thresholds at baseline (prior to injection), and at 3 hours (h) 24 h, 72 h, and 7 d, post injection. Immediately after threshold testing, mice were euthanized, and we collected ACC samples, allowing us to assess lactate levels, measured through a colorimetric lactate assay, and protein expression levels, assessed through western blot analyses. Temporary, continuous inflammatory pain was modelled through formalin injections into the left hindpaw, with vehicle injections serving as control. We used antisense oligonucleotides (AS-ODN) targeting monocarboxylate MCT4, which is exclusively expressed on astrocytes, to decrease the export of lactate out of astrocytes.

Results: We found that CFA injection causes rapid increases in lactate levels in the ACC of female and male mice, observed at 3h post injection. In contrast, male mice, but not female mice, showed increases in lactate levels after 7 days of inflammatory pain. Accordingly, chronic inflammatory pain increased monocarboxylate transporters (which bidirectionally transport lactate) and enhanced enzymes that increase lactate levels in the ACC of male but not female mice 7 days after CFA injection. Lastly, we found that disrupting astrocyte-neuronal lactate shuttling in the ACC reduces expression of pain behavior in in male, but not female mice. Our data thus shows that astrocyte-neuronal metabolic coupling is involved in chronic pain development in a sex-specific manner.

Conclusions: Our data indicates that there are sex differences in astrocyte-neuronal coupling during chronic pain development. Furthermore, our data indicate that disrupting astrocyte-neuronal lactate shuttling in the ACC reduces persistent pain in male mice.

Keywords: Neuron-Astrocyte Interactions, Chronic Pain, Sex-specific Effects, Mouse Models

Disclosure: Nothing to disclose.

P181. A Controlled Examination of Impairment in Delayed Recall in Persons Living With HIV: The Loewenstein and Acevedo Scales for Semantic Interference and Learning (LASSI-L)

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Background: Persons living with human immunodeficiency virus (PLHIV) may be at higher risk for progression of cognitive impairment, due to other comorbid psychiatric and medical disorders, as well as pre-existing nicotine and/or substance use disorders, and traumatic brain injury. There is a need for early identification of those with mild cognitive impairment not revealed by standard cognitive testing, e.g. the Montreal Cognitive Test (MOCA). The Loewenstein Acevedo Scales of Semantic Interference and Learning (LASSI-L) is a cognitive stress test that uniquely assesses subtle cognitive breakdowns, such as the failure to recover from proactive semantic interference effects (frPSI). We sought to determine whether PLHIV would exhibit reduced performance on the LASSI-L compared to controls, as the the LASSI-L has distinguished those with amnestic MCI (aMCI) and high amyloid load from MCI attributable to other non-Alzheimer's Disease (AD) conditions.

Methods: In a specialty mental health clinic, 34 persons with HIV aged 37 to 69 were matched with cognitively normal controls of similar age with no more than a 1-year age difference who also completed the LASSI-L cognitive test. Demographic characteristics, MoCA, and LASSI-L performance were compared between the two groups. Two sample t-tests compared the means of continuous variables; chi-square tests compared the distribution of categorical variables. Analysis of covariance (ANCOVA) was conducted to compare LASSI-L performance between the two groups while adjusting for covariates. The analyses were performed using the statistical software SAS 9.4 (Cary, NC).

Results: The mean age was 54.8 and 54.7 for the HIV and control group, respectively. There was no statistically significant difference in race distribution, Hispanic background, and testing language between the two groups. The HIV group had lower education levels (13.5 vs. 15.7 yrs, $p = 0.02$) and a higher proportion of men (71% vs. 35%, $p < 0.01$) compared to the control group. The MOCA scores were also similar between the two groups (25.6 vs 25.2, $p = 0.5$). Initial t-tests of the unadjusted means of the LASSI measures showed the HIV group performing below the control group in five LASSI measures of recall (List A-Cued Recall 2, List B - Free Recall, List B Cued Recall 1, List A - Free Recall 2 Short Delay, List A - Cued Recall, Delayed Free Recall) (all $p < 0.05$). After adjusting for gender and education using ANCOVA, only one LASSI measure (Delayed Free Recall) remained statistically significant with HIV group scored worse than control (15.5 vs. 19.4, $p = 0.03$).

Conclusions: The LASSI-L appears to be more sensitive than traditional cognitive tests in detecting subtle cognitive decline in HIV persons. A potentially faster and less expensive way to screen for subtle neurocognitive impairment, a larger study sample size, combined with laboratory assessment of functional capacity and observer perspective, can provide validation and utility of the LASSI-L in persons living with HIV.

Keywords: LASSI, HIV-Associated Neurocognitive Disorder, Mild Cognitive Impairment Due to AD

Disclosure: Nothing to disclose.

P182. Behavioral and Endocrine Responses to Stress and Alcohol Cues in Treatment-Seeking Alcohol Use Disorder Patients With and Without Comorbid Anxiety Disorders

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Background: There is a high prevalence of comorbidity between alcohol use disorder (AUD) and anxiety disorders, with as many as 50% of individuals seeking treatment for AUD also meeting criteria for an anxiety disorder. Research suggests that individuals with comorbid AUD and anxiety disorders are characterized by a faster transition from non-problematic alcohol use to an AUD, may exhibit increased withdrawal and increased anxiety levels during detoxification, and are more likely to relapse after treatment. Less

is known, however, about how these individuals respond to acute stress and alcohol-related cues, which may be major triggers to relapse. Laboratory challenges designed to evaluate behavioral and physiological responses to stress and alcohol cues, such as guided imagery and the Trier Social Stress Test (TSST), provide valuable information on variation in these responses among AUD individuals, and thus are useful tools in evaluating potential therapeutic interventions. The goal of the current study was to investigate the effect of comorbid anxiety on behavioral and endocrine responses to stress- and alcohol-cue guided imagery, and to a combined TSST/alcohol cue reactivity challenge, in individuals seeking inpatient treatment for AUD. We hypothesized that AUD patients with comorbid anxiety disorder would exhibit elevated anxiety, stress, and alcohol craving responses to these challenges compared to those with only AUD.

Methods: This study is a secondary analysis of data from three previously completed experimental medicine studies conducted by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) Intramural Clinical Research program. There were no significant medication effects on any of the outcomes currently analyzed, thus the data from the treatment and placebo control arms were combined. Data were analyzed from 55 treatment-seeking inpatients (34 males, 21 females; mean age 43.2 years) diagnosed with AUD using the Structured Clinical Interview for DSM-IV, of which 29 also had a comorbid anxiety disorder (AUD +Anx; PTSD was not included as an anxiety disorder) and 26 had no psychiatric diagnoses other than AUD (AUD-only). Individuals completed 1) the TSST combined with physical alcohol cue exposure (Trier/CR), and 2) personalized auditory guided imagery scripts relating stress and alcohol cue-associated stimuli. Behavioral responses were serially measured during each challenge using the Spielberger State Trait Anxiety Inventory (STAI-S), the Subjective Units of Distress scale (SUDS), and the Alcohol Urges Questionnaire (AUQ). Blood samples were serially collected during each challenge for measurement of serum cortisol and plasma ACTH. ACTH and cortisol responses were only analyzed for the Trier/CR, as the guided imagery scripts did not elicit any observable ACTH or cortisol responses in this AUD inpatient sample. Data analyses were conducted using linear mixed models controlling for study, treatment arm, and sex.

Results: Contrary to our hypotheses, there were no significant differences between the AUD+Anx and AUD-only groups in either anxiety (STAI-S) or stress (SUDS) responses to the stress-cue script, the alcohol-cue script, or the Trier/CR. There was a significant difference in alcohol craving (AUQ) responses to the alcohol cue and stress cue scripts ($p = 0.03$ and $p = 0.04$, respectively), however it was opposite from expected: alcohol craving responses were consistently lower in the AUD+Anx group compared to the AUD-only group in both contexts. There was no difference between groups in alcohol craving in response to the Trier/CR. However, ACTH response to the Trier/CR was significantly elevated in the AUD+Anx group compared to the AUD-only group. There was no significant difference between groups in the cortisol response to the Trier/CR.

Conclusions: Subjective anxiety and stress responses to laboratory stress and alcohol cue challenges did not differ between AUD inpatients with comorbid anxiety disorders and those with no comorbidity. AUD individuals with comorbid anxiety disorders did however exhibit a blunted alcohol craving response to both the stress and alcohol cue scripts. This finding suggests that the guided imagery procedure, which comprises listening to a recorded narrative of a personalized experience created from interviews conducted with each patient, may have diminished salience in AUD patients with comorbid anxiety, where the objective is to elicit a craving response. The higher overall ACTH profile observed in AUD patients with comorbid anxiety during the Trier/CR is consistent with other literature showing an enhanced ACTH response to the TSST in individuals with comorbid depression and anxiety disorders. The lack of differences between groups in subjective anxiety or stress responses to the Trier/CR suggests minimal correspondence between behavioral and physiological responses to acute stress in the comorbid group. Our findings may inform future studies utilizing laboratory stress and craving challenges in individuals with comorbid AUD and anxiety, with particular relevance for studies evaluating potential therapeutic interventions in this population.

Keywords: Alcohol Use Disorder, Anxiety, Stress, Craving, Co-Morbidity

Disclosure: Nothing to disclose.

P183. Diabetes Alters Economically Dissociable Decision-Making Algorithms Depending on the Salience of Reward Scarcity in the Environment

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Background: Those with diabetes mellitus are at higher risk of developing depression and other psychiatric disorders. Although diabetes is primarily characterized by chronic hyperglycemia, it remains unclear how impaired insulin function, which is known to have direct effects on neural activity within the reward circuitry, regulates motivated behavior or how motivated behavior may be dysregulated in diabetes.

Methods: We characterized value-based decision-making of an insulin-deficient diabetic mouse model on a naturalistic neuroeconomic foraging paradigm. 40 8-week old CB57BL/6J male mice were injected with either vehicle (VEH) or streptozotocin (STZ), an antibiotic that ablates insulin producing beta cells in the pancreas, to induce hyperglycemia. Hyperglycemia was confirmed via blood glucose and hemoglobin A1C% of tail blood before beginning training. STZ-treated mice that were not hyperglycemic were excluded. Mice were then tested across two months on the "Restaurant Row" task during which they foraged daily for their primary source of food while on a limited time budget. Mice learned to make serial decisions accepting or rejecting reward offers as a function of cost (delays cued by tone pitch) and subjective value (flavors cued by unique spatial contexts). Mice were trained on two different schedules during which the economic landscape (i) drastically or (ii) gradually progressed into an increasingly reward-scarce environment. Statistical analyses were carried out using Matlab and JMP. Two-sample t-tests, two-way ANOVAs, and repeated measures ANOVAs were used to analyze blood sugar and decision-making metrics between-group as well as within-group across longitudinal timepoints.

Results: STZ induced robust hyperglycemia in treated mice with an average pre-test hemoglobin A1C of 7.15% and significantly different from VEH treated mice (4.3%, $p < 0.0001$, t-test). Overall, STZ-treated mice earned less food but shifted meal consumption patterns in complex ways based on the revealed preferences of various flavors. Vicarious trial and error behavior, a proxy of deliberation, revealed decreased decision conflict for less-preferred flavors in STZ-treated mice. These findings were divorced from individual differences in economic choice policies, which were uniquely modulated in STZ-treated mice depending on their prior training schedules. Interestingly, we found that groups of mice valued the passage of time differently based on the type of choice being made. During change-of-mind decisions, mice became sensitive to the magnitude of time spent waiting, or

"sunk costs," in altering the probability of earning a reward but only after transitioning into a reward-scarce environment - except STZ-treated mice trained on a gradual schedule, who surprisingly never developed sensitivity to sunk costs.

Conclusions: Deliberative and re-evaluative choice algorithms, which have been previously shown to be processed in physically separable circuits in the brain, may be differentially perturbed in a mouse model of insulin-deficient diabetes. These findings suggest that complex relationships between glycemic regulation, the contrast of realized scarcity of the environment, and different types of opportunity costs interact to influence dissociable decision-making systems and fundamentally distinct behavioral computations underlying unique aspects of reward value.

Keywords: Value-Based Decision-Making, Diabetes, Neuroeconomics

Disclosure: Alkermes, Inc.: Contracted Research (Self). Ono Pharmaceutical Co, LTD: Contracted Research (Spouse/Partner).

P184. Reward Neurocircuitry Predicts Longitudinal Changes in Alcohol Use Following Trauma Exposure

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Background: Trauma is a risk factor for developing maladaptive alcohol use. Preclinical research has shown that stress alters the processing of striatal reward and motivation signals. The current study sought to identify reward-related neural mechanisms predicting increased alcohol use after trauma exposure.

Methods: Participants were recruited from emergency departments (ED) around the U.S. as part of the AURORA study (N = 286, 178 female). Trauma-related change in alcohol use was quantified as the change in 30-day total drinking at 8 weeks relative to pre-trauma as measured with the PhenX Toolkit. Using region of interest (ROI) and whole-brain voxel-wise analyses, reward-related neurocircuitry activation and functional connectivity (FC) were assessed 2 weeks post-trauma using fMRI during a monetary reward task using region of interest and whole-brain voxelwise analyses. Neuroimaging data were preprocessed using fMRIprep 1.2.2 and analyzed in SPM12.

Results: Increased alcohol use after trauma exposure was predicted by (1) greater ventral tegmental area (VTA) activation during monetary Gain>Loss trials measured 2 weeks post-trauma ($b = 1.06$, $t = 3.67$, $p < 0.001$), (2) greater cerebellum activation to monetary reward (MNI x = -16, y = -48, z = -28, PFWE = 0.03, Z = 5.46, KE = 184), and (3) greater seed-based FC between the VTA seed and lateral occipital cortex (MNI x = 28, y = -76, z = 50, PFWE < .001, Z = 4.33, KE = 607) and precuneus (MNI x = -20, y = -60, z = 18, PFWE = 0.014, Z = 3.93, KE = 290).

Conclusions: Altered peritraumatic VTA activation and FC identify vulnerability to increased alcohol use after trauma. These data pinpoint potential targets/mechanisms with early interventions to prevent alcohol use disorder after traumatic stress exposure.

Keywords: Alcohol Abuse, Human Neuroimaging, Monetary Reward, Stress and Trauma

Disclosure: Nothing to disclose.

P185. Brain N-Acetyl-Aspartyl-Glutamate (NAAG) is Associated With Cognitive Function in Virally-Suppressed People With HIV

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Background: Suppressive antiretroviral therapy has improved and extended the lives of people with HIV (PWH); despite these advances, cognitive impairment persists especially in the higher order domains of working memory (WM) and executive function (EF). One frontal-cortical brain circuit linked to these domains is regulated by the neurotransmitter N-acetyl-aspartyl glutamate (NAAG), the endogenous agonist of metabotropic glutamate receptor 3 (mGluR3). Activity of mGluR3 receptors in the dorsolateral prefrontal cortex has been shown to strengthen persistent firing and communication between neurons. The catabolic enzyme glutamate carboxypeptidase II (GCPII) regulates NAAG levels and is robustly upregulated in neuroinflammation. Inhibition of GCPII has been shown to increase brain NAAG and improve learning and memory in multiple rodent and primate models. As neuroinflammation is present in virally suppressed (VS)-PWH, we investigated if brain NAAG levels, measured by magnetic resonance spectroscopy (MRS), were associated with cognition.

Methods: We utilized 7-Tesla MRS data to examine relationships between regional NAAG levels in frontal white matter (FWM), left hippocampus (Hp), left basal ganglia (BG) and domain-specific cognitive performance in 40 VS-PWH after adjusting for depressive symptoms and premorbid function. All participants were >50 years of age, had no prior 3-month illicit-drug use, and were negative for affective and neurologic disorders. In a separate pilot study, we analyzed plasma NAAG levels via LC/MS in 20 VS-PWH (n = 10 cognitively impaired and n = 10 unimpaired). All study participants provided informed consent under a protocol approved by the institutional review board.

Results: We found higher NAAG levels in FWM were associated with better attention/WM ($r = 0.41$, $P = 0.01$) and processing speed ($r = 0.26$, $P = 0.05$). Higher NAAG in the Hp was associated with better EF ($r = 0.39$, $P = 0.05$) and higher BG NAAG levels related to better verbal fluency ($r = 0.26$, $P = 0.04$). Higher plasma NAAG was associated with poorer higher-order cognition. Specifically, higher NAAG levels were associated with verbal learning ($p = 0.04$) and recognition ($p = 0.04$) as well as executive function ($p = 0.03$). This significant effect is noteworthy given the small sample size of this pilot study.

Conclusions: Collectively, these data suggest brain and plasma NAAG may serve as biomarkers of cognition in VS-PWH, and modulation of brain NAAG could represent a novel therapeutic avenue.

Keywords: Cognition, 7T MRS, HIV-Associated Neurocognitive Disorder

Disclosure: Nothing to disclose.

P186. Infant Developmental Trajectories of Cingulum Bundle White Matter Predict 9-Month Infant Emotionality: Preliminary Longitudinal Analyses

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Background: Temperamental features/emotional behaviors, such as low positive emotionality (PE) and high negative emotionality (NE), are often precursors of subsequent behavioral and emotional problems. Thus, elucidating the underlying neural mechanisms of infant emotionality can identify tangible markers of risk linked to disease processes and inform interventions. Infant white matter development, including neuritic growth and myelination, progresses rapidly within the first postnatal year, and is associated with future cognitive functions and behavioral problems. The cingulum bundle (CB), a critical white matter pathway involved in emotion regulation, establishes connections within the default mode network (DMN), bridging temporal-parietal-frontal areas. Further, the CB connects central executive network and DMN anchors (dorsolateral prefrontal cortex and posterior cingulate cortex). Therefore, our aim is to investigate how early developmental trajectories of CB microstructure from 3 to 9 months predict early infant emotionality at 9 months.

Methods: The study sample comprised caregiver-infant dyads (males and females), with both neuroimaging of the infant and caregiver reports of infant emotional behavior at 3 and 9 months (n = 13). Participants were recruited from the community, including hospital postnatal wards, pediatric practices and a university research registry.

At 3 and 9 months of age, infants underwent an MRI scan on a Siemens 3 Tesla Skyra system using a 32-channel head coil during natural, non-sedated sleep. Diffusion-weighted imaging (DWI) data were acquired using a multi-shell diffusion scheme with 2mm slice thickness; two sequences were acquired, one with posterior to anterior phase encoding with 10 b0 volumes (TR = 2500 ms, TE = 80 ms, voxel size = 2.0× 2.0×2.0 mm3, FoV = 200 mm), and another with posterior to anterior phase encoding with 159 volumes with b-values of 750 (50 directions) and 2000 (100 directions) s/mm2 (TR = 3000 ms, TE = 97 ms, voxel size = 2.0×2.0×2.0 mm3, FoV = 200 mm).

DWI data were preprocessed using fMRI Software Library (FSL)'s TOPUP, BET, and EDDY tools. Using DSI Studio software, DWI data were reconstructed using a native-space generalized q-sampling imaging (GQI) approach. Tractography was performed for CB Bundle (e.g., left and right) using the Automated Fiber Tracking (AutoTrack) function with consistent parameters across infants. Only the frontoparietal/"standard" CB was included in the tractography. Mean fractional anisotropy (FA) and tract volume were extracted from each tract and averaged across hemispheres.

Infant emotionality was measured via caregiver report at 3 months using the Infant Behavior Questionnaire-Revised (IBQ-R). PE and NE composites were calculated using the raw IBQ-R data with a mean of the items from each of the relevant subscales (i.e., PE - smiling, laughter and high pleasure; NE - sadness, distress, fear and falling reactivity subscales). Caregivers also reported on household receipt of public assistance.

To assess the stability of PE and NE from 3 to 9 months, bivariate correlations (Spearman) were performed. Delta scores were calculated for CB volume and FA by subtracting 3-month from 9-month values. Initial General Linear Models (GLMs) were performed with 9-month PE or NE as the dependent variables (DVs, in separate models), and public assistance sum, delta CB volume, and delta CB FA as the independent variables (IVs) (all variables Z-scored) using a robust estimator. Secondary GLMs were performed using the model structure, but also included 3-month PE or NE as predictors to examine how results differed when accounting for 3-month emotionality.

Results: Stability: 3-month PE was not correlated with 9-month PE (rho = 0.037, p = 0.904), whereas 3-month NE was highly correlated with 9-month NE (rho = 0.703, p = 0.007). PE: Initial GLM Analyses revealed that delta CB volume significantly predicted 9-month PE (standardized beta = 0.607, p = 0.007), while delta CB FA did not (standardized beta = 0.273, p = 0.464). The effect of CB volume on 9-month PE remained significant when 3-month PE was added to the model (standardized beta = 0.623, p = 0.004). NE: Initial GLM Analyses revealed a trend toward delta CB FA predicting 9-month NE (standardized beta = -0.549, p = 0.061), with no effect of CB volume (standardized beta = -0.261, p = 0.480). The effect of CB FA on 9-month NE became significant when 3-month NE was added to the model (standardized beta = -0.314, p = 0.016).

Conclusions: Our preliminary findings revealed that the developmental trajectories of CB volume and FA predicted 9-month PE and NE, respectively, particularly when the model included 3-month emotionality. Greater change in CB volume predicted greater 9-month PE, and greater change in CB FA, or overall structural integrity, predicted lower 9-month NE. Taken together, our findings indicate that during this period of early infancy, more rapid development of CB microstructure may reflect greater emotional regulation capacity yielding more adaptive emotional behaviors.

Keywords: Infant Brain, Infant Emotionality, Infant Behavior, Cingulum, Longitudinal Study

Disclosure: Nothing to disclose.

P187. Prevalence and Association of Defense Mechanisms With Common Psychiatric Disorders: A National Study

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Background: Defense mechanisms, which can be defined as an individual's automatic psychological responses to internal or external stressors or emotional conflict, are central concepts in psychoanalysis, psychodynamic psychiatry and psychology. However, whether each psychiatric disorder (or category of disorders) is associated with a specific profile of defense mechanisms or defense mechanisms are general markers of severity of psychopathology is unknown.

Methods: We drew on data from a large nationally representative sample, the National Epidemiological Survey on Alcohol and Related Conditions (N = 43,093) to investigate the association of 12 defense mechanisms with mood, anxiety and substance use disorders. In supplementary analyses, we examined the odds of endorsing a defense mechanism when adjusting for age, sex and race/ethnicity. Logistic regressions were fit with mental disorder as the independent variable of interest, defense mechanism as the dependent variable, and respondent age, sex, and race/ethnicity as covariates.

Results: When considering the broad diagnostic categories, there was a clear ordering effect in which individuals with mood disorders had greater prevalence of all defense mechanisms than those with anxiety disorders who, in turn, were more likely to have greater prevalence of all defense mechanisms than those with substance use disorders. Individuals with no psychiatric disorders had the lowest prevalence for all defense mechanisms.

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Among anxiety disorders, prevalence of defense mechanisms decreased from social anxiety disorder to generalized anxiety disorder to panic disorder and specific phobia. Reversal to this pattern included psychotic distortion, acting out, devaluation and affect isolation, all of which were more common in panic than in

generalized anxiety disorder. Immature defenses, as a group, were also more prevalent in panic than in generalized anxiety disorder.

Consistent with findings in mood and anxiety disorders, substance use disorders showed ordering effects. Defense mechanisms were most prevalent in drug dependence, followed by drug abuse, alcohol dependence and alcohol abuse. Exceptions to this pattern were projection and withdrawal, both of which were more prevalent in alcohol dependence than drug abuse. Obsessive/controlling behavior fit an almost reverse pattern as it was least prevalent in drug dependence, followed by drug abuse, alcohol abuse and alcohol dependence (Table 4). Adjusting for age, sex and race/ethnicity did not change the pattern of these findings.

Conclusions: Broad diagnostic categories or individual psychiatric disorders were not associated with specific profiles of defenses. Rather, with very few exceptions, there was a general pattern in which disorders could be ordered by the prevalence of the defense mechanisms. Our findings further suggest that defense mechanisms and psychiatric disorders represent correlated but different dimensions of psychopathology, that may respond to different treatment approaches.

Keywords: Diagnosis, Psychopathology, Dimensions, Psychiatric Disorders, Dimensional Psychopathology

Disclosure: Nothing to disclose.

P188. Cross-Disorder Meta-Analysis of Endocannabinoid DNA Variations in Major Depressive Disorder, Bipolar Disorder, Attention Deficit Hyperactivity Disorder, Autism Spectrum Disorder, and Schizophrenia

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Background: The endocannabinoid system (ECS) is implicated in multiple mental disorders. In this study, we explored DNA variations in the ECS across major depressive disorder (MDD), bipolar disorder, attention deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), and schizophrenia by performing a cross-disorder genome-wide association study (GWAS) meta-analysis.

Methods: We obtained six datasets from the Psychiatric Genomics Consortium containing GWAS summary statistics from European cohorts (284,023 cases and 508,515 controls). Effective sample size weighted meta-analysis was performed for 2241 single nucleotide polymorphisms (SNPs) pertaining to gene bodies of 33 endocannabinoid genes using METAL, where an overall z-statistic is calculated for each marker based on a weighted sum of individual statistics. Heterogeneity was examined with I2 and X2 tests. MAGMA gene-based analysis was also performed.

Results: We identified nine SNPs significantly associated with a change in risk of having a mental disorder. The lead SNP was rs12805732 (Gene: Diacylglycerol Lipase Alpha; DAGLA). Four SNPs had substantial heterogeneity ($I2 > 60\%$). DAGLA had the strongest association with disease risk in gene-based analysis.

Conclusions: Our findings suggest that the ECS may be a shared pathway in mental disorders. Future studies validating these findings would contribute to the identification of biomarkers of disease risk across multiple mental disorders.

Keywords: Cross Disorders, Genomics, Endocannabinoid System

Disclosure: Nothing to disclose.

P189. The Association of Polygenic Risk for Coronary Artery Disease With Grey Matter Structure in Youth With Bipolar Disorder

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Background: Bipolar disorder (BD) is strongly associated with coronary artery disease (CAD) and is also characterized by anomalous neurostructural phenotypes. Adults with CAD exhibit significantly reduced total brain volume and regional grey matter volume and thickness. The adverse association of CAD with brain structure has typically been attributed to the negative effects of clinically manifested CAD. However, there is preliminary evidence that higher polygenic risk for CAD is associated with reduced whole brain volumes in adults with mild cognitive impairment. Here we examined the association of polygenic risk for CAD with grey matter structure in relation to youth BD. Due to significant sex differences in CAD, and increased standardized CAD mortality ratios for females vs. males with BD, we also examined for sex-specific associations.

Methods: Youth participants (mean age 17.1 years, males and females; $n = 66$ BD, $n = 45$ healthy controls [HC]) underwent T1-weighted magnetic resonance imaging. Polygenic risk scores for CAD (CAD-PRS) were calculated using PRS-CS-auto, based on independent adult genome-wide summary statistics. Covariate adjusted voxel-wise analyses examined the associations of CAD-PRS with cortical volume, thickness, and surface area [SA]) in the overall sample, and within BD and HC groups. Anterior cingulate cortex (ACC) and hippocampus were also examined as regions of interest (ROI). Exploratory sex-stratified analyses were also undertaken.

Results: In the overall sample, higher CAD-PRS was associated with lower right inferior temporal gyrus volume ($\beta = -0.32$, $p = 0.03$). Similar negative associations between CAD-PRS and brain structure were observed within the BD (5 clusters; $\beta = -0.54$ to -0.65, $p = 0.003$ to 0.04) and HC (1 cluster; $\beta = -0.41$, $p = 0.02$) groups. ROI analyses revealed a nominal association of higher CAD-PRS with lower ACC thickness in the overall sample ($\beta = -0.31$, $p = 0.05$). Within the BD group, sex-stratified analyses revealed significant findings for females, but not for males.

Conclusions: As hypothesized, higher CAD-PRS was associated with lower regional grey matter structure in youth in regions implicated in BD. Findings were more pronounced in the BD group, particularly among females. Future longitudinal studies are needed to examine the association of CAD-PRS with neurodevelopmental changes over time, and to discern mechanisms underlying the observed sex-specific findings.

Keywords: Bipolar Disorder, Cardiovascular, Adolescence, Human Neuroimaging, Gene-by-Sex Interaction

Disclosure: Nothing to disclose.

P190. Altered Neural Responsivity to Food Cues Among Youth With Avoidant/Restrictive Food Intake Disorder

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Background: Avoidant/restrictive food intake disorder (ARFID) is a serious, chronic psychiatric condition in which individuals severely restrict intake of food (either by volume and/or variety) due to fear of aversive consequences, low interest in food, or sensitivity to sensory aspects of food. Often emerging early in life, ARFID is as common as other eating disorders, affecting 3% of children/adolescents and 1-4% of adults. ARFID is associated with significant medical and psychological morbidity, including precipitous weight loss, nutritional deficiencies, dependence on tube feeding or nutritional supplements, and significant psychosocial impairments. Given ARFID's more recent addition to the DSM, understanding of the neurobiological mechanisms that contribute to the onset and maintenance of ARFID symptoms remains unclear. To address this gap, we conducted the first study investigating the neural pathways associated with ARFID in youth. Based on the role of neural circuits associated with fear (amygdala), hunger (hypothalamus), and sensory processing/attention (insula; anterior cingulate cortex, ACC), we tested hypotheses that (1) individuals with ARFID would exhibit differential activation to food cues in these regions relative to healthy controls; (2) individuals with each ARFID phenotype [ARFID-fear; ARFID-lack of interest (LOI); ARFID-sensory sensitivity (SS)] would exhibit differential activation in phenotype-specific brain regions; and (3) variation in ARFID phenotypes would be associated with activation.

Methods: Youth (age range: 10-23 years) with ARFID ($n = 75$; 40F/35M; mean age = 16.2; mean BMI z-score = -0.5) and healthy controls (HC; $n = 35$; 24F/11M; mean age = 17.3, mean BMI z-score = -0.01) completed a visit including an fMRI scanning session involving a food cue paradigm while undergoing fMRI scanning. In addition, participants completed the Pica, ARFID, and Rumination Disorder Interview (PARDI), a semi-structured diagnostic interview for ARFID. fMRI data were analyzed using SPM12 to examine (1) between-group (ARFID vs. HC) comparisons for the Food>Objects contrast; (2) between-group comparisons of HC compared to individuals meeting PARDI cutoffs for each phenotype (fear: >0.5; LOI: >1.125; SS: >0.625); (3) within each phenotype, regression models relating PARDI subscale scores with activation. Regions of interest (ROIs) were based on hypotheses outlined above: amygdala, hypothalamus, insula, ACC. Clusters within these ROIs meeting small-volume correction thresholds of $k > 10$ and p(FWE-corrected) < 0.05 are reported. Additionally, whole-brain analyses were reported if they met thresholds of $k > 20$ and p(FWE) < 0.05.

Results: Youth with ARFID exhibited elevated activation, relative to HC, in response to food cues (vs. objects) in the ACC [$t = 4.44$, p(FWE)=0.009]. Groups did not differ significantly in activation in the amygdala, hypothalamus, or insula. At the whole-brain level, there was hyperactivation in the ARFID (vs. HC) group in the somatosensory cortex [SSC; $t = 4.55$, p(FWE)=0.005] and supplementary motor cortex [SMC; $t = 3.8$, p(FWE)=0.041]. In comparison to the HC group, (a) those with ARFID-fear ($n = 20$) exhibited hyperactivation in the amygdala [$t = 3.41$, p(FWE)=0.042]; (b) those with ARFID-LOI ($n = 48$) exhibited no differences in the hypothalamus; (c) those with ARFID-SS ($n = 58$) exhibited greater activation in the ACC [$t = 4.7$, p(FWE)=0.005]. At whole-brain, those with ARFID-LOI [p(FWE) < 0.05] and ARFID-SS [p(FWE) < 0.03] showed relative hyperactivation (vs. HC) in the SSC. Within the ARFID-LOI group, activation to food cues in the hypothalamus was negatively associated with PARDI subscale score [$t = -3.33$, p(FWE)=0.028].

Conclusions: Results indicate generalized hyperactivation of the anterior cingulate cortex, somatosensory cortex, and supplementary motor cortex in response to visual food stimuli amongst a group of individuals with mixed-phenotype ARFID, indicating a novel neurobiological circuit associated with this restrictive eating disorder. When examined according to phenotype, hyperactivation of the amygdala typified those with ARFID-fear of consequences, consistent with the role of the amygdala in aversive conditioning and processing of fearful stimuli. Hyperactivation in regions associated with attention and cognitive interference (ACC) and multisensory perception and integration (SSC) appeared to be driven by those with disinterest in food and hypersensitivity to the sensory aspects of food, groups showed greater overlap in membership in this sample. Findings of this first fMRI study of youth with ARFID reveal key aberrations in networks associated with fear, attention, and sensory processing, providing insight into neural circuits to target in future interventional studies.

Keywords: Avoidant/Restrictive Food Intake Disorder, Functional MRI (fMRI), Eating Disorders, Food Cues, Adolescence

Disclosure: Nothing to disclose.

P191. Reduced Gastrointestinal Interoception in Anorexia Nervosa: Initial Behavioral and Neural Insights

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Background: Anorexia nervosa (AN) has among the highest mortality rates of any psychiatric illness and current treatments show limited efficacy, reflecting a crucial need to better understand the neural mechanisms driving the pathophysiology of this disorder. Abnormal gut-related sensations are a prevalent and poorly understood symptom feature of AN, suggesting that altered gastrointestinal interoception could contribute to the functional impairments observed in these individuals. Here we used a minimally invasive probe consisting of an ingestible vibrating capsule (Vibrant Ltd) to examine gut mechanosensory processing and the associated neural responses in AN. We hypothesized that, relative to HCs, individuals with AN would have reduced accuracy in detecting gastrointestinal sensations and diminished neural responses to vibratory capsule stimulations. We further hypothesized that gastrointestinal interoceptive accuracy would be associated with eating disorder symptom severity.

Methods: Data from this single-blinded randomized crossover study were collected in 40 females: 20 weight-restored inpatients with AN (mean age: 18 ± 3 years, mean body mass index (BMI): 21.7 ± 3.8) and 20 healthy comparisons (HC; mean age: 21 ± 4 years, mean BMI: 23.2 ± 3.4). Eating disorder severity was assessed via the Eating Disorder Examination Questionnaire (EDE-Q) at study onset. Participants fasted for 3 hours before the study to have a mostly empty stomach. Shortly after swallowing the capsule, participants performed the gastrointestinal detection task in a sitting position. Two blocks of vibratory stimulations (normal and enhanced) were delivered in a counterbalanced order, each including approximately 60 vibratory stimulations with a 3000 millisecond (ms) duration. Participants were told to press and hold a button as soon as they felt a sensation they ascribed to the capsule and to release the button once this sensation had ended. Perceptual accuracy was computed for each block using the normalized A prime (A') measure, a non-parametric signal detection analog of d prime for task with low trial numbers. Miss rates, response bias and reaction times were also calculated. Scalp electroencephalogram (EEG) was continuously recorded during the capsule vibration task using a 31-channel system. During offline processing, EEG data were band-pass filtered between 0.1 and 80 Hertz and referenced to the average of mastoid channels (TP9 and TP10). After artifact correction, EEG data were epoched in an event-related manner, from -200 to 3000 ms after the capsule vibration onset, which served as the temporal reference. For the

purpose of the present statistical analyses, we computed the average ERP amplitude (avERP) as the mean vibration-evoked signal across midline parieto-occipital electrodes (Cz, CP1, CP2, Pz, POz, O1, Oz, and O2) and time points (400-720ms after the vibration onset) that we recently identified as an EEG marker of gut mechanosensation (Mayeli et al., 2023). We implemented linear mixed effect models with group (AN, HC), block (normal, enhanced), age, and BMI as fixed variables, subject as a random factor, and perceptual performance measures (normalized A′, response time, response bias), avERP, and EDE-Q score as dependent variables.

Results: Relative to HCs, the AN group exhibited lower accuracy in detecting capsule vibrations across the two stimulation conditions (normal and enhanced), as evidenced by a lower normalized A′ ($p = .003$; $b = -0.13$; 95%CI [-0.22;-0.05]) and a higher miss rate ($p < .001$; $b = 0.14$; 95%CI [0.07;0.20]). There were no significant group differences in terms of reaction time ($p = .11$; $b = 0.08$; 95%CI: [-0.01;0.18]), or response bias ($p = .34$; $b = 0.08$; 95%CI: [-0.07;0.22]). Capsule stimulations induced EEG evoked responses with intensity-dependent increases in amplitude at the parieto-occipital electrodes near the midline ($p < .001$; $b = 0.86$; 95%CI: [0.47;1.24]). However, the avERP within the latency from 400-720 ms after the vibration onset did not differ between the groups ($p = .55$; $b = -0.21$; 95%CI: [-0.89;0.46]). Across the entire sample, the EDE-Q total score was inversely associated with normalized A′ ($p = .01$; $b = -0.06$; 95%CI: [-0.10;-0.02];) and positively associated with miss rate ($p < .001$; $b = 0.06$; 95%CI: [0.03;0.10]). The miss rate was also inversely associated with the avERP at a trend level ($p = .06$; $b = -0.03$; 95%CI: [-0.05;0.0001]).

Conclusions: These initial findings demonstrate that weight-restored inpatients with AN show reduced gastrointestinal interoception during the delivery of vibratory stimulations to the gut. The associations between gut mechanosensation and eating disorder symptom severity and neural evoked responses provide preliminary evidence that a disruption in gut-brain communication plays a role in the pathophysiological alteration of eating behaviors relevant to AN. These findings, if confirmed in a larger sample, could provide the basis for a new class of behavioural and neural markers of gastrointestinal interoception in AN.

Keywords: Eating Disorders, Anorexia Nervosa, Interoception, EEG/ERP Electrophysiology

Disclosure: Nothing to disclose.

P192. Aversive Value Response in Anorexia Nervosa Supports Striatum Mediated Food Avoidance

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Background: Anorexia nervosa (AN) is a severe psychiatric illness associated with food avoidance. Studies from Richard and Berridge (2013) showed that environmental ambience can trigger either appetitive (desire) or avoidance (dread) behaviors in animals via frontal cortex, nucleus accumbens and hypothalamus. Human functional imaging research by Plassmann et al. (2010) localized aversive and appetitive goals to the orbitofrontal cortex at the time of decision making. Previous research in eating disorders suggested that passive tasting of aversive high calorie taste stimuli activated the ventral striatal-hypothalamic circuitry supporting Berridge's model (Frank et al 2021). Here we tested the hypothesis that aversive goal values and food avoidance would be stronger related to frontal and ventral striatal activation in AN compared to controls, further supporting the involvement of the fronto-striatal circuitry in food avoidance.

Methods: We recruited adolescents and young adults with AN ($n = 19$, age M 18.2 ± 6 years) and healthy controls ($n = 30$, age M 21.4 ± 6 years). Study participants were carefully screened and assessed for eating and general psychiatric psychopathology. Participants then underwent functional magnetic resonance brain imaging (fMRI) during which they conducted a food avoidance task.

Prior to an fMRI scan, subjects rated a list of 35 foods typically available for snacks on a scale from 1 to 4 based on how much they wanted to avoid eating that particular snack food (4 being the highest avoidance). The day of the scan, subjects were told that they would play a game where they are asked to bet money in order to avoid certain snack foods, and that a snack from the list provided to the subject would be randomly chosen and they would have to eat that as their snack that evening. If a subject could bet correctly, they would avoid having to eat a food that they fear. In the fMRI scanner, they were given a total of $90 'play' money with the instructions that they could bet $1, $2, $3, or $4 depending on how much they wanted to avoid eating that food. The objective of the study was to bet money in order to avoid the chance of having to eat that food.

We estimated a general linear model including the regressors free bid at response, and forced bid at response. We calculated the contrasts 1) correlation with aversive goal values in free trials, 2) correlation with aversive goal values in forced-bid trials, and 3) correlation with aversive goal values in free versus forced trials. Regional data were extracted for dorsolateral prefrontal cortex, medial orbitofrontal cortex, anterior cingulate cortex, insula, striatum, and nucleus accumbens. Group contrasts were calculated using MANCOVA. Potential confounding variables such as age, comorbidity was included in the statistical model. Brain imaging data were non-normally distributed and ranked data were used for analysis.

Results: The AN group (18 ± 6 years) was slightly younger than the HC group (21.4 ± 6 years) and had lower body mass index (16.1 ± 1) compared to the HC group (20.7 ± 2). The AN group placed on average higher bets on high calorie food items ($\$3.2 \pm 1$) versus the HC group ($\$2.0 \pm 1$). The AN group showed greater engagement during aversive value processing during freed bid trials for the bilateral ventral anterior cingulate (R: $F = 6.205$, $p = 0.017$; L: $F = 6.335$, $p = 0.016$), caudate head (R: $F = 8.187$, $p = 0.007$; L: $F = 16.967$, $p < 0.001$) and nucleus accumbens (R: $F = 5.661$, $p = 0.022$; L: $F = 5.584$, $p = 0.023$), and left dorsolateral prefrontal cortex ($F = 4.673$, $p = 0.0.037$) and insula ($F = 4.592$, $p = 0.038$). For the contrast free minus forced bids, AN engaged higher the head of the bilateral caudate in AN compared to the HC group (R: $F = 5.253$, $p = 0.027$; L: $F = 5.576$, $p = 0.023$). Free bid caudate nucleus activation in AN was associated with state anxiety (R: $r = -0.477$, $p = 0.039$; L: $r = -0.583$, $p = 0.009$) and intolerance of uncertainty (R: $r = -0.530$, $p = 0.020$; L: $r = -0.793$, $p = 0.009$).

Conclusions: Food avoidance has not been directly studied previously on a neural level. This study indicates that the frontal and cingulate cortex as well as striatal caudate head and nucleus accumbens regions have a particular role in aversive value computation in AN. Those regions have been identified in animal models as part of a circuitry that drives dread and avoidance in negatively biased or fear conditioned circumstances. Those regions could be central to the core psychopathology of AN.

Keywords: Anorexia Nervosa, Brain Imaging, fMRI, Value-Based Decision-Making

Disclosure: Nothing to disclose.

P193. Weight Gain, But Not Behavioral Traits or Treatment Duration Predict 6-Month Body Mass Index in a Large Sample of Patients With Anorexia Nervosa

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Background: Anorexia nervosa (AN) is a severe psychiatric illness with a mortality rate twelve times higher than all causes of death for females 15-24 years old. AN is associated with severe emaciation, food avoidance, and a perception of being overweight despite very low body weight. The principal diagnostic criterion of AN is low body weight relative to height or body mass index (BMI). Long-term outcome studies of adults have found that at 20- to 30-year follow-up, 19-26% had an eating disorder while 64-66% had fully recovered. Identifying factors that predict illness outcome is critical to help allocate limited resources and improve treatments. Prior studies investigating predictors of outcome in AN have had limited congruence. This study aimed to use machine learning in a large data set of individuals with AN who underwent intensive treatment and to identify variables predicting BMI six months following intensive treatment.

Methods: Data were drawn from a naturalistic treatment outcomes study of patients admitted to a partial hospitalization program (PHP) with adolescents (n =178) or adult (n =154) patients meeting the criteria for AN, with a BMI < 18.5 upon admission.

Outcome Variable BMI: Height and weight measured on admission and discharge were used to calculate BMI (kg/m2), respectively. In addition, BMI at the 6-month follow-up was calculated from the self-reported weight. Supporting this methodology, objective BMI and BMI derived from self-reported weight were strongly correlated at admission (r(284) = .91, p

Predictor Variables: Patient Demographics. age, gender, length of illness, history of treatment, and psychotropic medications prescribed at admission. Eating Disorder Examination – Questionnaire (EDE-Q), Beck Depression Inventory-II (BDI), State-Trait Anxiety Inventory, Difficulties in Emotion Regulation Scale (DERS), Behavioral Inhibition/Behavioral Activation System Scales (BIS/BAS).

Data Analysis: Analyses used established machine learning procedures, including the caret (version 6.0-91;41 package in R (version 4.1.2; R Core Team, 2021) via multiple, parallel, computationally efficient algorithms, e.g., support vector machine learning (function svmRadial), random forest prediction (function rf), and the elastic net regularization (function glmnet) algorithm. Algorithms were trained with a hold-out set reserved for testing via an "exploratory/confirmatory" approach, minimizing the risk of overfitting. To mitigate bias in any one algorithm, models were combined into a single, predictive model via caret's ensemble algorithm (function caretEnsemble), which generates a combined prediction by additive weight by each model included. Items with any potential for circularity were removed, i.e., items probing diagnosis or weight, as BMI was the primary outcomes variable. A total of 142 predictors remained after data wrangling. Variable importance was calculated via the varImp function and reflected variance explained by a predictor relative to all other predictors, where all variable importance values sum to 100. Machine learning parameterization was set according to data characteristics prior to analysis.

Results: There were 160 participants who followed up at six months, 120 were randomly allocated to the training set, and 40 were assigned to the test set. Secondary analyses predicted BMI on admission (N = 369, n train = 277, n test = 92) and at discharge (residualized for admit BMI, N = 368, n train = 276, n test = 92).

Predicting Self-Reported BMI 6 Months After Discharge: The models predicting self-reported BMI 6-month follow-up explained 35.9% variance in the training set (n = 120). The model also had excellent performance in predicting 6-month BMI in the test dataset (n = 40), with predicted BMI significantly correlating with actual BMI (r(38)=0.39, p = 0.01; figure 1). The top 10 most predictive variables are displayed in Table 2., demonstrating that the change in BMI by discharge was the most important predictor, with change in BMI by discharge strongly correlating with self-reported BMI at six-month follow-up (r(118)=0.60).

Predicting Admission BMI: The models could not predict admission BMI, with predictors only explaining 4.6% variance in the training dataset (n = 277). The best-fitting model also failed to predict admission BMI in the test dataset (r(90)=0.11, p = 0.28).

Predicting Discharge BMI: Models significantly predicted discharge BMI, explaining 51.2% of the variance in the training sample (n = 276) and 47.6% of the variance in the test dataset (r(90)=0.69, p < 0.01; Figure 2). Of all predictors, greater length of stay was most predictive of greater discharge BMI (r(274)=0.60, p < 0.01).

Conclusions: This study, using an agnostic machine learning approach in the largest to-date sample of individuals with anorexia nervosa, suggests that achieving weight gain goals in treatment predicts longer-term weight-related outcomes. However, treatment duration, personality, mood, and cognitive-emotional eating disorder symptoms do not. These results may have important implications for treatment decisions.

Keywords: Anorexia Nervosa, Machine Learning, Body Mass Index, Clinical Outcome Prediction

Disclosure: Nothing to disclose.

P194. Negative Affect Influences the Computations Underlying Food Choice in Bulimia Nervosa

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Background: Negative affect is a common precursor to binge eating among individuals with bulimia nervosa (BN). In addition, reactivity to negative emotions, or "negative urgency," has been shown to predict more frequent binge eating in BN. However, the mechanism underlying the tight link between emotion and eating in BN remains poorly understood. In a laboratory assessment, we found that negative affect did not alter food choices made by women with BN. Whether neutral or negative affect was induced, the BN group made food choices that were ultimately restrictive. In the current study, we reanalyzed the data from this food choice task with computational modeling to evaluate whether negative affect influenced the decision-making process in more subtle ways. We then assessed whether these decision-making parameters were connected to self-report measures.

Methods: Data were previously collected in a randomized crossover design, where women with BN (n = 25) and healthy controls (HC; n = 21) completed a food choice task after both a neutral affect induction and a negative affect induction. During the first phases of the food choice task, participants used a 5-point scale to rate the healthiness and tastiness of 43 foods. Independent of these ratings, researchers categorized each food as low-fat or high-fat. In the subsequent Choice phase of the task, one food that was rated neutral by participants for both health and taste was used as a Reference Item, and participants made choices between this Reference Item and the other food items. Participants were informed that one trial would be randomly selected for payout at the end of the experiment and they would receive the chosen food item on that trial. Participants also completed self-report measures, including the UPPS-P Negative

Urgency subscale. A time-varying diffusion decision model was fit to participants' behavioral data to study how the perceived healthiness and tastiness of food influenced decision dynamics. Specifically, our analyses focused on the subjective weights assigned to health and taste attributes of foods (i.e., the differing degrees to which they influenced the evidence accumulation rate) and the relative time at which health and taste attribute information entered the decision process. Model parameters were estimated using a Bayesian hierarchical framework implemented in JAGS. The medians of the individual-level posterior distributions were used in parametric tests assessing the relationship between model parameters and negative urgency.

Results: Across affective states, the BN group put more weight on health information (μ_(HC-BN)=-1.80,95% HDI [-3.32,-0.77]), whereas HC put more weight on taste information (μ_(HC-BN) =1.25,95% HDI [0.72,1.79]). The HC group consistently accumulated taste information before health information across food types (τ_(High-Fat)=-0.54,95% HDI [-0.83,-0.32]; τ_(Low-Fat) =-0.54,95% HDI [-0.83,-0.32]), and negative affect had no effect on any HC decision parameters (ρ = -0.07,95% HDI [-0.39,0.16]). In contrast, the BN group accumulated taste information first only for high-fat foods (τ_(High-Fat)=-0.65,95% HDI [-0.94,-0.40]; τ_(Low-Fat)=-0.01,95% HDI [-0.24,0.23]), and this start time bias toward taste information was exaggerated after the negative affect induction (ρ = -0.20,95% HDI [-0.42,-0.03]). A group-by-negative urgency interaction indicated that this negative-affect induced delay in the onset of health information was particularly exaggerated among women with BN who reported the highest levels of impulsive behavior in response to negative affect (β = -0.61,t = -2.80,p = .006). We also observed an interaction between group and negative urgency on attribute weights, such that across affective states, higher levels of negative urgency were associated with more weight on taste in the BN group compared to the HC group (β = 1.67, t = 2.41, p = 0.018).

Conclusions: Although individuals with BN strongly weight health information, negative affect delays its entry into the evidence accumulation process. With this longer delay, decision-makers have more time to accumulate taste information, potentially biasing them towards high-fat food choices before health information can come online. Moreover, individuals with greater negative urgency had an even stronger affect-induced delay in accumulating health information. These dynamics may provide insights into the factors that induce intermittent binge episodes, such as why binge eating is more likely during periods of high negative affect, but dietary restriction is more likely during periods of low negative affect.

Keywords: Computational Models of Decision-Making, Eating Disorders, Bulimia Nervosa, Computational Psychiatry, Binge Eating

Disclosure: Nothing to disclose.

P195. Using Computational Modeling to Identify the Underpinnings of Restrictive Food Choice in Anorexia Nervosa

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Background: Maladaptive, persistent restriction of food intake is a core feature of anorexia nervosa (AN). Food choice paradigms capture this behavior: in food choice tasks patients with AN show a reduced preference for subjectively less healthy (high-fat) foods relative to healthy control peers (HC). Among HC, when health attributes of food are considered earlier in decision making (relative to taste), choices are influenced by subjective healthiness to a greater degree. Aberrancies in the timing at which healthiness and tastiness are processed during decision-making may therefore contribute to restrictive food intake in AN. In this study, we apply a drift diffusion modeling approach to test the hypothesis that individuals with AN consider healthiness first, while HC consider tastiness attributes before those of healthiness, when deciding what to eat.

Methods: Data from four existing studies of food choice in AN were combined to yield a total sample size of 193 individuals with AN and 97 HC, all of whom were female and aged between 12 and 60 years. Each participant completed a food choice task, in which they rated 76 food items varying in fat and energy content for healthiness and tastiness. In a binary choice phase participants completed 75 trials, indicating whether they would rather eat a reference item (rated neutrally for healthiness and tastiness, and constant across the trials), or each of the other 75 foods. Participants had 4000 ms to make each choice, and were aware that they would be required to eat one of their food choices (that of a randomly selected trial) after completing the task. A time-varying drift diffusion model (DDM) was fit to participants' choice and reaction time data using hierarchical Bayesian Markov Chain Monte Carlo methods (separate models for the two diagnostic groups). The model allows decision evidence to update according to the healthiness and tastiness of food choices and the weight an individual places on healthiness and tastiness when deciding what to eat. The model also specifies a relative start time parameter that denotes the period of time in which one attribute (i.e., healthiness or tastiness) is considered before the other begins to influence choice as well. In fitting the DDM, six parameters were estimated for each individual: the time at which healthiness begins to influence choice relative to tastiness (positive values indicating healthiness is considered after tastiness, negative values indicating healthiness is considered before tastiness); the influence of healthiness on drift rate (or the weight placed on healthiness when making food choices); the influence of tastiness on drift rate; bias (towards selecting the non-reference food item); response threshold; and non-decision time. These parameters were compared between AN and HC groups using multiple linear regression analysis (models were adjusted for age).

Results: Outcomes of the computational modeling procedure indicated that food choices were influenced more by healthiness and less by tastiness among individuals with AN compared to HC. For the influence of healthiness on drift rate: mean (SD) HC = 0.01 (0.24), mean (SD) AN = 0.49 (0.49), B for effect of AN = 0.47, 95% CI [0.36, 0.57], $p < 0.001$. For the influence of tastiness on drift rate: mean (SD) HC = 0.84 (0.66), mean (SD) AN = 0.61 (0.40), B for effect of AN = -0.25, 95% CI [-0.38, -0.12], $p < 0.001$. Patients with AN also considered healthiness attributes sooner in the choice process relative to HC. For the relative time at which healthiness attributes are considered during food choice (compared to tastiness): mean (SD) HC = 0.08 (0.01), mean (SD)AN = 0.00 (0.01), B for effect of AN = -0.08, 95% CI [-0.07, -0.08], $p < 0.001$.

Conclusions: Patients with AN tended to consider healthiness and tastiness in parallel, whilst HC considered tastiness first, when making food choices. The early timing at which food healthiness is considered during food choice decision making in AN may exacerbate the excessive influence of healthiness on food valuation, to further promote caloric restriction. Earlier consideration of healthiness may also explain why the choice to eat energy-dense foods requires extended deliberation among patients with AN: this deliberation potentially allows additional attributes and considerations to enter the choice process. Using computational modeling to probe food choice dynamics in a fine-grained manner yields decision process variables that may be targeted in novel interventions. Our findings suggest that choice slowing, and shifting attention away from food healthiness when deciding what to eat, should be targets of AN treatment.

Keywords: Anorexia Nervosa, Decision Making, Computational Modeling

Disclosure: Nothing to disclose.

P196. Spatial-Molecular Sex Differences in the Human Ventromedial and Arcuate Hypothalamus

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Background: The hypothalamus (HYP) coordinates core physiology and behaviors central to survival, such as growth, mating, parenting, aggression, appetite, and metabolism. Given these roles, it is unsurprising that the HYP is also a nexus of morphologic and functional sex differences that have been robustly studied in model species. An exemplary area of HYP with established morphological and functional sex differences is the ventromedial hypothalamus (VMH), which regulates appetitive behaviors in both canonical and abstract (e.g., social motivation) senses. In rat, the VMH is approximately 25% larger in males; moreover, rodent VMH contains neurons expressing estrogen receptor alpha (Esr1), which are activated by social encounters in different patterns depending on interactor and interactee sexes. Complimentary roles are played by the adjacent arcuate nucleus (ARC), which integrates and dispatches signals regarding peripheral nutritional states and metabolism. Intriguingly, estrogen receptor regulation in ARC and VMH appears to occur in opposing directions in the adult rat.

Despite the thorough profiling of molecular and sex-differentiated functions of HYP in rodents, little is known about how well these features correspond to human HYP. To characterize the molecular anatomy of the human HYP, including potential sex differences, we performed spatial RNA-sequencing using the Visium platform (10x Genomics) to identify marker genes, spatial gene expression patterns, and sex-differential expression in post-mortem, neurotypical human VMH and ARC from males and pre-menopausal females.

Methods: Post-mortem human HYP ($n = 4$ per sex) was collected from donors with neither obesity (i.e., body mass index (BMI) < 30) nor neurologic, psychiatric, or endocrinologic disorders. Female donors were pre-menopausal and not pregnant. In situ hybridization was performed on cryosectioned tissue to detect VMH and ARC marker genes, NR5A1 and NPY, respectively. Upon locating the VMH and ARC, a 10 µm section for each sample was mounted directly onto a Visium array for imaging, RNA-seq library preparation, and sequencing.

RNA-sequencing data were demultiplexed, mapped to spatial coordinates on accompanying histological images, and compiled into a SpatialExperiment object for analysis in R. Quality control removed fewer than 10% of spots due to low numbers of unique molecules and/or genes detected. Spatially variable genes (SVGs) were identified using the nnSVG package and mean ranks calculated across samples. The top 1828 ranking SVGs were selected as features for dimension reduction in HARMONY to correct for batch (sample) effects. The spatial clustering algrothim BayesSpace was used to define 15 spatial domains, the approximate number of anatomic divisions near the VMH using reference brain atlases.

Results: Our findings highlight that both VMH and ARC in human HYP share key molecular signatures with rodent and primate species—such as a preponderance of excitatory neurons in VMH and of inhibitory neurons in ARC. We also identified surprising divergences from model species: for example, while LAMP5 is generally associated with inhibitory neurons, we find here is highly and specifically expressed in human VMH. We additionally identify ANKRD34B as a VMH-specific marker unique to humans.

We observed a relative paucity of molecular sex differences in the VMH relative to ARC in our cohort. More broadly, we find that despite differences in the number of significant genes, VMH and ARC share molecular sex signatures across the autosomes with a strong positive correlation of log fold-change values. Intriguingly, an autosomal gene, PWP2, was sex-differentially expressed at a magnitude comparable to Y chromosome genes (>30-fold higher in male ARC and VMH).

Male-upregulated genes across these two HYP areas are especially enriched for genes linked to neurodevelopmental and autism spectrum disorders (NDD; ASD) in a curated gene-disease database, DisGeNET. Female upregulation was also observed for individual, well-characterized ASD risk genes, including SHANK3. As a set, however, female-upregulated genes did not show enrichment for NDD/ASD genes, but rather those linked to brain tumors. However, cancers arising from the HYP are more common in childhood and lack sex differences in incidence.

Conclusions: Despite its importance in behavior and disease, the HYP has not been spatially characterized in humans at a molecular level. Here, we identify genes that specifically mark VMH and ARC in the human HYP, contrast them with marker genes in model species, and reveal novel spatial subdivisions of gene expression in these brain areas. Fittingly, we find that these HYP areas driving sex-differential physiology and behavior likewise have (broadly shared) sex-differential gene expression patterns. Surprisingly, this effect of sex includes expression differences in a large group of NDD/ASD genes. These findings suggest that dysregulated gene expression in VMH and ARC may contribute to social and/or metabolic disruptions (e.g., obesity) across syndromic NDDs to a greater extent than previously appreciated. Finally, these discovery-based findings identify priority genes for future cellular resolution assays aimed at characterizing cell type-specific and single-molecule features of the HYP.

Keywords: Hypothalamus, Sex Differences, Spatial Transcriptomics, Postmortem Brain Tissue Gene Expression

Disclosure: Nothing to disclose.

P197. Estradiol Deficiency in Anorexia Nervosa as a Driver of Central and Peripheral Inflammation: Evidence From Peripheral Blood-Based Biomarkers, in Vivo Imaging of the Brain Barrier, and Extracellular Free Water

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Background: Estradiol is a neuroprotective hormone that alters inflammation in both the peripheral and central nervous systems. While inflammation is widely implicated across psychiatric disorders, the role of inflammation in anorexia nervosa (AN) etiology and symptom maintenance is complex. Estradiol deficiency, however, is common in acute AN (~60%) and may

help explain the presence of peripheral and central inflammation during the acute phases of the disorder. Across three multi-modal studies of 108 adolescents with AN and healthy controls (HC), we aimed to: quantify an inflammatory state in AN, and their relationship with estradiol deficiency (Study 1),and explore how estradiol deficiency may drive neuroinflammation using two in vivo correlates of neuroinflammation: imaging of the brain barrier (Study 2) and extracellular free water (Study 3). Across all studies, we hypothesized that greater central and peripheral inflammation would be associated with defects in estradiol exposure.

Methods: Data were drive from two parent studies that assessed food motivation pathways as mediators of eating disorder trajectories. Subjects were recruited between April 2014 and January 2021. In Study 1, we applied a targeted proteomics approach to quantify 92 inflammation proteins in AN and HC. We explored relationships between estradiol exposure over the preceding 9 months and inflammation protein expression levels, while controlling for age and age + body mass index. In Study 2, we segmented high resolution T1 MRI scans of the choroid plexus (ChP), a brain structure that plays a crucial role in peripheral-central inflammation cross-talk, using a deep learning algorithm (Multi-Dimensional Gated Recurrent Units) confirmed with manual annotation, the gold standard for ChP segmentation. Associations between ChP volume and estradiol exposure were assessed. In Study 3, we applied a free water imaging pipeline to characterize extracellular free water (FW) from diffusion tensor images and its association with endogenous estradiol exposure.

Results: Estradiol exposure was significantly decreased across all studies in AN compared to HC (prange 0.001 - 0.01). Study 1 identified nine proteins significantly different between AN/HC (lower levels: CXCL1, HGF, IL-18R1, TNFSF14, TRANCE; higher levels: CCL23, Flt3L, LIF-R, MMP-1; FDR prange 0.001 - 0.05), with 3 estradiol dependent proteins (higher: LIF-R; lower: TRANCE, IL-18R1). In Study 2, ChP enlargement was evident from both the deep learning and manual annotation methods in patients with AN compared to HC ($p < 0.0001$) and within the AN group, was negatively associated with estradiol exposure ($p = 0.002$). Study 3 we found significantly increased FW, reflecting greater neuroinflammation, among individuals with AN and HC who had no estradiol exposure compared to those with high estradiol exposure ($p = 0.03$).

Conclusions: Estradiol deficiency may drive the inflammatory state seen in some patients with AN both centrally and peripherally. In Study 1, we found that AN is associated with an inflammatory state, driven by 9 proteins; three of which are estradiol dependent. In Study 2, we found that left CP volume was significantly higher in AN compared with HC (independent of total gray matter volume), and inversely associated with endogenous estradiol exposure in AN. In Study 3, our results of higher FW related to endogenous estradiol exposure, rather than AN diagnosis, highlight the importance of studying estradiol when investigating the effects of AN on WM, as well as other conditions involving sex hormone changes. Taken together, our results highlight the therapeutic potential of estradiol replacement in anorexia nervosa treatment.

Keywords: Eating Disorders, Anorexia Nervosa, 17-β-estradiol, Novel Therapeutics, Neuro-Inflammation

Disclosure: Nothing to disclose.

P198. FTO rs9939609 A Allele is Associated With the Evolution of Body Weight, Ghrelin and Brain Function Following Laparoscopic Sleeve Gastrectomy for Obesity Treatment

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Background: Obesity is a consequence of the imbalance between energy intake and expenditure combined with genetic susceptibility. The fat mass and obesity-associated gene (FTO) is a well replicated locus of obesity across different ages and populations. The A allele of rs9939609 (FTO intron 1) is consistently associated with higher body mass index (BMI). Carriers of the risk-conferring variant displayed an attenuated suppression of the orexigenic hormone ghrelin and impaired central nervous system satiety processing, food reward processing and inhibitory control of eating, thereby enhancing the neural sensitivity to food stimulation and increasing food intake. Laparoscopic sleeve gastrectomy (LSG), one of the most effective procedures for treating obesity, produces sustained weight-loss and reduces craving for high-calorie food after surgery. However, a small proportion of obese carriers of the rs9939609 A allele regain more weight after the surgery than non-carriers. It remains unclear whether FTO variants are associated with alterations in ghrelin levels and brain function following surgery, as well as with weight loss. We hypothesized that changes in ghrelin levels and neural activation in regions involved with reward processing and inhibitory control of eating following surgery had a negative impact on weight loss for FTO variant carriers, and these variables could be used to predict long-term outcomes of bariatric surgery.

Methods: Forty-two individuals with obesity were recruited for LSG surgery including 16 carriers with one copy of the rs9939609 A allele (AT) and 26 non-carriers (TT). Resting-state functional magnetic resonance imaging (RS-fMRI) and cue-reactivity fMRI task with high- (HiCal) and low-caloric (LoCal) food cues were performed pre-surgery (PreLSG) and 1, 6, and 12 months (PostLSG-1, -6, -12) after surgery to evaluate the impact of rs9939609 A allele on brain activity and food cue-induced activation. Fasting blood samples were taken to measure plasma ghrelin levels. All of the participants were followed at 24, 36, 48, 60 months (PostLSG-24, -36, -48, -60) after surgery and reported their BMI. A two-way repeated measures ANOVA was implemented to model the effects of group (AT, TT) and time (Baseline, 1 month) on brain function, plasma ghrelin, food craving and weight loss response.

Results: There were significant interaction (group × time) effects on BMI ($F = 5.25$, $P < 0.001$) and the percentage of excess BMI loss (EBMIL) ($F = 5.17$, $P < 0.001$). Post-hoc tests showed TT relative to AT group has greater EBMIL ($t = 2.26$, $P = 0.029$) and lower BMI ($t = -2.43$, $P = 0.020$) at 12-months after LSG. Both AT and TT groups showed significant weight regain at more than 36-months after surgery, however, there was lower EBMIL in AT group. LSG significantly decreased fasting plasma ghrelin ($F = 37.97.63$, $P < 0.001$) and HiCal food craving ($F = 12.63$, $P < 0.001$), but there were no significant interaction or group effects. The ANOVA showed significant interaction effects on resting-state brain activity (ALFF) in posterior cingulate cortex (PCC) (PFWE < 0.05, cluster-level correction). Specifically, LSG increased PCC activity in TT group at PostLSG-1, -6 and -12. Conversely, the AT group showed decreased activity in the PCC at PostLSG-12. There was no significant interaction but group effects on brain responses to HiCal vs. LoCal food-cues, and AT compared to TT group showed greater activation in dorsolateral prefrontal cortex (DLPFC), dorsomedial prefrontal cortex (DMPFC), insula, supplementary motor area (SMA) and precentral gyrus (PostCen) group (P FWE < 0.05, cluster-level correction). In AT group, basal plasma ghrelin levels were negative correlated with reduced BMI at PostLSG-12 ($r = -0.57$, $P = 0.020$). There were also negative correlations between HiCal food craving following 12 months and the EBMIL. Reduced BMI in AT group following 12 months were negatively correlated with PCC activity ($r = -0.52$, $P = 0.038$) and food cue-induced activation in DLPFC ($r = -0.56$, $P = 0.022$), insula

(r = -0.55, P = 0.027) and DMPFC (r = -0.57, P = 0.021). However, there were no significant correlation of weight loss response with ghrelin levels, HiCal food craving and brain function in TT group.

Conclusions: These findings indicate that FTO rs9939609 A allele is associated with the evolution of body weight before and after bariatric surgery, highlighting the critical role of LSG-induced ghrelin, food craving and brain function changes in maintaining long-term weight-loss and improving eating behaviors in obese carriers of the rs9939609 A allele.

Keywords: Obesity, Bariatric Surgery, MR Imaging, Food Cues, Genetic Variation

Disclosure: Nothing to disclose.

P199. Assessing the Heritability of Body Size Estimation and Body Dissatisfaction in Monozygotic Twins and Age-Matched Control Pairs Using a Digital Avatar: Somatomap 3D

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Background: Body image disturbance (BID) is a key characteristic of eating disorders such as anorexia nervosa and body dysmorphic disorder. BID has contributions from body size distortion (inaccurate body size estimation) as well as body dissatisfaction. The influence of genetic and environmental factors on BID, in general, remains uncertain. However, the relative contribution of these factors may help establish and evaluate risk factors associated with the development of body image-related disorders and better understand their underlying causes. Somatomap 3D is a digital avatar tool that can be used to quantify body size estimation accuracy and body dissatisfaction. Somatomap 3D has been demonstrated to be sensitive to detecting abnormalities in body size estimation accuracy and body dissatisfaction in a clinical sample with AN (less accurate than controls) and a non-clinical sample of fashion models (more accurate than controls). The current study examines the heritability of body size estimation accuracy and body dissatisfaction in healthy monozygotic twin and non-twin healthy controls. Based on previous studies, we hypothesized that there would be within-twin correspondence in body size estimation accuracy and body dissatisfaction.

Methods: Participants were female healthy monozygotic twins (n = 24) and healthy age-matched control pairs (n = 58) recruited from the community in Sweden as part of the Comprehensive Risk Evaluation for Anorexia Nervosa in Twins (CREAT) Study. Participants used Somatomap 3D to engage with a digital avatar; they adjusted 21 different body parts to create representations of a) their current body, and b) what their ideal body would look like. To assess body size estimation accuracy, a researcher took physical measurements of five corresponding body parts (bust girth, hip size, thigh girth, upper arm girth, and waist girth) and subtracted these physical measurements from the current body avatar. Body dissatisfaction was calculated by subtracting current avatar measurements from the ideal for all 21 body parts. Intraclass correlations coefficient (ICC) estimates were computed based on mean-rating (k = 2), absolute agreement, 2-way random effects models for monozygotic twins and age-matched control pairs separately. Since age-matched pairs were not siblings, randomization resampling was used to get all possible age-matched combinations. The analyses were adjusted for age due to the age-matched pairs not being the same age as the twins as well as height, weight, and BMI in both groups.

Results: Among monozygotic twins, the reliability of body size estimation accuracy varied from poor (ICC < .50) to moderate (.5 < ICC < .75) [bust girth ICC = .29, hip size ICC = .32, thigh girth ICC = NaN, upper arm girth ICC = .69, waist girth ICC = .17]. Notably, body size estimation accuracy for upper arm girth was significant between monozygotic twins [ICC = .69, p = .039]. However, for age-matched control pairs, body size estimation accuracy was found to be poor or anticorrelated, and nonsignificant, indicating a lack of consistent agreement. Body dissatisfaction for twin pairs showed moderate and significant relationships for bust girth [ICC = .66, p = .024], and upper arm girth [ICC = .61, p = .040]. The remainder of the body parts for dissatisfaction within the twin pairs had poor reliability or were inconclusive due to the small sample size and large variance. For the age-matched control pairs, for body dissatisfaction these were poor or anticorrelated and nonsignificant.

Conclusions: With a limitation of a modest sample size, the results suggest that body size estimation accuracy and body dissatisfaction for some body parts may be influenced by heritable factors. These findings provide further understanding of possible biological and nonbiological contributions to BID. Larger sample sizes will aid in more fully evaluating the factors influencing BID.

Keywords: Body Image Disturbance, Twins, Body Dissatisfaction, Heritability

Disclosure: NOCD, Inc.: Consultant (Self).

P200. An Anorexia Nervosa Network Derived From Human Brain Lesions

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Background: Anorexia nervosa (AN) is a life-threatening disease characterized by significantly low body weight in combination with avoidance of weight gain. In this study, we used the human connectome to test whether brain lesions associated with AN map to a common brain network.

Methods: We systematically searched the literature for cases of lesion-induced AN. Next, we mapped AN lesion locations to a common brain template and computed their functional connectivity using the human connectome (n = 1000). Functional connections common to AN lesions were identified and tested for specificity versus control lesions not associated with AN (n = 490). Connectivity to brain regions implicated in eating, reward, obsessive-compulsive behavior, and face/body recognition was determined using Neurosynth. Alignment with published neurosurgical ablation and deep brain stimulation (DBS) sites that improve AN was assessed.

Results: AN lesions (n = 15) were heterogeneously distributed across the brain. However, all AN lesion locations were functionally connected to lateral hypothalamus. Functional connectivity to lateral hypothalamus was both sensitive and specific to AN versus control lesions (p < 0.05). An AN network defined by functional connectivity to lateral hypothalamus included positive connectivity to brain regions previously implicated in reward, eating, fear, and obsessive-compulsive behavior, and negative connectivity to regions implicated in body/face recognition (p < 0.05). Neurosurgical ablation and DBS sites that improve AN aligned with positive connections of this AN network.

Conclusions: Lesions associated with AN map to a common brain network defined by functional connectivity to lateral

hypothalamus. This AN network may have therapeutic relevance as a brain stimulation treatment target for AN.

Keywords: Anorexia Nervosa, Lesions, Human Connectome, Functional Connectivity, Neuromodulation

Disclosure: Nothing to disclose.

P201. Activational Differences in the Lateral Habenula of Rats With Varied Preferences for Palatable Food

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Background: The overconsumption of highly palatable food (high fat, sugar) is believed to significantly contribute to the development of various maladaptive feeding behaviors (ie, binge eating). Identification of brain substrates that inhibit the increased intake of palatable food can contribute to our understanding of excessive food preferences and how to control that behavior. The lateral habenula (LHb) is a brain region that has been previously identified as a component that exerts control of feeding.

Methods: Female Sprague Dawley rats (n = 7-8/group, 250-300g) underwent nine feeding tests, to characterize feeding phenotypes as BEP or BER based on their consumption of palatable food (PF, high fat, sugar pellets). The median 4-hour PF intake was utilized to establish the upper, middle, and lower tertiles where animals were characterized as high preferring (HP), low preferring (LP), or neutral. After the phenotypes were identified, animals were transcardially perfused and processed for c-Fos immunoreactivity at two different levels from bregma (-3.2mm and -3.6mm). A control group of rats with only regular chow and no PF exposure were included in the study.

Results: Data show that PF intake was significantly higher in HP versus LP rats ($p < 0.05$) on feeding test days. A significant increase in c-Fos immunoreactivity when comparing chow only versus HP rats ($p < 0.05$); however, this significance was not observed when comparing chow only versus LP rats at -3.2mm from bregma. At -3.6mm from bregma, there was a trend towards significance ($p = 0.07$) in c-Fos immunoreactivity when comparing chow only versus HP rats. No trend towards significance in c-Fos immunoreactivity was observed when comparing chow only versus LP rats at -3.6mm from bregma.

Conclusions: Preliminary analyses reveal that rats with prior exposure to PF have higher expression of c-Fos versus control rats in the LHb. In particular, the LHb is more activated in rats with increased consumption of a high-fat, sugar diet. Differential activation of c-Fos immunoreactive cells was observed in the more rostral portion of the LHb versus the caudal portion. These data will help to guide ongoing investigations to delineate functional circuit connectivity of the LHb and other regions that regulate hedonic and maladaptive feeding states.

Keywords: Maladaptive Feeding, Lateral Habenula, Behavioral Model

Disclosure: Nothing to disclose.

P202. Nos1 Neurons Regulate Feeding by Signalling the Homeostatic Salience of Food

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Background: Alterations in food intake can be influenced by a number of factors aside from energy balance, including cognitive, emotional or environmental. The insular cortex is a brain region that has been proposed to mediate non-homeostatic control of feeding, in particular through learned associations with environmental cues (e.g., conditioned overconsumption and conditioned taste aversion). The insula is also proposed to be a critical part of a "salience network" which determines the homeostatic relevance of environmental stimuli in order to provide adaptive decision-making. Despite this, the neurobiological underpinnings of the "salience network" have not yet been identified. Here we propose that insular cortex Nitric-oxide synthase 1 neurons may serve this function and regulate food intake by signaling the homeostatic salience of food.

Methods: Nos1-Cre mice (n = 6-10) were injected with a cre-dependent virus expressing the inhibitory DREADD, hM4Di or the activating DREADD, hM3DQ into the insular cortex and tested for learned feeding behaviors, anxiety tasks, and homeostatic feeding. 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 neural activity was examined with either Fiber Photometry or Miniscope imaging in the same tasks. Nos1-Cre mice (n = 4) were examine for inputs using monosynaptic Rabies tracing and whole brain clearing. A subset of Nos1-Cre mice (n = 4-6) were also crossed with Prkcd-Flp or Sst-Flp mice in order to examine dynamics of Nos1 to CeA circuits in salience detection and feeding.

Results: We found that inhibition of Nos1 neurons specifically disrupts feeding in tasks that involved learned associations, but do not regulate homeostatic feeding, even of palatable food. Calcium recording/imaging experiments further suggest that Nos1 neurons specifically encode the homeostatic relevance of food in the environment, in that activity is higher when mice are fasted than fed ad lib, and also when food is novel or highly palatable. Accordingly, activation of Nos1 neurons increases food intake in homeostatic conditions, likely by increasing the salience of food. Inputs to Nos1 neurons arrive from a number of areas, including basolateral amygdala, somatosensory cortex, the endopeduncular nucleus and areas of the brainstem. Moreover, activation of Nos1 neurons can modulate activity within defined central amygdala populations.

Conclusions: We have found that Nos1 neurons specifically regulate non-homeostatic feeding behaviors, likely by signaling the homeostatic salience of food in the environment. Sensory inputs regarding the salience of food may arrive from upstream regions, which will be tested in the future. We have thus uncovered a potential circuit for salience in the insular cortex, which may be altered in diseases and syndromes that involve maladaptive feeding.

Keywords: Insular Cortex, Eating Disorders, Salience

Disclosure: Nothing to disclose.

P203. Food Restriction Influences Action Control Strategies and Dopamine Release in the Dorsal Striatum of Mice

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Background: Diverse factors influence how we control our actions. For example, reinforcement schedules bias control strategies via distinct action-outcome contingencies. However, non-learning related factors have equally important influences on behavior and must be studied in combination with reinforcement

principles to understand how animals learn and control their actions.

One such factor is food deprivation – a condition often experimentally induced to enhance task engagement and learning rate in operant training. While restricting caloric intake is known to affect general states such as motivation and stress, whether and how it specifically changes action control strategies is not well understood.

Methods: The goal of this study is to - using instrumental conditioning, fiber photometry, ex vivo slice voltametry and immunoblotting - determine the neural mechanisms that underlie the influence of food restriction on action control strategies.

Results: 1)We first induced different levels of food restriction in male and female mice and showed that stricter food restriction during training on a random interval schedule results in behaviors that are more sensitive to extinction. This finding indicates food restriction promotes a goal-directed strategy and suggested that dorsomedial (DMS) and dorsolateral (DLS) striatum dopamine may be differentially affected.

2)To test this hypothesis, we measured dopamine release in striatal slices using voltammetry. We found that food restriction increased dopamine release in DMS but not in DLS. This result suggested that food restriction promoted changes in the regulation of dopamine release at axon terminals.

3)Similarly, in vivo fiber photometry recordings using the dopamine sensor dLight revealed that food restriction modulated dopamine release in DMS, but not DLS. Surprisingly, only the response to sucrose consumption was increased, whereas the responses to randomly delivered external stimuli were not affected by food restriction. This finding raised the question: how could terminal regulation differentially impact dopamine released in response to distinct external events?

Our voltammetry and fiber photometry data suggest that modulation by food restriction state change terminal release in a way that is only apparent when large amounts of dopamine are released. Thus, we hypothesized that food restriction changes the expression of the dopamine transporter (DAT), which normally works to blunt the size of large dopamine transients and controls re-uptake dynamics.

4)Consistent with our hypothesis, we found that expression of a phosphorylated version of DAT (T53) was reduced specifically in DMS under food restriction conditions.

Conclusions: Thus, our study shows that food restriction fundamentally affects behavioral control strategy and the balance of dopamine release in dorsal striatum. Specifically, our data suggest that food restriction reduces the efficiency of dopamine re-uptake by DAT via phosphorylation of the T53 residue specifically in DMS. This leads to enhanced dopamine release in DMS compared to DLS, thus promoting DMS-dependent goal-directed control of actions.

We are currently performing several experiments to test predictions that stem from these observations and to determine whether DAT phosphorylation, enhanced dopamine release and behavioral changes are causally linked.

Keywords: Dopamine, In Vivo Fiber Photometry, Dorsal Striatum

Disclosure: Nothing to disclose.

P204. Binge Eating as Self-Medication? Excessive Food Intake Normalizes Hedonic Tone in Female Rats via an Orexin (Hypocretin) Circuit

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Background: It has been proposed that persons of higher weight binge eat to 'self-medicate' against depressive/dysphoric states. The neural mechanisms underlying this phenomenon remain understudied, in part because of a lack of preclinical studies examining binge-like eating in animal models of obesity. Here, we asked whether diet-induced obesity (DIO) in rats predisposes to depression-like behaviors, and whether these are ameliorated by binge-like eating. We also examined whether DIO alters the functioning of orexin neurons and their projections to ventral tegmental area (VTA), and whether these changes can be reversed by binge-like eating.

Methods: In female Long Evans rats, DIO was induced by maintaining rats on a high fat diet (45% fat) for 8w; control rats ('lean') were fed chow over the same period. Binge-like eating was then promoted in DIO and lean rats by providing intermittent, restricted access to sweetened fat for 4w. Hedonic tone was measured before and after dietary/binge interventions using intracranial self-stimulation ($n = 6$-7/group) and social preference ($n = 8$/group) assays. We also measured how binge-like eating affected the expression of 'addiction-like' food behaviors in lean ($n = 16$) vs. DIO ($n = 20$) rats by measuring sucrose demand, sucrose seeking during periods of signaled food non-availability, and reinstatement of extinguished sucrose seeking. In another group of rats, orexin release in VTA was measured using fiber photometry recordings of the OxLight1 sensor ($n = 5$-6/group). In a final group of DIO rats ($n = 17$), we tested if binge eating and 'addiction-like' behaviors could be reversed by chemogenetically inhibiting orexin neurons.

Results: DIO rats had higher ICSS thresholds ($p = 0.035$) and lower social preference ($p = 0.001$) compared to lean controls. DIO rats exhibited greater escalation of binge-like eating ($p = 0.041$), which partially normalized ICSS thresholds ($p = 0.032$) and social preference ($p = 0.054$) in these rats. Binge-like eating promoted higher 'food addiction' behaviors in DIO but not lean rats (all p's < 0.042). In lean rats, food-associated stimuli elicited orexin signaling in VTA as measured by OxLight1 signal; this was blunted in DIO rats but partially restored by binge-like eating ($p = 0.045$). Chemogenetic inhibition of orexin neurons reduced binge eating ($p = 0.045$) and 'food addiction' behaviors (all p's<0.05).

Conclusions: DIO in female rats promotes depression-like outcomes and a deficit in reward-associated orexin signaling in VTA. Binge-like eating partially normalizes these depression outcomes, but also promotes 'food addiction' behaviors; these can be blocked by inhibiting orexin neuron signaling. This supports the notion that binge eating in persons of higher weight might become governed by negative (rather than positive) reinforcement processes and that these are mediated by the orexin system.

Keywords: Binge Eating Disorder, Obesity and Eating Disorders, Orexin, Ventral Tegmental Area (VTA), Depression Model

Disclosure: Janssen Pharmaceuticals: Employee (Spouse/Partner).

P205. Novel Role of Nucleus Accumbens D2 MSNs During Hedonic Feeding

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Background: Appropriate feeding behavior is essential to survival and well-being. Both negative and positive reinforcement signals in the brain and body motivate food seeking. The striatum, particularly the nucleus accumbens (NAc), critical regulates motivated behavior and reinforcement learning. While

multiple studies demonstrate its important role in regulating homeostatic and hedonic feeding, overarching mechanistic explanations are lacking. Creating a cohesive framework requires consideration of numerous parameters governing eating in distinct situations that relate to the organism's internal state and experiential history.

Methods: High fat intake. Mice were grouped housed with ad libitum access to standard chow and water. For exposure, individual mice were placed into the same transparent cage without bedding with a 20 min habituation period prior to high fat access. Weight matched mice were randomly assigned to each experimental condition. A single, pre-weighed high fat pellet was provided to the mice in their own cage daily for 20 min at the same time each day for a given experiment. Intake of the high fat diet within that period was measured. All mice with appropriate expression and targeting were included in data presentation and analyses.

Chemogenetics: Transgenic mice expressing Cre under a D1 promoter (D1-Cre) or A2a promoter (D2-Cre) underwent intracranial injections of AAVs encoding either a are-dependent DREADD (inhibitiory hM4Di, activating hM3Dq) or eGFP control virus into the NAc bilaterally (bregma coordinates: angle 10°, anteroposterior 1.6, mediolateral ±1.5, dorsoventral −4.4) at a rate of 0.1 µl/min. Needles were removed 5 min after infusions were complete. DCZ injections (3 ug/kg, Tocris) were administered intraperitoneally ~30 min prior to the start of a limited-access exposure on days 2-4. All mice were habituated with a saline injection in the morning for the 2 days prior to experimental manipulations.

Fiber photometry: Transgenic mice expressing Cre under an A2a promoter (D2-Cre) underwent intracranial injections of AAVs encoding GCaMP6m into the NAc unilaterally. Optic fibers (ferrules) were implanted above the injections (bregma coordinates: angle 10°, anteroposterior 1.6, mediolateral ±1.5, dorsoventral −4.2). Calcium signals were collected by a fiber photometry system that uses two light-emitting diodes (405 and 465 nm), coupled to an optical fiber. Signal analysis was performed with custom-written MATLAB software to measure $Ca2+$ event activity and average amplitude during bouts of feeding. To ensure scientific rigor, all experiments will be conducted in a blinded manner.

Results: We find that bidirectional modulation of MSN subtype activity had opposing effects on high fat intake (measured in cal/g), We confirmed a subset of these results in a similar limited access paradigm using the homecage FED device. Here we examined D2 MSN activity in a control and high fat exposure context and find increased activity of D2 MSNs on the fourth day of exposure compared to day 1. Current investigations are examining the magnitude of population activity events during bouts of intake. Finally, we observed a significant basal sex difference in intake between male and female mice. Further investigation into basal sex-specific differences of high fat intake behavior will be prioritized to gain insight into how hedonic feeding is uniquely regulated between sexes.

Conclusions: Consistent with the numerous studies examining how modulation of MSN activity regulates reward-related behavior, we find bi-directional modulation of MSN subtypes has opposing effects on high fat intake. Previous work showed an increase only in D1 MSN population activity during bouts of high fat intake in sated, single housed male mice, however, chronic exposure to a "cafeteria diet" results in a down regulation of striatal D2 receptor. Our results are in line with hypothesis that the activity of both MSN subtypes is involved in hedonic feeding as soon for other motivated behaviors and motor activity. Still unclear is the neural basis underlying our basal sex differences in high fat intake. Further investigations will be prioritized to gain insight into how hedonic feeding is uniquely regulated between sexes.

Keywords: Motivated Behaviors, Nucleus Accumbens, Feeding Behavior

Disclosure: Nothing to disclose.

P206. Increased Prevalence of Parkinson's Disease in Families of Individuals With Eating Disorders: Importance for Identifying Neural Circuits for Eating Disorders

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Background: Effective treatments for eating disorders (ED), including pharmacological treatment for anorexia nervosa (AN) have been elusive, due to a lack of a clear mechanistic understanding. Recent imaging, medication, and genetic studies have suggested that striatal neural pathways or dopamine (DA) play a role in ED. This potential link raised our curiosity when several patients with AN noted that a close relative had Parkinson's Disease (PD). To explore this, we designed a study investigating the incidence of PD in families of those with ED.

Methods: We assessed 2 groups of subjects. 1) 483 male and female ED subjects applying for an admission to the UCSD ED treatment program, and 2) 724 females from the community (healthy controls and those with an ED). Subjects completed a brief study screen with a trained masters-level clinician regarding eating disorder diagnosis, weight, and psychiatric and medical history and presence of a family history of PD.

Results: Considered together, for 727 individuals with ED, 7.2% had a relative with PD. This was significantly more ($p = 0.01$) than 408 healthy control women, of which 3.4% reported having a relative with PD. Participants in the UC San Diego Eating Disorder Center for Treatment and Research met criteria for varying eating disorder diagnoses (359 individuals with an AN-spectrum disorder; 98 individuals with BN; 26 individuals with BED). The community sample consisted of 408 healthy control females and 244 females with a similar mixture of ED diagnoses. Rates of family PD were similar for individuals with an ED that were treatment seeking (7.5%); or the individuals from the community with an ED (6.2%).

Conclusions: There is an increased prevalence of a family history of PD among individuals with an ED compared to community controls suggesting that ED and PD may share some vulnerability. Importantly, a recent study (Smeland 2023) using GWAS data found common genetic risk between AN and PD. Given the difference in course and behaviors, a relationship between ED and PD may seem preposterous, however it is well known that anxiety and weight loss, which is common in AN, occurs early in the course of PD and some literature suggests ED and PD have similar premorbid temperament. Considered together, these findings may shed new light on mechanisms underlying reward and punishment, anxiety, feeding, weight loss, and motor activity in ED. That is, consider whether disturbances of the striatum, which performs a computation on sensorimotor, cognitive, and emotional/motivational information to facilitate the selection of an appropriate action out of a collection of possibilities, {Martinez, 2003; Hassler, 1978} contributes to developing an ED. For example, data suggests that striatal alterations (Kaye 2020) may contribute to impaired initiation or disturbed motivation to eat, and thus play a role in restricted eating. Much is known about the neurobiology of PD, and this knowledge could help accelerate insights into puzzling AN behavior, neural function, and new approaches to treatment for ED.

Keywords: Brain, Dopamine, Adaptive Behavior, Learning, Anorexia Nervosa, Eating Disorders, Parkinson's Disease

Disclosures: Compass Pathways: Grant (Self). EDCare: Advisory Board (Self).

P207. Examining the Effects of Methylphenidate on Resting Brain Function: Role of Dopamine

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Background: Stimulant medications such as methylphenidate (MP), which enhance dopamine (DA) and noradrenergic signaling in the brain, are first line treatments for ADHD but also have rewarding effects, especially when injected. The effects of MP on the amplitude of low-frequency fluctuations (ALFF) and global functional connectivity density (gFCD) and their dynamics, two fMRI derived metrics used to assess brain function, are largely unknown. In this study, we utilize simultaneous PET/MRI to investigate how dynamic changes in striatal dopamine induced by MP influence ALFF and gFCD metrics. Since DA modulates neuronal activity, we hypothesized that changes in DA levels triggered by MP would affect the fMRI signals in basal ganglia and cortical regions where DA receptors are located but also in regions to which they project.

Methods: Twenty healthy adults (36.1 ± 9.6 years old; 9 females) underwent 90-min simultaneous PET/fMRI scans collected in 3 randomly ordered sessions (placebo, oral-MP, and intravenous IV-MP) under resting conditions, using oral-MP and IV-MP (ClinicalTrials.gov Identifier: NCT03326245). In each session, each participant received an oral pill (60mg-MP or placebo) 30 min before injection of the PET tracer ([11C]raclopride), followed 30 min after the tracer by an IV injection (0.25mg/kg-MP or placebo, ~30-second bolus). Differences in standardized uptake value ratio (SUVr) between placebo and MP conditions were used to estimate striatal DA increases during IV-MP and oral-MP, and sliding window analysis was used to assess the dynamics of ALFF and gFCD from echoplanar imaging time series at 1min temporal resolution. Multiple linear regression analysis was used to assess the neurovascular coupling of ALFF and gFCD with the amplitude and rate of the DA increases in putamen (striatal region with strongest signal), and a linear mixed-effects model was employed to assess the statistical significance of neurovascular coupling.

Results: MP significantly increased DA in putamen, which was associated with decreases in ALFF in subcortical regions (nucleus accumbens, putamen, caudate, pallidum, thalamus, amygdala, hippocampus, midbrain, and cerebellum) and in occipital cortex, both for IV- and oral-MP conditions [PFDR < 0.05; 0.7 < Cohen's d < 1.6 (oral) or 1.9 (IV)]. The rate (but not the amplitude) of DA increases in putamen was associated with stronger gFCD in putamen, posterior thalamus, and anterior cerebellum, and moderate in nucleus accumbens, caudate, PFC, insula and parahippocampal gyrus (PFDR < 0.05; 0.7 < Cohen's d < 1.6). In subcortical regions, but not in cortical regions, the slope of the linear association between gFCD and the rate of DA increases was stronger for IV- than oral-MP (P < 0.03). Across individuals, ALFF and gFCD demonstrated significant positive temporal correlation in occipital and parietal cortices during oral-MP (PFDR < 0.05; Fig 5A) and placebo conditions. However, during IV-MP, there was a strong negative correlation observed in the remaining brain regions.

Conclusions: Our study employing simultaneous PET-fMRI revealed distinct dopaminergic modulation of brain activity in humans following fast (IV) and slow (oral) challenges with MP. Increases in striatal DA levels induced by MP administration were accompanied by a prominent reduction in ALFF at lower frequencies (0.01-0.03Hz) compared with higher frequencies (0.08-0.10Hz), which suggests that, when injected, stimulant drugs have the potential to disrupt neurovascular processes in brain regions that are not usually regarded as the primary DA targets. Notably, IV-MP administration resulted in increased gFCD in basal ganglia regions, thalamus, anterior cerebellum and anterior cortices that were associated with the rate of DA increases. These findings show that MP promotes increased brain connectivity while reducing ALFF, which could reflect a combination of DA's neuronal effects and of vascular effects that could also reflect MP noradrenergic actions. Our results also highlight the differential effects of fast and slow changes in DA signaling on brain activity.

Keywords: Methylphenidate, Dopamine, Resting-State fMRI, Brain Connectivity, Reward

Disclosure: Nothing to disclose.

P208. Comparing Mechanistically Distinct Interventions That Alter Inter-Temporal Choice Behavior Using Cortical-Striatal Local Field Potentials

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Background: Maladaptive decision making (e.g., too impulsive or risky) is linked to poor outcomes in many psychiatric conditions and is a significant risk factor for suicide, violence, and risky substance use. Animal models used to study this important trans-diagnostic domain of function, its neural underpinnings, and evaluate potential interventions rely on behavioral readouts that may not always capture the overarching domain of interest or pertinent subdomains. Performance metrics obtained from inter-temporal choice tasks provide quantifiable estimates of the overarching domain of choice impulsivity. These metrics also reflect subdomains, such as reward motivation, reward sensitivity and temporal sensitivity, each underpinned by distinct neural processes. Mechanistically distinct interventions may modulate the function of unique subdomains and lead to similar changes in task performance. Here, we evaluated the stimulant methylphenidate at 2 doses and brain stimulation targeting three regions of the cortical striatal network that regulate inter-temporal choices. We simultaneously recorded local field potentials (LFPs) from the same brain regions. We then used machine learning to determine if changes in LFPs induced by an intervention were predictive of intervention outcomes, generalized across intervention types (identifying mechanistic similarities) or distinguished between interventions that caused the same outcome (identifying mechanistic differences).

Methods: Sprague-Dawley rats (N = 89, 44 male, 45 female) were trained in a delay discounting task (DDT) using either an ascending (N = 64) or descending (N = 25) delay order. Rats were implanted bilaterally with electrodes targeting the nucleus accumbens (NAc) shell and core as well as the infralimbic (IL) and orbitofrontal cortex (OFC). LFP recording was paired with video and operant behavioral events (MedPC) during delay discounting task (DDT) sessions. Once DDT performance was stable, interventions were tested (brain stimulation [7 sessions] targeted to the IL, NAc core or OFC; pharmacological manipulations [3 sessions] with 1 or 3 mg/kg of methylphenidate). Custom code written in Matlab was used to extract LFP features of power and imaginary coherence (connectivity between brain regions) across 6 established frequency bands (delta, theta, alpha, beta, low gamma, and high gamma). The 216 LFP features were then used as predictors in machine learning (lasso) models to classify intervention outcomes (increase, decrease [>2 SD change] or no

change in DDT performance). Model performance was determined using the area under the receiver operator characteristic curve (AUROC) from 100 iterations of train:test data sets. We determined if models built from all LFP features significantly outperformed models estimating chance through permutation testing and calculated $p = b + 1/m + 1$. Here, b is the number of permuted model performances greater than the real models mean AUROC and m is the total number of iterations. If $p < 0.05$, then an exhaustive evaluation of each LFP feature (simple regression) as well as permute and re-learn feature importance testing was carried out.

Results: The different types of interventions and delay presentation orders tested produced either binomial outcomes (no change, change in one direction) or multinomial outcomes (significant change in either direction or no change) across animals. As reported by others, we found that methylphenidate increased rat choices for the delayed lever when delays ascended in the session and reduced choices for the delay lever when the delays descended through the session. We found that NAc (AUROC = 0.92, $p < 0.05$); IL (AUROC = 0.97, $p < 0.05$), and methylphenidate (AUROC = 0.94, $p < 0.05$) outcomes could be predicted based on LFP feature changes. Single feature models and permute and re-learn feature importance testing revealed the nature of information content; for example, the model predicting IL stimulation outcomes had many LFP features that contained information and permuting all frontal or all striatal features only reduced model performance by 21%, while permuting all left NAc features reduced model performance by 11%. No specific frequencies carried unique information for this model. Additional modeling was used to determine the nature of similarities and differences between interventions or between ascending and descending delay orders within an intervention. As an example, common intervention-induced changes in LFP features were able to classify both IL and NAc outcomes with a single model (AUROC = 0.74, $p < 0.01$) suggesting some shared mechanisms between these two intervention types.

Conclusions: Intervention induced changes in LFPs were able to predict intervention induced changes in DDT performance. Models built from datasets across intervention types or delay presentation order provided insight into mechanistic similarities and differences underlying intervention outcomes. These data suggest that neural oscillations hold potential as biomarkers to guide the development and implementation of therapeutic approaches to change maladaptive decision making.

Keywords: Local Field Potentials, Impulsive Behavior, Brain Stimulation, Behavioral Pharmacology, Machine Learning Classification

Disclosure: Nothing to disclose.

P209. Single-Cell Transcriptomic Evaluation of a Stress Vulnerability Brain State

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Background: Increased vulnerability to stress is a major risk factor for the manifestation of several mood disorders, including major depressive disorder (MDD). Although MDD is a significant contributor to global disability, much has yet to be understood regarding the complex integration of genetic and environmental factors that contribute to such disorders. Recent advancement in both human and rodent models suggest that a brain-wide network approach is needed, taking into account the complex interplay of cell types spanning multiple brain regions. Many thorough studies have used rodent models of chronic psychosocial stress in order to uncover the underlying gene expression architecture of susceptibility and resilience to stress. An important unanswered question that remains is whether such genes are compensatory in response to stress or pre-existing prior to the stress. We have previously used local field potential oscillations and machine learning to identify an electrical brain network that is indicative of a predisposed vulnerability state. Here we conduct transcriptomic profiling at the single-cell level in prefrontal cortex of animals with high and low electrical brain activity corresponding with the predisposed vulnerability brain state.

Methods: This study combines single cell RNA-sequencing (scRNA-Seq) with multi-site in vivo neurophysiology in freely behaving mice in order to dissect the cellular and molecular architecture underlying a stress vulnerability brain network. We used a previously identified network of stress vulnerability, Electome Factor 1 (EF1), to discriminate between mice with high and low stress vulnerability network activity. Differential gene expression analysis was carried out for each cell type comparing high EF1 and low EF1 groups. Pathway analyses (gene ontology and gene set enrichment analysis) were used to identify functional biological pathways that support stress-resistant and stress-vulnerable brain states. Follow up analyses include use of MAGMA software to compare differentially expressed genes (DEG) against human GWAS risk genes for a range of brain disorders. Finally, weighted gene coexpression analysis (WGCNA) was used to identify differential networks and highly interconnected genes between high and low vulnerability states.

Results: Using local field potential (LFP) metrics collected from five brain regions across 12 C57BL/6 mice (male and female), we clustered brain network activity by stress vulnerability score (EF1 score), which resulted in high, medium, and low vulnerability groups. We then compared gene expression across cell types between animals in the high and low stress vulnerability network activity (EF1) groups. Using a flexible zero-inflated negative binomial model (ZINB-WaVE), we identified a number of genes in each cell type that were significantly differentiated expressed (FDR < .05): Glutamatergic neurons, with 838 genes significantly more highly expressed with high EF1 scores, 548 more highly expressed in mice with low EF1 scores; GABAergic neurons, 242 genes more highly expressed in the high EF1 mice, 140 genes highly expressed in the low EF1 group; astrocytes, 341 genes with high EF1, 221 genes with low EF1; microglia, 46 genes more highly expressed in the high EF1 group. Across neuronal and astrocyte cell types, pathway analysis indicated mice with high EF1 scores were significantly enriched for biological processes involved in respiratory and mitochondria related processes. Low EF1 score mice had enrichment in synaptic biology pathways. In order to assess which, if any cell type had the strongest relationship with EF1 activity, we used a generalized additive model (GAM) with the top two principal components of gene expression and found statistically significant ($p < .05$) relationships with GABA-ergic neurons for both PC1 and PC2 and for astrocytes PC1. Using WGCNA, we identified 15 network modules. We identified one module in particular with significant trait-module correlation for EF1 activity in GABA and glutamatergic neurons and astrocytes, all padj(< .05). This module was particularly strong for mitochondrial gene pathways. Finally, we find that genes identified as differentially regulated with vulnerability network activity significantly overlap with genes identified as having significant SNPs by human GWAS with depression but not non-psychiatric brain disorders such as Alzheimer's Disease.

Conclusions: Taken together these data provide the gene expression architecture of a novel stress vulnerability brain state, demonstrating that at least four major cell subtypes contribute to this state including both glutamatergic and GABAergic neurons,

astrocytes and microglia. Several themes emerge that are consistent with other clues from the literature regarding the pre-stressed vulnerability brain state. These include genes related to activation of microglia as well as mitochondrial electron transport chain functional pathways across all cell types. We determined that gene expression in GABAergic cells and astrocytes plays a particularly unique role in stress vulnerability brain activity. Comparison of these data to human GWAS data for psychiatric disorders suggests that the DEGs identified here have biological significance relevant to human disease. These data provide the first evidence of transcriptional regulatory support for dynamic brain-wide activity and a stress vulnerability brain state.

Keywords: Stress, Transcriptomics, Brain Networks

Disclosure: Nothing to disclose.

P210. Multi-Omics Profiling of Human Hippocampus Neurogenic Niche to Decipher the Neurobiology of Major Depression

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Background: The existence of human adult hippocampal neurogenesis remains debated despite the 1998 finding that administering BrdU to live patients resulted in its incorporation into dividing cells that expressed neuronal markers postmortem and were located in the subgranular zone of the dentate gyrus (DG). BrdU-labeling has demonstrated adult neurogenesis in rodents and non-human primates but cannot be replicated in humans due to ethical concerns. Immunohistological and recent RNA sequencing studies of the adult human neurogenic niche had inconsistent results. To address this problem, we applied single-nucleus RNA sequencing (snRNA-seq) and assay for transposase accessible chromatin with sequencing (snATAC-seq), together with spatial RNA sequencing, to profile cells of the human adult hippocampus, and investigate molecular features that could confirm or refute the presence of immature cell populations. Because the DG was found to be smaller in Major Depressive Disorder (MDD), and neurogenesis appears necessary to confer stress resilience in mice, we further assessed cellular and molecular differences between MDD and neurotypical controls.

Methods: We used Chromium Single-Cell Multiome Assay, for gene expression and open chromatin profiling, and Visium (10x Genomics) to resolve the spatial transcriptome in intact tissue, following manufacturer's instruction. Tissue was obtained from the Brain Collections of the New York State Psychiatric Institute at Columbia University and NIMH. We assayed the hippocampus proper, including DG and cornu ammonis regions, (2-3 biological replicates) from 9 neurotypical (age 40 + /-12 yrs) and 7 untreated MDD (age 48 + /- 10 yrs) males. A subsample (3 MDDs; 3 controls) were used for Visium (2 biological replicates, 12 capture areas total). All samples had RIN > 7 (7.9 + /- 0.4). Libraries were sequenced (NovaSeq 6000 Sequencer, Illumina) to an average sequencing depth of 62,000 reads/cell for RNA libraries, 33,000 reads/cell for ATAC libraries, and 67,000 reads/spot for Visium libraries with a total of 41,878 spots. Each sample's RNA and ATAC data were preprocessed using Seurat, Signac, and DoubletFinder. Batch effects for merged RNA and ATAC objects were removed using Harmony. Multimodal data for 293,236 cells were integrated using Weighted Nearest Neighbor analysis in Seurat. Cell types were annotated using canonical gene expression, enriched genes identified by a Wilcoxon rank-sum test (Bonferroni-adjusted p-value), and prediction scores from integration with spatial data implemented with TransferData function in Seurat. Gene ontology (GO) terms were generated using clusterProfiler (FDR-adjusted q-value). To test for differentially expressed genes (DEGs) in MDDs vs. neurotypicals, we implemented a likelihood ratio test accounting for the bimodal expression distribution of single-cell data using MAST with latent.vars set to total number of RNA molecules detected within the cell and donor identity.

Results: We identified the hippocampus canonical cell types: granule neurons (GN), non-granule excitatory neurons (ExN), inhibitory neurons (InN), astrocytes, oligodendrocytes, microglia, vasculature, ciliated epithelial cells, choroid plexus cells, Cajal-Retzius cells, and oligodendrocyte progenitor cells (OPC).

We discovered a neural progenitor cell cluster (NPC) enriched for EGFR ($p = 1.68E-35$), BMPR1B ($p = 1.25E-31$), NOTCH2 ($p = 4.43E-11$), and DLL3 ($p = 4.48E-21$). Top GO terms in NPC included 'regulation of nervous system development' ($q = 2.11E-05$) and 'cell fate commitment' ($q = 2.11E-05$). We identified a distinct cluster of immature granule neurons (ImN) enriched for PROX1, DCX, CALB2 ($p < 2.23E-308$), that also expressed GAD1 ($p < 2.23E-308$). Biological process GO terms unique to ImN included 'central nervous system neuron axonogenesis' ($q = 9.11E-05$), 'excitatory synapse assembly' ($q = 0.004$), and 'ephrin receptor (involved in nervous system development) signaling pathway' ($q = 0.024$).

ATAC data showed NPC and ImN had increased chromatin accessibility of ASCL1, involved in neuronal commitment and differentiation ($p = 0.00013$ and $p < 2.23E-308$ respectively).

Integration of spatial data with single-cell data confirmed the expected location of GN, ExN, oligodendrocytes, astrocytes, and ImN clusters.

In MDD vs. controls, DEGs included: upregulated interferon inducible genes (IFI44, IFI6, IFIT1, etc.) in GN, ExN, InN, ImN, astrocyte, microglia, choroid plexus, OPC ($p < 0.01$), and down-regulated transthyretin (TTR), which transports thyroid hormone, in GN, ExN, InN, astrocyte, microglia, vasculature, and ciliated epithelial cells ($p < 4.06E-05$).

Conclusions: This is the first multiome single cell and spatial study of adult human hippocampus from neurotypical and MDD subjects. We first detected NPCs enriched in genes involved in cell fate determination and developmental processes. Genes involved in neuronal migration and modulation of excitability characterized ImN. In addition, GAD1 expression in ImN explains their known inhibition of mature GNs. Results provide evidence of a neurogenic niche in the adult human hippocampus and support a role of neuroinflammation in MDD. Integration of single nuclei and Visium data provided further validation of cell type annotation. Molecular landscape of canonical and immature cell populations in MDD vs. controls will point to druggable targets to support cell viability and regeneration in MDD. Studies including females and a wider age range are being conducted.

Keywords: Hippocampal Neurogenesis, Single-Cell Genomics, Spatial Transcriptomics, Major Depressive Disorder (MDD)

Disclosure: Nothing to disclose.

P211. The Astrocyte-Specific Extracellular Matrix Gene Htra1 Sex-Specifically Regulates Susceptibility to Stress

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Background: The exact pathophysiology of major depressive disorder (MDD) remains poorly understood, but emerging evidence from both pre-clinical and clinical research suggests that MDD is associated with compromised structural plasticity in crucial limbic regions. Astrocytes, pivotal in synapse regulation and influenced by chronic stress, likely play a significant role in driving these changes. Delving into the interplay between astrocytes, synaptic plasticity, and chronic stress holds promise for gaining crucial insights into the underlying mechanisms of MDD.

Methods: We analyzed the transcriptional profiles of astrocyte-related genes in the nucleus accumbens (NAc) of postmortem brain tissue from both males and females with major depressive disorder (MDD) and mice displaying depression-like behavioral abnormalities after 21 days of chronic variable stress (CVS) exposure. After identifying common dysregulated astrocyte-specific genes across species, we validated their cell-type specificity using in situ hybridization in male and female mice exposed to CVS. To establish causal relationships, we employed viral vectors to overexpress or knockdown target genes in a cell-type specific manner and evaluated behavioral reactivity to sub-threshold stress exposure. In a separate group of mice, we examined changes in the electrophysiological properties of both D1 and D2 subtypes of dopaminergic (DA) medium spiny neurons (MSN). These MSNs were recorded ex vivo following cell-type specific manipulation of target genes combined with sub-threshold stress exposure. Additionally, to verify any alterations in MSN activity observed ex vivo, we employed fiber photometry during behavioral responses after cell-type specific manipulation of target genes combined with sub-threshold stress exposure.

Results: We found that Htra1, an astrocyte-enriched secreted serine protease that targets the extracellular matrix (ECM), was significantly down-regulated in the NAc of males and up-regulated in females across both species. Notably, we found that selectively manipulating the Htra1 gene in astrocytes within the mouse NAc had bidirectional effects on susceptibility to stress, exhibiting a sex-specific pattern. Moreover, direct manipulation of Htra1 in NAc astrocytes, combined with sub-threshold stress exposure, had a sex-specific impact on MSN activity confirmed both with ex vivo recordings and by fiber photometry recordings during behavior. Additionally, our investigation revealed that CVS exposure resulted in sex-specific alterations in perineuronal net (PNN) intensities in the NAc. Strikingly, when we specifically manipulated Htra1 in astrocytes along with sub-threshold stress exposure, we observed a recapitulation of the changes in PNN intensity that were initially observed with CVS, once again demonstrating sex-specific effects.

Conclusions: Our study uncovers the crucial involvement of astroglia and the brain's ECM in influencing stress vulnerability, with significant sex-specific implications. These findings open up new avenues for exploring innovative therapeutic strategies for treating MDD.

Keywords: Chronic Stress, Major Depressive Disorder (MDD), Astrocyte, Sex Differences, Extracellular Matrix

Disclosure: Nothing to disclose.

P212. Identification of Psychedelic Signaling Signatures Across the Serotonin GPCROME

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Background: Since psychedelics have been designated with "breakthrough therapy" status by the FDA, a "psychedelics renaissance" is aimed at drug discovery at serotonin (5-HT) G protein-coupled receptors (GPCRs). GPCRs signal via canonical and non-canonical G proteins or β-arrestins to elicit their multi-dimensional signaling. However, key questions remain into which receptors or signaling effectors are required for psychedelic potential. In fact, interest in this question is at an all-time high, both at academic and industrial levels, because uncovering key receptor signaling pathways will lead to next-generation neuropsychiatric medicines.

Methods: We designed new selective 5-HT2A agonists using structure-based design and docking, and synthesized these toward the discovery of β-arrestin-biased agonists. We optimized and employed kinetically-sensitive BRET platforms capable of detecting signaling transducer functional activity for 5-HT2A and all of the serotonin receptors. We then formed a multi-dimensional structure-activity relationships (SAR) for this series and performed molecular dynamics to uncover a mechanism for β-arrestin-biased agonism. We use rodent head-twitch response (HTR) to assess psychedelic potential and mouse PCP-hyperolocomotion assays to assess anti-psychotic potential.

Results: Here we show traditional psychedelics show poly-pharmacology across the 5-HT GPCRome with little selectivity for the 5-HT2A receptor. We also show all tested psychedelics have little preference for 5-HT2A Gq/11 and β-arrestin2 signaling pathways, making it unclear which receptor(s) and signaling pathway(s) are responsible for psychedelic potential versus fast-acting antidepressant effects. Toward this problem, we developed 5-HT2A-selective ligands with various efficacies for Gq-mediated signaling, resulting in several β-arrestin-biased ligands. We show that the mechanism for β-arrestin-biased agonism is due to steric and aromatic interactions with a key binding pocket residue, W6.48, whereby the bulkier biased agonists "push" on this switch to prevent G protein activation states. Importantly, we show that 5-HT2A β-arrestin-biased agonists induce 5-HT2A internalization, downregulation, and show tachyphylaxis in a repeated treatment head-twitch experiment with psychedelics. Finally, we show 5-HT2A β-arrestin-biased agonists can block mouse PCP-hyperlocomotion similar to other antipsychotics.

Conclusions: These results establish 5-HT2A-Gq signaling efficacy is important for psychedelic-like effects. We also establish a structural mechanism for 5-HT2A β-arrestin-biased agonists and for 5-HT2A selectivity. Finally, we demonstrate unique profiles for β-arrestin biased compounds including the ability to internalize and downregulate the 5-HT2A receptor, and cause tachyphylaxis for psychedelics. Overall, serotonin GPCR signaling properties can be exploited for next-generation rapid-acting neuropsychiatric drugs.

Keywords: Psychedelic Medicine, Serotonin 5-HT2A Receptor, β-arrestin, Tolerance, Antipsychotics

Disclosure: Nothing to disclose.

P213. Loss of Neuronal Glycine Decarboxylase is Associated With Glycine Abundance, Mitochondrial Dysfunction, and Decreased Motivated Escape Behavior

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Background: Chemical modifications on transfer RNAs (tRNAs) are critical for maintaining tRNA regulatory functions in protein synthesis yet largely unexplored in the adult brain. We recently

showed that there is a potent effect of Nsun2 tRNA methyltransferase depletion, via loss of tRNA cytosine methylation, on codon-specific tRNA expression and glycine amino acid levels, producing proteomic shifts that impair synaptic transmission, cognition, and depressive-like behavior. Because dysregulation of glycine via alteration of the glycine cleavage system (for example, Glycine decarboxylase; Gldc) can have a large impact on cellular function via changes in metabolic activity, we sought to explore whether this may be a mechanism by which loss of Nsun2 alters behavioral outcomes.

Methods: We used two Cre-driven conditional knockout mouse lines to postnatally delete 1) Nsun2 or 2) Gldc and used western blot and glycine ELISA to confirm protein abundance and glycine levels in mutant mice. We used fluorescent-activated cell sorting (FACS), cell-type specific proteomics, and Riboseq to assess the proteome and translatome respectively ($n = 2$-3/group). We performed a battery of behavioral tests on male and female mice to assess cognition, anxiety-like behavior, and motivated escape behavior. Data was analyzed using two-tailed t-tests and two-way ANOVAs with Bonferroni posthoc tests when appropriate.

Results: We found a significant loss of Gldc in Nsun2 knockout mice that corresponded to a large increase in glycine amino acid. An unbiased proteomics screen of Nsun2 knockout neurons revealed massive dysregulation of proteins involved in mitochondrial function, which is in line with altered translation efficiency of these genes found in Riboseq. Gldc knockout mice also showed an upregulation of glycine amino acid ($p < 0.01$) and similar proteomic changes, which may contribute to decreased motivated escape behavior in the tail suspension ($p < 0.05$) and forced swim tests ($p < 0.01$).

Conclusions: Alterations to the glycine cleavage system following epitranscriptomic deficits may be a crucial mechanism for phenotypic changes relating to psychiatric disease. Future studies will identify how Gldc-driven molecular changes to mitochondria contribute to behavioral phenotypes and may provide insight into novel areas of therapeutic intervention for psychiatric disease.

Keywords: Epigenetics, Glycine, Behavioral Despair, Mitochondria

Disclosure: Nothing to disclose.

P214. In Vivo [11C]ER176 Brain and Non-Brain Translocator Protein (TSPO) PET Binding in Major Depressive Disorder and Healthy Volunteers

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Background: Major depressive disorder (MDD) has been associated with inflammation through brain and peripheral blood-based markers. The presence of neuroinflammation is consistent with studies of translocator protein (TSPO) binding assessed by positron emission tomography (PET). The relationship between brain and peripheral inflammation in general, and in MDD specifically, however, remains unclear. We used PET imaging with the TSPO-specific radiotracer [11C]ER176 to compare brain and non-brain TSPO binding in individuals with MDD and healthy volunteers (HVs). Brain and non-brain TSPO binding were also compared with peripheral blood markers of inflammation (C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-alpha)) and the relationship of all three sets of indices with depression severity was examined.

Methods: Dynamic PET scans with concurrent arterial blood sampling were obtained in currently-depressed, medication-free participants with MDD ($n = 36$) and HVs ($n = 16$). Brain tracer total volume of distribution (VT) was quantified using a metabolite-corrected arterial input function and Logan graphical analysis in 11 a priori brain regions, and regional VT values were averaged and weighted by region size to obtain one whole-brain VT for each participant. In each scan, non-brain TSPO VT was quantified by applying k-means clustering to partition the entire PET field of view into 30 clusters of voxels encompassing the head and neck areas. Each of these 30 clusters contained voxels with similar time activity curves (TACs) throughout the entire PET scan duration. The cluster whose average TAC had the greatest [11C]ER176 tracer uptake was identified, and non-brain VT was quantified in that cluster using Logan graphical analysis and an arterial input function without metabolite correction. This non-brain cluster included bilateral parotid glands and possibly neck lymph nodes. To assay peripheral blood markers, a thawed aliquot of serum obtained at the time of the PET scan was processed according to the Meso Scale Discovery V-Plex protocol. Linear regressions with either brain or non-brain TSPO binding as the model outcome assessed relationships: (1) across diagnoses, (2) with peripheral blood markers, and (3) with depression severity (Beck Depression Inventory (BDI) score). A model was also fit with brain TSPO binding as the outcome and non-brain TSPO binding and diagnosis as predictors to assess the association between brain and non-brain TSPO across diagnoses. Lastly, a model was fit with each peripheral blood marker as the outcome and diagnosis as a predictor. Age, sex, body mass index, and rs6971 genotype were included as covariates in all models.

Results: Neither brain nor non-brain TSPO binding differed between MDD and HV groups. A positive correlation between brain and non-brain TSPO binding was present in MDD, but not in HVs (estimate (B)=0.81, standard error (SE)=0.14, model adjusted R-squared = 0.62, $p < 0.001$, and $B = 0.36$, $SE = 0.21$, R-squared = 0.39, $p = 0.11$, respectively). Brain and non-brain TSPO binding were both unrelated to CRP, TNF-alpha, or IL-6 in either diagnostic group. In participants with MDD, brain TSPO binding correlated positively with depression severity ($B = 0.012$, $SE = 0.004$, R-squared = 0.55, $p < 0.01$), whereas no such relationship was observed with non-brain TSPO binding ($B = 0.008$, $SE = 0.005$, R-squared = 0.18, $p = 0.15$). CRP, TNF-alpha, or IL-6 did not differ between MDD and HV groups and no correlations were found between these peripheral blood markers and depression severity in participants with MDD.

Conclusions: This is the first study to compare brain and non-brain TSPO binding assessed by PET. A positive correlation between TSPO binding in the brain and a non-brain TSPO cluster containing the parotid glands and possibly neck lymph nodes was found in MDD but not in HVs. This indicates a potential pathologic effect that impacts both brain and non-brain TSPO binding in MDD. Further, in MDD, brain TSPO binding was the only marker related to depression severity. One possible hypothesis is that a common effect activated both brain microglia/astrocytes and non-brain parotid gland macrophages, increasing comparably expression of TSPO. There is mixed support for the hypothesis that peripheral inflammation can affect the brain through a leaky blood brain barrier (BBB) in MDD. This may explain why non-brain TSPO correlated with brain TSPO in MDD and not HVs, and brain TSPO correlated with depression severity in MDD, but indicates that it is the brain effect that causes the depression and not peripheral inflammation. In support of that model, neither non-brain TSPO binding level nor the blood cytokine levels correlated with depression severity. Future studies need to determine precisely what role brain TSPO plays in the pathogenesis of depression and to determine if treatment of brain or peripheral inflammation can have antidepressant effects.

Keywords: PET Imaging, Translocator Protein (TSPO), Major Depressive Disorder (MDD), Peripheral Blood Marker

Disclosure: Nothing to disclose.

P215. Chronic Stress-Induced Astrocyte Dysfunction and Neurovascular Remodeling in the Prefrontal Cortex Contributes to Sex-Dependent Deficits in Cognition

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Background: Astrocytes form an integral component of the neurovascular unit, ensheathing brain blood vessels with projections high in aquaporin-4 (AQP4) expression. These AQP4-rich projections facilitate interaction between the vascular endothelium, astrocytes, and neurons, and may help stabilize vascular morphology. Studies using preclinical models of psychological stress and post-mortem tissue from patients with major depressive disorder (MDD) have reported reductions in AQP4, loss of astrocytic structures, and vascular impairment in the prefrontal cortex (PFC). Though compelling, the role of AQP4 in mediating stress-induced alterations in blood vessel function and behavior remains unclear. Here, we address this, alongside potential sex differences in chronic unpredictable stress (CUS) effects on astrocyte phenotype, blood-brain barrier integrity, and behavior.

Methods: In initial studies, adult male and female mice were subjected to 14-days of CUS, followed by testing for exploratory behavior (open field), stress coping behavior (forced swim), and working memory (temporal object recognition, spontaneous alternation; n = 16-20/group). In one cohort, astrocytic coverage of blood vessels- and blood-brain barrier integrity- in the PFC was assessed using confocal microscopy (n = 7-8/group). In a separate cohort, astrocytes from the frontal cortex were isolated using fluorescence activated cell sorting (FACS) and characterized using gene expression analyses (n = 8-12/group). In follow-up experiments, astrocytic AQP4 was knocked down in the PFC (AAV5-shRNA) in male mice. These animals were exposed to a truncated CUS paradigm (7-days) to assess stressor susceptibility, with similar methods used to analyze astrocyte phenotype, neurovascular function, and behavior (n = 7-8/group).

Results: 14-days of CUS led to pronounced shifts in stress-coping behavior and working memory deficits in male –but not female– mice. We found that CUS increased various transcripts associated with blood vessel maintenance in astrocytes from males, including Bfgf, Angpt1, and Agt, but either had no effect on- or decreased- expression of these genes in females. Furthermore, CUS caused astrocyte atrophy, reduced levels of vascular-localized AQP4, and elevated extravasation of a small molecule fluorescent reporter (Dextran) in the PFC exclusively in males. CUS-induced reductions in astrocytic structures correlated with vascular-AQP4 loss and working memory dysfunction in males. Subsequent mechanistic studies indicate that knockdown of AQP4 in the PFC disrupted astrocyte phenotype in males, lowering transcript levels of S100b, Vegf, and Agt. Loss of AQP4 in the PFC increased susceptibility to deficits in working memory following 7 days of CUS.

Conclusions: These studies uncovered a number of sex-dependent stress effects on astrocyte phenotype and neurovascular integrity in the PFC. Moreover, our findings indicate that diminished AQP4 levels in astrocytes promote stress-induced alterations in prefrontal function, cognition, and behavior in males. These results align with recent transcriptomic reports suggesting a stronger role for astrocytes in MDD in men as compared to women.

Keywords: Astrocytes, Sex Difference, Stress, Neurovasculature, Blood Vessel

Disclosure: Nothing to disclose.

P216. Brain Derived Neurotrophic Factor Scales Presynaptic Calcium Transients to Modulate Excitatory Neurotransmission

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Background: Brain derived neurotrophic factor (BDNF) plays a critical role in synaptic physiology, as well as mechanisms underlying various neuropsychiatric diseases and their treatment. BDNF acts on its high affinity receptor, Tropomyosin receptor kinase B (TrkB), to enact downstream signaling cascades during development and in mature brain. In addition to its role as a growth factor, BDNF is widely accepted to be a key modulator of synaptic plasticity, though with controversy regarding the exact mechanism. BDNF also plays a critical role in human disease – multiple lines of evidence have found that the pathophysiology of neuropsychiatric conditions and its treatment mechanisms involve BDNF, such as antidepressant therapies. Despite the numerous studies examining its role in the brain, there is little information about its impact on neurotransmission at the single synapse level. This information is essential to understand endogenous BDNF function as well as how BDNF plays a role in the pathophysiology of disease and treatment of psychiatric disorders.

Methods: In this study, we used primary hippocampal neurons transfected with iGluSnFR, a novel glutamate sensing fluorescent probe, to resolve glutamatergic events at single synapses. For the TrkB KO cultures, we used previously generated floxed TrkB mice to make dissociated hippocampal cell cultures, and infected these cultures with lentivirus expressing Cre recombinase tagged to GFP to conditionally knock out (cKO) the TrkB gene. We then transfected these neurons with a glutamate or calcium sensing optical probe to examine both evoked and spontaneous release in cTrkB KO and wild type cultures. Based on previous, similar experiments performed in our lab, we estimated that a minimum of 2 independent cultures with 3 coverslips (with ~50-100 boutons per coverslip analyzed) per experimental group was enough for significance testing. All sexes were included in this study. A Welch's t-test was used to compare effects in pairwise datasets obtained from synapses or neurons under distinct conditions. For parametric analysis of multiple comparisons, two-way analysis of variance (ANOVA and one-way ANOVA) with Tukey post hoc analysis were used. Differences among experimental groups were considered statistically significant when a p value ≤ 0.05 was reached.

Results: We find that exogenous BDNF applied over 30 minutes selectively increases evoked excitatory neurotransmission ($p < 0.05$) without affecting spontaneous neurotransmission. However, acutely blocking endogenous BDNF has no effect on evoked nor spontaneous release, demonstrating that different approaches to studying BDNF may yield different results. When we suppressed BDNF-TrkB activity chronically over a period of days to weeks using a mouse line enabling conditional knockout of TrkB, we found that evoked glutamate release was significantly decreased ($p < 0.05$) while spontaneous release remained unchanged. Moreover, chronic blockade of BDNF-TrkB activity selectively downscales evoked calcium transients without affecting spontaneous calcium events.

Conclusions: Our findings suggest that BDNF modulates evoked glutamate release in hippocampal neurons, and that this effect is dependent on the timescale of its activity as well as whether endogenous or exogenous BDNF is manipulated. Collectively, these data start to provide insight into how the loss of TrkB receptors impacts presynaptic function, and ultimately have broad implications for neuropsychiatric disorders and their treatment.

Keywords: BDNF, Optical Imaging, Glutamate, Calcium Imaging, Neuronal Culture

Disclosure: Nothing to disclose.

P217. Metabolic and Inflammatory Changes as Biological Mechanisms Underlying the Accelerated Pace of Aging in Bipolar Disorder

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Background: Bipolar Disorder (BD) has been associated with accelerated epigenetic aging and metabolic dysregulation, although the potential link between these findings is unknown. We hypothesized that accelerated aging in BD is associated with a worse metabolic and inflammatory profile.

Methods: An epigenetic estimate of aging pace (DunedinPACE), as well as DNA methylation-based surrogates of metabolic, anthropometric, and inflammatory markers, were estimated in blood from controls (n = 39, 71.2% female) and individuals with BD (n = 86, 71.4% female) matched for age, sex, and race/ethnicity. We also computed a 'metabolic index' and an 'inflammatory index' consisting of 14 and 3 markers, respectively, corresponding with metabolic dysfunction and inflammation. Specifically, varimax rotation was applied after computing Kaiser-Meyer-Olkin and Bartlett's test of sphericity. Finally, the patient group was subdivided (median split) into those with high (n = 43) and low DunedinPACE (n = 43) for downstream comparisons.

Results: Individuals with BD showed significantly higher DunedinPACE compared to controls when controlling for age, sex, genetic ancestry, and smoking ($p = 0.046$). Mediation analyses showed significant indirect effects for the composite index of metabolic dysfunction (ab = 0.22, BC 95% CI = 0.1077 to 0.3616) and inflammation (ab = 0.06, 95% CI = 0.035 to 0.1013) on the relationship between the diagnosis and DunedinPACE. High DunedinPACE was associated with a significantly higher metabolic dysfunction index in both groups ($p < 0.001$). Specifically in patients, those with high DunedinPACE had significantly higher levels of c-peptide, insulin-like growth factor-binding protein-4, hepatocyte growth factor, adrenomedullin, TIMP metallopeptidase inhibitor 1, plasminogen activator inhibitor-1, growth/differentiation factor (GDF)-15, GDF-8, cystatin C, beta-2-microglobulin, waist-to-hip ratio, body mass index, body fat, interleukin-6, transforming growth factor-α, and C-reactive protein, as well as lower levels of insulin receptor, leptin, HDL cholesterol, and GHR compared to those with low DunedinPACE ($q<0.001$ for all).

Conclusions: Worsening metabolic parameters are associated with accelerated pace of aging in BD, suggesting them as important targets for prevention of aging acceleration and its consequences in patients.

Keywords: Bipolar Disorder, Accelerated Aging, DNA Methylation, Metabolism, Inflammation

Disclosure: Nothing to disclose.

P218. A Zinc Finger Transcription Factor Enhances Social Behaviors by Controlling Transposable Elements and Immune Response in Prefrontal Cortex

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Background: KRAB zinc finger proteins (KZFPs) are the largest family of transcription factors (TFs) in the mammalian genome, and are noted to repress genomic transposable elements (TEs). 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 KZFP gene family, Zfp189, as the top transcript responsible for manifesting transcriptional networks in the prefrontal cortex (PFC) unique to stress 'resilient' rodents. CRISPR-mediated activation of Zfp189 in PFC rescued social deficits in stress-exposed mice, establishing PFC Zfp189 as a molecular mediator of resistance to stress-induced social deficits.

Methods: To uncover the biological mechanisms through which ZFP189 regulates social behaviors, we created three synthetic ZFP189 TFs: 1) ZFP189-WT which is identical to endogenous ZFP189; 2) ZFP189-VPR wherein the endogenous, repressive KRAB domain is replaced with the transcriptional activator VP64-p65-Rta (VPR); and 3) ZFP189-NFD in which any regulatory domain is removed, which serves as a control. We tested the gene-regulatory function of these ZFP189 TFs in mouse Neuro2a cells (n = 3-6 wells in triplicate). To study influence of ZFP189 TFs on behaviors, we packaged in herpes simplex virus (HSV) and injected bi-laterally to PFC (15°; +1.8 AP; ±0.75 ML; -2.7 DV). Both male and female C57Bl/6J mice, aged 8-12 weeks, were used. We performed three-chamber social interaction and novel object recognition in distinct cohorts mice three days post viral surgery. In a separate cohort, we performed bulk RNAseq of manipulated PFC (n = 5-10 mice per group). Following processing and alignment, TE metagenes were identified via the TEtranscripts R package and were calculated alongside canonical genes. We generated differentially expressed genes (DEGs) relative to the control HSV-ZFP189-NFD treatment (5% FDR adjusted) and employed Qiagen ingenuity pathway analysis (IPA) to identify potential upstream regulators of these DEG lists.

Statistics: Sample sizes are 10-15 mice per group and statistical analyses were performed with one- or two-way ANOVAs, with Bonferroni post-test comparing the test group (HSV-ZFP189-WT or HSV-ZFP189-VPR) and the control group (HSV-ZFP189-NFD). Statistical analysis of RNAseq was performed using DESeq2.

Results: In Neuro2a cells expressing a ZFP189 luciferase gene and ZFP189 TF, we see that ZFP189-VPR induces gene activation ($P < 0.0001$), ZFP189-WT induces gene repression ($P < 0.05$), and ZFP189-NFD exerts no regulatory control ($P > 0.8$). In three chamber social testing, mice expressing ZFP189-NFD or ZFP189-WT show normal preference for social interaction ($P < 0.001$), whereas mice with ZFP189-VPR intra-PFC show no preference for social interaction for a mouse vs. object ($P > 0.5$) or for a novel vs. familiar mouse ($P > 0.9$). In novel object recognition, all groups were able to recognize novel objects (Discrimination index >65%, $P > 0.5$). In RNAseq annotating TEs, we see that ZFP189-VPR, but not ZFP189-WT, up-regulates the expression of TE metagenes

(ZFP189-VPR: 105 TEs upregulated, 0 downregulated). The top predicted upstream regulators of the DEG lists were attributed to opposite function immune factors, such as of tumor necrosis factor and Interferon γ, among others (|activation z-score| > 4).

Conclusions: By synthetically inverting the gene-regulatory function of ZFP189 in the brain, we discover that ZFP189 coordinates social behaviors via regulation of genomic TEs and subsequent immune response in PFC. This data complements growing evidence for a co-evolution of brain immune functions and the maintenance of proper social behavior, and implicates TF-mediated control of TEs in this mechanism.

Keywords: Social Behavior, Artificial Transcription Factors, Immune Responses

Disclosure: Nothing to disclose.

P219. Selective Antagonism of GluN2A Receptors Produces Pharmacodynamic Effects in Vivo

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Background: Major depression is a prevalent mental disorder that impairs the ability to function in society. Although existing medications can relieve symptoms and prevent relapse, approximately one-third of patients do not respond to typical antidepressant treatments. Selective antagonism of GluN2B-containing NMDARs produces rapid symptom improvement in TRD patients with a side-effect profile necessitating medical oversight upon administration. Based on the distinct functional properties and distribution of NMDAR family members, we hypothesize that selective GluN2A antagonism may provide efficacy with an improved side effect profile. Here, we report the discovery of Compound A, an orally available, brain-penetrant, GluN2A selective antagonist, and present its pharmacodynamic effects in preclinical in vitro and in vivo assays.

Methods: Compound A's potency and selectivity against diheteromeric NMDARs were established with $Ca2+$-based fluorescent assays conducted in CHO cells expressing NMDARs. Broader selectivity was determined in binding assays against G-protein-coupled receptors and ion channels, as well as radiometric kinase assays. Potency in triheteromeric GluN1/2A/2B receptors was tested in oocytes using two-electrode voltage-clamp. The affinity of Compound A for neuronal receptors was assessed by a competition radioligand binding assay in hippocampal (HP) membranes. An in vivo dose-occupancy relationship was established by administering Compound A to rats and measuring brain slice radioligand binding. The effects of Compound A on prefrontal cortex (PFC) monoamine levels were measured using microdialysis. Compound A's effects on synaptic plasticity were assessed by testing its effect on theta burst potentiation of field potentials in the CA1 region of the rat HP. Long-term effects on dendritic complexity and synapse number were evaluated in primary rat HP cultures, as were α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) mediated miniature excitatory postsynaptic currents (mEPSCs) in patch-clamp studies. In vivo, the effects of Compound A on AMPAR mEPSCs in rat layer five cortical neurons were also tested. Tolerability was assessed in a 4-day rat study, including evaluations of mortality, clinical observations, body weight, clinical pathology, insulin, toxicokinetics, gross pathology, histopathology, and gene expression. Cardiovascular safety was tested in anesthetized guinea pigs and telemetered rats, and neurotoxicity was also assessed in rats.

Results: Compound A blocked GluN1/2A receptors with an IC50 of 44 nM and exhibited exquisite selectivity: >500-fold against GluN1/2B, 2C, and 2D receptors, >200-fold against a panel of ion channels and GPCRs, and >20-fold against a panel of kinases. Compound A blocked GluN1/2A/2B receptors with an IC50 of 140 nM but showed a reduced efficacy of 60%. In neuronal membranes, it had a Ki of 66 nM. In vivo, both oral (PO) and subcutaneous (SC) administration resulted in plasma and brain exposure, as well as dose-dependent receptor occupancy (RO). At a dose of 60 milligrams per kilogram (mpk) SC, Compound A achieved 70% RO in the HP for 1 hour, and increased levels of prefrontal cortex (PFC) norepinephrine, serotonin, and dopamine in wild type, but not GluN2A knockout mice. At this dose, it also blocked theta burst-induced long-term potentiation (LTP) in the CA1 region of the hippocampus. Compound A also produced an increase in dendritic complexity and synapse number in vitro, as well as an increase in mEPSC frequency. Furthermore, 24 hours after dosing with 60 mpk of Compound A, mEPSC frequency was increased in layer V rat cortical neurons. Compound A was well tolerated over 4 days at 200 and 500 mpk/day (PO), with minor clinical observations that tolerated with repeat dosing. No Olney's lesions were observed, and cardiac conduction was unaffected by Compound A, but acute increases in heart rate and blood pressure were observed in telemetered rats.

Conclusions: Compound A is an orally available and brain penetrant GluN2A antagonist with sufficient selectivity to fully differentiate between GluN2A and GluN2B receptors in vivo. Compound A effectively blocks theta burst-induced LTP. Notably, 24 hours after dosing, Compound A induces synaptogenesis in layer V of the cortex. Compound A is generally well tolerated but does produce hemodynamic effects in telemetered rats. Progression of this compound is hindered by its moderate potency and generation of reactive metabolites. Nevertheless, these findings clearly illustrate that selective GluN2A antagonism elicits effects on the central nervous system and suggest that molecules with this mechanism of action hold promise for patients with treatment-resistant depression.

Keywords: GluN2A Receptor Subunit, Pharmacokinetic and Pharmacodynamic, Preclinical Pharmacology, NMDA Antagonists, Treatment Resistant Depression

Disclosure: Janssen R and D, LLC: Employee (Self).

P220. Allosteric Site Mediates Inhibition of Tonic NMDA Receptor Activity by Low-Dose Ketamine

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Background: Ketamine, a general anesthetic, has rapid and sustained antidepressant effects when administered at lower doses. At anesthetic doses, ketamine causes a drastic reduction in excitatory transmission by lodging in the centrally located hydrophilic pore of the NMDA receptor, where it blocks ionic flow. In contrast, the molecular and cellular targets responsible for the antidepressant effects of ketamine remain controversial.

Methods: We use single-molecule and whole-cell electrophysiology recordings from recombinant NMDA receptors, molecular dynamics simulations and kinetic modeling to examine the ketamine dose -NMDA receptor response relationship over an extended range of concentrations and experimental paradigms.

Results: Results show functional and structural evidence that, at nanomolar concentrations, ketamine interacts with membrane-accessible hydrophobic residues to stabilize desensitized receptors and cause an incomplete, voltage- and pH-dependent reduction in NMDA receptor activity. This allosteric mechanism

spares brief receptor activations (such as synaptic transmission) and reduces preferentially currents from tonically active receptors.

Conclusions: The hydrophobic site is a promising target for safe and effective therapies against acute and chronic neurodegeneration.

Keywords: NMDA Receptors, Dose Response, Electrophysiology, Mechanism, Extrasynaptic Glutamate

Disclosure: Nothing to disclose.

P221. Ribosomal Dysregulation: An Evolutionarily Conserved Stress Mechanism Reactivates in Human Depression

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Background: Mood disorders exist on a spectrum, with nebulous boundaries separating depression subtypes. Despite advancements made in our understanding of depressive disorders fueled by experimental models of chronic stress, there is a critical gap in our understanding of the disorder due to the poor connection between the aspects of depression explained by these models and clinical endpoints. We hypothesize that comparative analysis of molecular data from different experimental systems of chronic stress and major depressive disorder (MDD) to identify stress related orthologous genes can facilitate bridging this gap.

Methods: To find orthologous genes, we compared the transcriptomic profiles of the dorsolateral prefrontal cortex in human postmortem MDD datasets (females: control = 9, MDD = 13; males: control = 13, MDD = 13) and the prefrontal cortex of mice exposed to chronic variable stress (CVS; males: control = 10, stressed = 9; females: control = 9, stressed = 10). The human genome nomenclature database was used to identify orthologous genes. Identified orthologous genes were confirmed through the analysis of multiple independent cohorts. Using the identified ortholog genes as seeds, we generated seeded-gene co-networks, allowing us to highlight similarities and differences in the stress-induced functional alterations that these orthologs coordinate across both species and sexes. We conducted further in vitro experiments with glucocorticoid-stressed primary neuron cultures (control = 12, stressed = 12, glucocorticoid antagonist = 12) and in silico experiments designed to validate the association of the identified orthologous genes with stress, including their variations in stress-resilient or susceptible phenotypes and in response or non-response to antidepressants. A q-value of less than 0.05 was utilized for all functional analyses. Both the sample size and sequencing depth met the power calculation of 80%.

Results: Serving as stress-related orthologs, ribosomal protein genes (RPGs) were found to be downregulated, while the RP pseudogenes were upregulated in both MDD and CVS samples. Functional enrichment analysis showed that these dysregulations were predominantly found in neurite-related pathways. Seeded gene co-expression analysis with the altered RPGs common to both MDD and CVS indicated that the downregulated RPGs homeostatically regulated synaptic alterations across both phenotypes in a species- and sex-specific manner, likely via an RP pseudogene-driven mechanism. In vitro analysis showed that the RPG dysregulation was a glucocorticoid-driven endocrine response to stress. Analysis of independent postmortem transcriptomic datasets from subjects in an episode and in remission showed that the directionality of RPG and RP-pseudogene dysregulation reversed during remission from MDD. Additionally, bioinformatics analysis of transcriptomic data from mice treated with ketamine and imipramine showed that the dysregulation was selectively attenuated by ketamine, but not by imipramine.

Conclusions: This study provides the first evidence of ribosomal dysregulation, a stress response extensively studied in simpler organisms like bacteria and yeast, being conserved in human MDD and mice subjected to CVS. The functional assembly of ribosomes is tightly controlled by the gene dose (i.e., mRNA levels) of RPGs. As such, observed RPG downregulation can result in sub-stoichiometric production of ribosomal proteins, that contrary to the usual assumption of ribosomes as a homogeneous entity translating the transcriptome uniformly, can lead to the formation of heterogeneous ribosomes that lack certain RPs. We hypothesize that heterogeneous ribosomes can impact the synthesis of alternate proteins, particularly in neurites, shaping neuronal information input and output which manifests during mood disorders. The diversity of RPGs, heterogeneous assembly and mobility across neurites gives numerous permutations to explain the nuances of mood spectrum.

Keywords: Orthologs, Major Depressive Disorder, Chronic Variable Stress, Ribosome-Heterogeneity

Disclosure: Nothing to disclose.

P222. Microglial Nr3c1 Depletion Alters Stress Effects on Synaptic Density and Behavior in a Sex-Dependent Manner

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Background: Several studies show that chronic stress influences neuron-microglia interactions in the prefrontal cortex (PFC) in a sex-specific manner, leading to divergent effects on synaptic plasticity and behavior. In particular, male, but not female, mice have reduced synapse density in the PFC and display cognitive impairments after 14 days of chronic unpredictable stress (CUS). Other studies using pharmacological and broad genetic manipulations demonstrate that glucocorticoid receptor (GR; Nr3c1) signaling is necessary for stress-induced synapse loss in the PFC and associated behavioral consequences. Despite this, the cell type-specific role of GR signaling in the neurobiology of stress and how this pathway contributes to sex differences in stress responses have not been explored.

Methods: In this study, male and female mice with inducible microglia-specific depletion of GR (Cx3cr1Cre/+:Nr3c1fl/fl) and their genotype controls (Cx3cr1Cre/+:Nr3c1 + /+) were exposed to 14 days of CUS or handled intermittently as controls. Initial studies used flow cytometry, fluorescence-activated cell sorting, and immunohistology to assess microglia phenotype and gene expression. To examine the neurobiological and behavioral effects of microglial Nr3c1 depletion, we systemically injected AAV(PHP.eB)-hSyn1-tdTomato into mice and assessed discrimination in the temporal object recognition.

Results: Similar to prior work, we found that CUS exposure reduced body weight gain in both male and female mice, which was unaffected by genotype. Initial gene expression analyses confirmed effective Nr3c1 depletion in sorted microglia from both male and female mice. Interestingly, Nr3c1 depletion in microglia decreased transcript levels of the phenotypic marker P2ry12, but not Csf1r or Tgfbr1. After CUS exposure we also found that P2ry12 expression was diminished in mice lacking microglial Nr3c1 and genotype controls. These phenotypic alterations coincided with increased microglia-mediated neuronal remodeling and impaired working memory in male mice exposed to CUS, but these endpoints were not affected by microglial Nr3c1 depletion. In

contrast, microglial Nr3c1 depletion in female mice led to increased microglial phagocytosis of neuronal elements in the PFC and reduced discrimination in the temporal object recognition following CUS exposure.

Conclusions: In conclusion, these findings indicate that microglial GR signaling regulates the neurobiology of stress in a sex-dependent manner, with female mice being more susceptible to stress when this regulation is disrupted. Further studies are necessary to determine the exact mechanisms underlying these effects and their potential relevance to stress responses and microglial regulation in humans.

Keywords: Microglia, Glucocorticoid Receptor, Chronic Unpredictable Stress, Neuronal Remodeling, Cognitive Impairment

Disclosure: Nothing to disclose.

P223. Longitudinal Assessment of Extracellular Levels of Mitochondrial DNA in Late-Life Depression: Implications for Health Outcomes and Cognitive Decline in Older Adults

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Background: Late-life depression (LLD) is a prevalent and disabling mental illness affecting 1% to 5% of the elderly population. The pathophysiology of LLD involves various biological processes, including pro-inflammatory cascades, mitochondrial dysfunction and increased oxidative stress (OS). The mitochondria are the primary source of ROS, being key players in the oxidative stress cascade. Endogenous or exogenous cellular stressors can disrupt mitochondrial function, leading to increased ROS production and damage to mtDNA. This oxidized and fragmented mtDNA can be released from mitochondria either as circulating cell-free mitochondrial DNA (ccf-mtDNA) or encapsulated within extracellular vesicles, which act as mediators of intercellular communication. The most well-characterized subtype of extracellular vesicle is the exosome (EXs). EXs typically range in size from approximately 40 to 160 nm in diameter and originate from endosomes. Formed within the cytoplasm of cells, they are subsequently released into the extracellular space. The free mtDNA molecules in the cytosol, including ccf-mtDNA and engulfed in EXs (EX mtDNA), can activate inflammatory pathways, leading to the release of pro-inflammatory cytokines and increasing vulnerability to adverse health outcomes. Additionally, the free cytosolic mtDNA can be released from cells and detected in extracellular fluids, such as blood. Therefore, the ccf-mtDNA and EX mtDNA can be viewed as potential markers for cellular stress involving mitochondrial dysfunction and oxidative stress. This study aimed to do a longitudinal exploratory analysis of the levels of ccf-mtDNA and EX mtDNA and verify whether they were associated with more severe depressive symptoms, poorer health status and cognitive decline in LLD compared to healthy controls (HC).

Methods: A total of 90 individuals (48 LLD and 42 HC) were assessed at the baseline and were followed for 18 months (13 LLD and 17 HC) and 30 months (12 LLD and 14 HC). All participants underwent comprehensive clinical assessments (e.g. MoCA, MADRS, FRAIL questionnaires). Blood was collected and centrifuged to obtain the plasma-free platelet. We used size exclusion chromatography to isolate the exosomes. DNA was separately extracted from both plasma and EXs, and the quantification of two different mitochondrial genes (ND2 and ND4) was performed using RT-qPCR. To assess the association between LLD diagnosis, ccf-mtDNA, EX mtDNA, and clinical features, generalized linear models were employed, incorporating sex, age, and tobacco (packs per year) as covariates. Additionally, we examined correlations between ccf-mtDNA, EX mtDNA, and clinical measures using Spearman's or Pearson's tests.

Results: The levels of ND2 and ND4 in both ccf-mtDNA and EX mtDNA were consistently and significantly positively correlated across all time points. In the baseline assessment, individuals diagnosed with LLD showed higher levels of ccf-mtDNA ND2 compared to HC ($F = 5.492$, $p = 0.021$). These higher levels were significantly correlated with more severe depressive symptoms ($r = 0.330$, $p = 0.002$), increased frailty ($r = 0.296$, $p = 0.005$), and higher burden ($r = 0.279$, $p = 0.008$). When considering both mitochondrial genes ND2 and ND4, LLD individuals still exhibited higher levels of ccf-mtDNA compared to HC ($F = 4.064$, $p = 0.047$). These higher levels were significantly correlated with more severe depressive symptoms ($r = 0.269$, $p = 0.05$) and increased frailty ($r = 0.269$, $p = 0.011$), but not with burden ($r = 0.161$, $p = 0.132$). There was no significant correlation between ccf-mtDNA levels at the baseline and measures of cognitive decline. Regarding EX mtDNA levels, no significant differences were found between groups when considering each gene individually or both ND2 and ND4 together. However, higher levels of EX mtDNA ND2 were negatively correlated with MoCA scores ($r = -.242$, $p = 0.022$), indicating that increased EX mtDNA ND2 was associated with cognitive decline in the context of LLD. At the 18-month and 30-month follow-ups, there were no significant differences in ccf-mtDNA and EX mtDNA levels between HC and LLD groups when considering each gene individually or both ND2 and ND4 together. However, negative correlations with MoCA scores were observed for ccf-mtDNA at the 18-month (ND4: $r = -.449$, $p = 0.019$; both ND2 and ND4: $r = -.484$, $p = 0.014$) and at the 30-month follow-ups (ND2: $r = -0.446$, $p = 0.025$; ND4: $r = -0.484$, $p = 0.014$; both ND2 and ND4: $r = -0.400$, $p = 0.047$), and EX mtDNA at the 30-month follow-up (ND2: $r = -0.420$, $p = 0.05$; ND4: $r = -0.517$, $p = 0.020$; both ND2 and ND4: $r = -0.468$, $p = 0.037$) when considering individual mitochondrial genes ND2 and ND4 or both together.

Conclusions: These preliminary findings provide valuable insights into the association between ccf-mtDNA and EX mtDNA levels and their relevance to clinical features. Further research is warranted to validate and understand the underlying mechanisms of these correlations, which may have implications for early detection and targeted interventions in the management of LLD and its associated cognitive impairments.

Keywords: Circulating Cell-Free Mitochondrial DNA, Exosome, Mitochondria, Late-Life Depression

Disclosure: Nothing to disclose.

P224. Effects of Chronic Stress and Prefrontal Cortical REDD1 Overexpression on Attentional Set Shifting Behavior in Mice

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Background: Cognitive (behavioral) flexibility, the ability to adapt behaviors in response to changes in the environment is an essential element for everyday life, with deficits commonly observed in neuropsychiatric disease states and reducing resilience to negative life events such as stress. The rodent prelimbic cortex (PrLC) plays a critical role in processing information necessary for optimal cognitive flexibility and is known to undergo structural and functional changes following prolonged stress exposure, thus PrLC dysfunction represents a likely substrate for stress-induced deficits in cognitive control. We have recently shown that chronic unpredictable stress (CUS)

produces an enduring dysfunction in PrLC physiology and impaired cognitive flexibility using an operant-based attentional set shifting in male but not female mice. However, what adaptations drive these deficits remains unclear. To gain more insight into this, our studies chose to focus on the protein REDD1 (regulated in development and DNA damage responses-1; aka DDIT4, RTP801, Dig2) as it is increased in post-mortem dorsolateral prefrontal cortex (dlPFC) tissue from individuals diagnosed with depression. We hypothesize that overexpression of REDD1 in the PrLC will produce deficits similar to chronic stress in cognitive flexibility in male mice.

Methods: Adult C57/B6 male mice were given a bilateral prelimbic infusion of a REDD1-expressing (AAV9-CamKII-REDD1-mcherry) or a control viral vector (AAV9-CamKII-mcherry). Once they recovered from surgery, they were tested for cognitive flexibility. Cognitive flexibility was assessed using the operant based attentional set shifting task (ASST) procedures and performed in sound-attenuating boxes (Med Associates, Inc.) After CUS or recovery from REDD1 overexpression surgery, mice were food deprived to 90% of their body weight. First, mice were trained to press two levers for a food reward. After the mice acquired lever-press behavior, a time limit is introduced, in which the subjects have 10-s to register a lever press after the lever is presented. If there was no response within 10-s, the lever retracted and the trial was counted as an omission. Once a mouse showed an omission rate lower than 6% on 2 consecutive days, a lever bias test was used to assess the lever preference. Following lever bias test, mice underwent a visual cue test, where the reinforced (correct) lever is always under right or left visual cue light that is illuminated for 3 seconds and resulting in a reward. The criterion for the rest of the tests is ten consecutive correct responses in a row with a minimum of 25 trials or until 150 trials are conducted. After the mice met this criterion, they underwent an extradimensional (ED) shift test where the visual cue needs to be ignored. The cue is presented but the correct response is always the less preferred lever as established in the bias test, regardless of cue location. This was followed by reversal testing, in which the reinforcement switches to the other lever.

Results: In line with the chronic stress effects on cognitive flexibility findings, we found that there is an increase in REDD1 expression ($t(13) = 2.15$, $p = 0.021$) and a decrease in Raptor phosphorylation (one of the key elements of the mTORC1 complex; $t(10) = 2.33$, $p = 0.026$) in the PrLC after CUS, suggesting disrupted mTORC1 function. To determine if REDD1 overexpression is sufficient to produce deficits in attentional set shifting, we used a viral vector to overexpress REDD1 in the PrLC of male mice. Relative to control mice, REDD1 mice required more trials to pass the extradimensional shift (control, $n = 16$; Redd1, $n = 14$; CUS, $n = 15$; ANOVA $F(43) = 5.02$, $p = p = 0.001$) testing criterion that was equivalent to that produced by CUS. Notably, neither CUS or REDD1 overexpression impacted acquisition of lever training ($F(43) = 2.53$, $p = 0.09$), performance in the visual cue discriminative learning test ($F(43) = 2.18$, $p = 0.15$), reversal learning test ($F(43) = 0.44$, $p = 0.65$) or measures of motivation for non-drug reward. Following behavioral assessments, we performed whole-cell recordings in acute slices and found that REDD1 overexpression produced a trend towards reduction in the frequency (control, $n = 8$; REDD1, $n = 9$; $t(15) = 1.92$, $p = 0.07$) and amplitude ($t(15) = 3.39$, $p = 0.005$) of miniature excitatory post synaptic current (mEPSC) signaling, as well as overall excitatory drive (frequency x amplitude; $p = 0.02$). Current clamp recordings also showed a significant reduction in firing frequency at higher current amplitudes (Two-way RM ANOVA: interaction: $F(19,285) = 14.56$, $p < 0.001$).

Conclusions: The observation that CUS and REDD1 overexpression produced deficits in attentional set shifting in male mice likely has relevance for understanding a number of stress related disorders. Future research will assess the cell-type localization of REDD1 increases following stress in males, determine whether female mice are similarly affected by REDD1 overexpression and/or is upregulated in females following CUS, chronic CORT effects on attentional set shifting and examine the necessity of disrupted mTORC1 for stress effects.

Keywords: Depression, Prefrontal Cortex, Cognitive / Behavioral Flexibility, Chronic Unpredictable Stress, REDD1 (DDIT4, RTP801, Dig2)

Disclosure: Nothing to disclose.

P225. Effectiveness of Conventional Sequential Bilateral Repetitive Transcranial Stimulation Versus Bilateral Theta Burst Stimulation for Patients With Treatment-Resistant Depression: A Randomized Non-Inferiority Clinical Trial

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Background: Major depressive disorder is a common mental disorder, but approximately one-third of patients do not respond to standard antidepressant medications and are referred to as suffering from treatment-resistant depression (TRD) (Gaynes et al., 2011). Repetitive transcranial magnetic stimulation (rTMS) has emerged as a promising therapeutic approach for TRD. In particular, bilateral rTMS over the dorsolateral prefrontal cortex (DLPFC) exhibits the highest efficacy among several rTMS treatment protocols (Mutz et al., 2019). However, each session of this treatment protocol takes nearly one hour, posing a significant burden on patients. Bilateral theta burst stimulation (TBS) is a viable alternative to bilateral rTMS, which takes only five minutes for each session and is highly effective. However, no study has compared the efficacy for TRD across a wide range of age between them. Therefore, we conducted a randomized non-inferiority clinical trial comparing the effectiveness between bilateral TBS and bilateral rTMS for patients with TRD.

Methods: This clinical trial was approved by the Keio Certified Review Board and registered in the Japan Registry of Clinical Trials (jRCT: 2071210072). This trial was conducted at Keio University Hospital between November 2018 and June 2023. Participants in this study were 18 years of age or older with a diagnosis of major depressive disorder in the DSM-5. In addition, participants were required to show depression severity with a score of 18 or higher on the Montgomery Åsberg Depression Rating Scale (MADRS) at the time of entry, even with optimal antidepressant treatment. In this study, to confirm medication resistance and further reduce the confounding effect of various antidepressant medications on TMS treatment efficacy, the antidepressant was changed and adjusted to a venlafaxine monotherapy during the lead-in period prior to induction of TMS treatment. After a one-month lead-in observation period, the MADRS score was reassessed, and only those participants whose MADRS score was still 18 or higher at that time and whose score had improved by less than 50% from the time of entry were eligible for randomization. Participants were randomly assigned in a 1:1 ratio to either bilateral sequential rTMS or bilateral sequential TBS. Bilateral rTMS consisted of 1-Hz stimulation targeting the right DLPFC, followed by 10-Hz stimulation over the left DLPFC. Bilateral TBS involved the right-sided continuous TBS (triplet burst pulses at 50 Hz, repeated at 5 Hz for 600 pulses over 40 seconds) followed by the left-sided intermittent TBS (triplet burst pulses at 50 Hz, repeated at 5 Hz, 2 seconds-on, 8 seconds-off, for 600 pulses over 3 minutes and

12 seconds). Treatment targets were identified using a neuronavigation system that utilized individual T1-weighted magnetic resonance imaging data. Participants received 20-30 treatment sessions over 4-6 weeks. The primary outcome was a change in MADRS scores from baseline to the assessment at 6 weeks analyzed with a mixed model for repeated measures. The non-inferiority margin was set at 3.86. A minimum sample size of 143 treatment completers was necessary to achieve 80% power at $\alpha = 0{\cdot}05$ with this margin (Blumberger et al., 2016). To account for the dropouts in the lead-in period and failure to meet eligibility criteria before randomization of TMS intervention, we anticipated that a total sample size of 180 participants would be needed.

Results: A total of 180 participants with TRD were enrolled. Prior to randomization, 21 participants were excluded during the lead-in observation period. The remaining 159 participants were randomly assigned to receive either treatment A (82 [51.6%]) or treatment B (77 [48.4.0%]). As of August 2023, when the abstract was submitted, the allocation of specific interventions is not disclosed. Of the participants, 5 (6.10%) in the treatment A group and 4 (5.19%) in the treatment B group discontinued treatment before completing 20 sessions (Odds Ratio = 1.17; $p = 1.00$). Evaluation of the estimated marginal mean changes in MADRS scores from baseline to the assessment at 6 weeks indicated -2.42 points difference in MADRS scores between the groups, favoring treatment B. The lower one-tailed 95% CI reached 4.27 points, exceeding the non-inferiority margin of 3.86 points. Conversely, the upper one-tailed 95% CI was -0.662 points, within the non-inferiority margin. A two-tailed test indicated that the estimated marginal mean changes in MADRS scores were significantly greater in the treatment B group compared with the treatment A group (mean difference: -2.42, 95% CI -0.324 to -4.51).

Conclusions: This clinical trial was the first study to evaluate the non-inferiority of bilateral TBS compared with bilateral rTMS in participants with TRD across a wide range of age. Currently, as the treatment labels remain undisclosed, we cannot confirm whether bilateral TBS is non-inferior to bilateral rTMS for TRD. Previous studies compared the effectiveness of the left-sided rTMS and the left-sided TBS in adult participants with TRD (Blumberger et al., 2018), as well as the effectiveness of bilateral rTMS and bilateral TBS in late-life depression, noting significant non-inferiority of TBS in comparison with rTMS with no remarkable difference in tolerability and safety (Blumberger et al., 2022). The disclosure of the data of the current study awaits confirmation as to whether bilateral TBS has non-inferiority compared to bilateral rTMS.

Keywords: Repetitive Transcranial Magnetic Stimulation (rTMS), Major Depressive Disorder (MDD), Theta-Burst Stimulation, Non-Invasive Neuromodulation, Treatment Resistant Depression

Disclosures: Sumitomo Pharma, Eisai, Takeda: Speakers Bureau (Self). Nakatani Foundation, Takeda Science Foundation: Grant (Self).

P226. Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of CYB003, a Deuterated Psilocybin Analog in Patients With Major Depressive Disorder

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Background: Available treatments for Major Depressive Disorder (MDD) have limited efficacy, require daily dosing, are associated with dose-limiting side effects, and do not address the maladaptive patterns of thinking underlying these conditions.

Clinical studies have demonstrated that short-term administration of psilocybin has the potential for long-lasting therapeutic benefits in MDD and may therefore meet a significant need in this area. While psilocybin is efficacious and inherently safe, there is significant variability between patients in their psychedelic and side-effect experiences. This variability is likely due to psilocybin acting as a pro-drug that requires dephosphorylation to the psychoactive metabolite, psilocin. CYB003 is a novel deuterated analog of psilocybin that may offer benefits over psilocybin.

The aim of this clinical study was to determine safety, tolerability, and pharmacokinetics (PK) of ascending doses of CYB003 and to evaluate the efficacy of CYB003 in treating symptoms of MDD.

Methods: A seamless Phase 1/2 study (www.clinicaltrials.gov; ID: NCT05385783) was designed to evaluate safety, tolerability, PK, psychedelic effects, and therapeutic efficacy of ascending doses of CYB003. In this study, healthy participants were enrolled in the three lower dose cohorts (doses up to 10 mg), primarily to assess safety and PK. At doses > 10 mg, patients suffering from moderate to severe MDD, scoring ≥21 on the Montgomery-Åsberg Depression Rating Scale (MADRS), who were inadequately responding to their ongoing antidepressant treatment and were not treatment resistant, were enrolled in a double-blind, randomized, placebo-controlled manner across three cohorts with 12 patients per cohort. MDD patients were allowed to remain on a stable dose of their antidepressant medication during the trial. 36 MDD-Patients were randomized to Placebo or CYB003 at a 1:3 ratio for the first dose, with all patients receiving CYB003 as the second dose. Doses were administered one week apart for the healthy participants and 3 weeks apart for the MDD patients. All participants received psychological support based on Cybin's EMBARKTM psychotherapy framework. MADRS scores were collected at baseline and up to 12 weeks after the first dose to assess acute and long-term efficacy. Dose escalations were possible between doses within a cohort and between cohorts, based on a decision of a Safety Review Committee. Dose selection for MDD-patients was based on PK-PD modelling [Visual Analogue Scale any drug effect (VAS), Revised Mystical Experience Questionnaire (MEQ-30), Hallucinogen Rating Scale (HRS), 5-Dimensional Altered States of Consciousness Rating Scale (5D-ASC), Persisting Effects Questionnaire (PEQ)].

The primary endpoint for the efficacy assessment is the change from baseline to Day 21 (MADRS total score). A responder analysis (improvement of at least 50%) and the number of subjects going into remission (MADRS scores of 10 or below) will be performed after unblinding.

Results: The data from 2 completed cohorts show a rapid onset of psychedelic effects which peaked for a short duration, and a favorable PK profile that was found to be approximately dose proportional.

CYB003 demonstrated a favorable safety and tolerability profile. Side effects for all doses (1 mg to 12 mg) were mild or moderate and mostly self-limiting, and no severe or serious AEs occurred. The most common AEs reported were headache and nausea. Transient and self-limiting increases in systolic and diastolic blood pressure, which were not clinically significant, were noted.

A blinded review showed a rapid onset of psychedelic effects. 24 MDD patients were treated in two cohorts of 12 patients. 18 patients received a single active dose of 12 mg and 6 were given matching placebo. 18 patients reported a psychedelic experience. VAS scores above 70 mm were achieved in 11 patients, and scores on the MEQ-30 above 60% were recorded in 14 patients. This resulted in a rapid statistically significant clinical improvement of large magnitude based on the MADRS scores on Day 21.

Unblinded data, including Day 42 assessments as well as the results of an additional cohort of 12 MDD patients to be treated with 16 mg, will be presented at the conference.

Conclusions: CYB003 was shown to be safe and well tolerated with a rapid onset of a clinically meaningful improvement.

These data support the potential of CYB003 to have superior properties to psilocybin and to offer considerable clinical benefits.

Keywords: Major Depressive Disorder (MDD), Randomized Double-Blind, Psilocybin Analog, Efficacy and Safety

Disclosure: Sebastian Krempien: Employee (Self).

P227. Impact of AXS-05 (Dextromethorphan-Bupropion) on Depressive Symptoms, Anxiety, and Quality of Life in Patients With One Prior Treatment Failure: Results From the Evolve Long-Term, Open-Label Study

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Background: In STAR*D, after failure of initial SSRI therapy, switching to an alternative monoaminergic treatment resulted in only a ~20% remission rate (Rush AJ, et al. J Clin Psychiatry. 2020;81(5):19m12949). Most approved oral antidepressants act primarily via monoaminergic mechanisms and are associated with a prolonged time to clinically meaningful response.

AXS-05 [dextromethorphan-bupropion (Auvelity® extended-release tablet)] is a novel, oral, NMDA receptor antagonist and sigma-1 receptor agonist approved by the FDA for the treatment of MDD in adults. In this study, we evaluated the impact of AXS-05 on depressive symptoms, anxiety, and quality of life in people with depression who had been treated with at least one prior treatment in their current major depressive episode (MDE).

Methods: EVOLVE was an open-label, US trial, in which patients were treated with AXS-05 (dextromethorphan HBr 45mg-bupropion HCl 105mg) twice daily for up to 15 months. Eligible patients had either rolled over 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 ≥1 prior antidepressant in the current MDE. A total of 186 patients were enrolled, consisting of 35 roll-over and 146 directly enrolled patients. Efficacy endpoints included MADRS, HAM-A, and the Quality of Life Enjoyment and Satisfaction Questionnaire – Short Form (Q-LES-Q-SF). 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: Mean change in MADRS total score from a baseline of 32.2 were -9.1 ± 7.64, -13.3 ± 8.58, and -20.4 ± 7.79 points at Weeks 1, 2, and 6, respectively ($p < 0.001$ for all). Remission on the MADRS (≤10) was achieved by 5.7%, 16.2%, and 46.0% of patients at Weeks 1, 2, and 6, respectively. Remission rates increased over time and was 82% at Month 12.

Mean baseline HAM-A scores were 15.6. Reductions from baseline to Weeks 1, 2, and 6 were 3.4 ± 5.34, 5.5 ± 5.81, and 8.6 ± 5.75, respectively ($p < 0.001$ for all). Response on the HAM-A (≥50% improvement) was achieved by 18.4%, 27.9%, and 62.1% of patients at Week 1, 2, and 6, respectively. Response rates continued to improve through Month 12 (77.1%).

Mean Q-LES-Q-SF score at baseline was 42.9. Improvement from baseline to Weeks 1, 2, and 6 were 6.4 ± 11.45, 10.4 ± 13.14, and 18.6 ± 13.52 ($p < .001$ for all). Improvements were sustained through Month 12 (24.5 ± 16.96, $p < 0.001$).

Long-term treatment with AXS-05 was generally 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: In a naturalistic setting, treatment with AXS-05 improved depression symptoms, anxiety, and quality of life in patients who failed one prior antidepressant in the current MDE. These data support the long-term efficacy and safety of AXS-05 in this difficult-to-treat patient population.

Keywords: AXS-05, NMDA Receptor Antagonist, Major Depressive Disorder (MDD), Quality of Life (QoL), Depression and Anxiety

Disclosure: Axsome Therapeutics: Employee (Self).

P228. Identification and Prospective Replication of an EEG Biomarker for Predicting the Antidepressant Effect of ALTO-300 in Patients With Major Depression: Results From a Large Phase 2a Study

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Background: Major Depressive Disorder (MDD) is a markedly heterogeneous diagnosis, the result of which is that antidepressants developed in broad populations either fail to differentiate from placebo or demonstrate small effect sizes. Identifying likely responders through objective biological markers may yield larger effects by virtue of targeting the right patient subpopulation for a given drug. Our group has shown that brain activity, such as measured using resting state electroencephalography (rsEEG), has shown an ability to predict antidepressant response in MDD across a wide variety of interventions (i.e., SSRI, rTMS, etc). Here we sought to determine whether a machine learning-based analysis of rsEEG data could predict treatment outcome with ALTO-300, a novel antidepressant with a distinct pharmacological profile, when used adjunctively to an antidepressant to which patients had an inadequate response. We then tested whether the biomarker prediction could be prospectively replicated in an independent group of patients.

Methods: 239 participants with MDD were enrolled in 8-week open-label trials of ALTO-300 as an adjunctive treatment to a current antidepressant to which they had an inadequate response (site based study: NCT05118750 and decentralized study: NCT05157945). A portion of these patients underwent EEG, either at clinic sites or in their homes. The analysis set focuses on 105 patients (77 female) with moderate to severe MDD (baseline Montgomery-Asberg Depression Rating Scale [MADRS] > 20 and Patient Health Questionnaire - 9 [PHQ-9] > 10) that completed baseline rsEEG. Analyses were conducted by developing an rsEEG predictive biomarker in a training subset ($n = 60$) and then prospectively testing its ability to predict outcome in a separate holdout set ($n = 45$), which had been locked and was inaccessible to study analysts during biomarker development. After defining a specific rsEEG biomarker that predicted a larger antidepressant response to ALTO-300 in the training set, this biomarker was applied to the holdout set to assess its prospective performance. Mixed Models for Repeated Measures (MMRM) were used to evaluate the efficacy of ALTO-300 in those with the biomarker compared to those without the biomarker with respect to change in MADRS scores from baseline. Replication was assessed based on the effect size needed to achieve a target drug vs. placebo Cohen's $d = 0.40$ in a biomarker-enriched population. Statistical significance was evaluated with two-sided p-values in the training set and one-sided p-values in the holdout set, given directional hypotheses.

Results: Cross-validated machine learning analyses on the training set identified a rsEEG biomarker (52% of the training

sample) that predicted the reduction in depressive symptoms with ALTO-300 (week 6 Cohen's d = 0.60, two-sided p-value = 0.01). The rsEEG biomarker was then prospectively applied to the blinded and locked holdout set. Results in the holdout set indicated successful replication of the biomarker-based treatment prediction across timepoints for assessment of antidepressant response. The week 6 effect size was d = 0.51 (p = 0.03) and week 8 effect size was d = 0.63 (p = 0.03). In the overall sample, 52% of patients were classified as being in the biomarker positive group. It was similarly effective among those taking an SSRI (N = 70; week 4 d = 0.42) or SNRI (N = 19; week 4 d = 0.64).

Conclusions: These findings provide strong evidence that ALTO-300 is significantly more effective at treating symptoms of depression among MDD patients with a machine learning-identified rsEEG biomarker than those who do not have the biomarker. Based on these findings, a prospective, biomarker-stratified, placebo-controlled phase 2b efficacy study is underway (NCT05922878).

Keywords: Precision Psychiatry, Major Depressive Disorder (MDD), Depression Subtypes, EEG Biomarkers, Phase II Clinical Trial

Disclosures: Alto Neuroscience: Employee, Stock / Equity (Self). Johnson and Johnson: Stock / Equity (Self).

P229. Identification and Prospective Replication of a Cognitive Biomarker for Predicting the Antidepressant Effect of ALTO-100, a Novel Pro-Plasticity Drug, in Patients With Major Depression: Results From a Large Phase 2a Study

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Background: Major Depressive Disorder (MDD) is a markedly heterogeneous diagnosis, resulting in antidepressants developed in broad populations either failing or demonstrating small effect sizes. Identifying likely responders through objective biological markers may yield larger effects by virtue of targeting the right patient subpopulation for a given drug. One such clinically significant subgroup in MDD is identifiable based on the presence of a specific objectively measured cognitive profile. Approximately 25-50% of MDD patients have significant cognitive impairments, with deficits across multiple cognitive domains. Cognitive impairment in MDD is associated with greater chronicity, disability, social and occupational dysfunction, and suicidal ideation and behaviors. It is seen in children and first episode patients, relates to genetic risk for the disorder, and persists between mood episodes. Standard of care antidepressants are less effective at treating depressive symptoms for patients with poor cognition. Thus, novel interventions are needed to address the unmet clinical need of this underserved subset of people with MDD. Animal models suggest that reductions in neuroplasticity can drive both cognitive and mood-like abnormalities. ALTO-100 is a novel, first-in-class compound that acts by increasing neuroplasticity and has the potential to ameliorate depressive symptoms in those demonstrating cognitive impairment. The current study seeks to define, and prospectively replicate, a response-predictive biomarker for the treatment of MDD with ALTO-100. Doing so would identify a target population for subsequent placebo-controlled efficacy studies.

Methods: A total of 243 participants with MDD and/or Posttraumatic Stress Disorder (PTSD) were enrolled in an 8-week open-label trial of ALTO-100 (NCT05117632). Here, our analysis set focuses on 123 (88 female) patients with moderate to severe MDD (baseline Montgomery-Asberg Depression Rating Scale [MADRS] > 20 and Patient Health Questionnaire - 9 [PHQ-9] > 10). Patients were either taking ALTO-100 as monotherapy (n = 45) or adjunctive to an antidepressant to which they had an inadequate response (n = 78). Patients underwent a comprehensive, computerized neurocognitive battery at baseline that assesses executive function, learning and memory, and processing speed and attention. Analyses were performed by first developing a candidate poor cognition predictive marker in a training subset (n = 30) and prospectively testing its ability to predict clinical outcome in an independent holdout subset (n = 93), which had been locked and was inaccessible to the trial analysts during biomarker development. After identifying a specific biomarker of cognitive impairment that predicted a larger antidepressant response to ALTO-100, this biomarker was tested in the holdout set. Mixed Models for Repeated Measures (MMRM) were used to evaluate the efficacy of ALTO-100 in those with the biomarker compared to those without the biomarker with respect to change in MADRS scores from baseline. Replication was assessed based on the effect size needed to achieve a target drug vs. placebo Cohen's d = 0.40 in a biomarker enriched population. Statistical significance was evaluated with one-sided p-values in the holdout set given directional hypotheses.

Results: Analyses on the training set revealed that patients with evidence of poor cognition (54% of the sample) experienced a greater reduction in depressive symptoms with ALTO-100 than participants without the biomarker (week 6 Cohen's d = 0.96, two-sided p = 0.02). This biomarker was then prospectively applied to the blinded and locked holdout set. Results in the holdout set indicated successful replication of the biomarker-based treatment prediction across timepoints for assessment of antidepressant response. The week 6 effect size was d = 0.58 (p = 0.01) and the week 8 effect size was d = 0.61 (p = 0.01). In the overall sample of subjects, 47% of patients were classified as being in the poor cognition group. The biomarker similarly predicted ALTO-100 response when given as monotherapy (week 6 d = 0.66, p = 0.03) or as adjunctive therapy (week 6 d = 0.56, p = 0.01).

Conclusions: These data provide compelling evidence that ALTO-100, a first-in-class compound that increases neuroplasticity, is considerably more effective at treating symptoms of depression among MDD patients with a poor cognition biomarker profile than those without. This result stands in contrast to standard-of-care antidepressants, which are less effective in patients with the poor cognition biomarker profile. Based on these results, a prospective, biomarker-stratified, placebo-controlled phase 2b efficacy study is underway (NCT05712187).

Keywords: Precision Psychiatry, Depression Subtypes, Phase II Clinical Trial, Cognitive Biomarkers

Disclosure: Alto Neuroscience: Employee (Self).

P230. Long-Term Follow-Up of Participants in Ketamine Clinical Trials for Mood Disorders

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Background: Since initial studies were published over 20 years ago, there has been an explosion of interest in ketamine as a rapid-acting antidepressant. The uptake of ketamine's off-label use for depression in community clinics and even at-home administration has occurred at a rapid pace unusual in typical drug development. Nevertheless, questions remain about ketamine's potential long-term side effects and misuse potential. Participants who received ketamine at the National Institute of Mental Health

(NIMH) were some of the first to receive ketamine for depression in controlled clinical trials, providing a unique opportunity to assess long-term outcomes. This analysis sought to evaluate the relationship between participating in a highly regulated ketamine clinical trial and subsequent use of ketamine and/or esketamine years after leaving the research setting, as well as current symptomatology.

Methods: Participants seen within the NIMH Experimental Therapeutics and Pathophysiology Branch for psychiatric research and clinical trials from 2002-2022 were contacted for follow-up online assessment and telephone interview. Participants had taken part in research protocols of neurobiological procedures and/or clinical trials of experimental antidepressant medications, including, but not limited to, ketamine. 203 individuals with mood disorders agreed to follow-up assessments out of 998 contacted, including questions about whether they had used ketamine/esketamine since leaving the research setting as well as their current depressive symptoms, dissociative symptoms, hallucinations, seeking non-prescribed ketamine and ketamine craving.

Results: Of the 203 participants in follow-up assessments (55% female, average time since leaving the NIMH =9.04 years), 52 had received ketamine at the NIMH: these 52 individuals were more likely to receive ketamine and/or esketamine in the years post-discharge (22/52 or 42%) than those individuals who did not receive ketamine at NIMH (24/151 or 16%) (OR = 0.25, p < .001). Participants who reported using ketamine/esketamine in the years post-discharge reported more depressive symptoms than those who had not (p < .001). Receiving ketamine at NIMH was not associated with ketamine-related side effects at follow-up, including dissociation and hallucinations; nor was it associated with dependency-related variables including obtaining access to non-prescribed ketamine. Three participants reported mild cravings for ketamine; all were currently receiving ketamine treatment and looking forward to their next administration.

Conclusions: Patients who received ketamine as part of a NIMH clinical trial were more likely to receive ketamine/esketamine in the years post-discharge, but none reported symptoms indicative of dependence. Participants who sought out ketamine/esketamine post-discharge reported increased depressive symptoms suggestive of a more treatment-refractory depression. Results underscore the critical need for long-term follow-up of patients receiving ketamine, esketamine, and other similar rapid-acting antidepressants.

Keywords: IV- Ketamine, Depression, Clinical Trials

Disclosure: Nothing to disclose.

P231. Lumateperone Treatment for Major Depressive Episodes With Mixed Features in Major Depressive Disorder and Bipolar I or Bipolar II Disorder

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Background: The Diagnostic and Statistical Manual of Mental Disorders 5 (DSM-5) and 5-text revision (DSM-5-TR) define mixed features in major depressive disorder (MDD) or bipolar depression as having subsyndromal manic or hypomanic symptoms nearly every day during the majority of days of a major depressive episode (MDE). Mixed features are common in MDD and bipolar depression (25%-35%) and patients with mixed features have more severe symptoms, more comorbidities, increased suicide risk, and poorer treatment response than patients without mixed features.

Lumateperone is an FDA-approved compound to treat schizophrenia and depressive episodes associated with bipolar I or bipolar II disorder. This randomized, double-blind, placebo-controlled, multicenter trial (NCT04285515) investigated the efficacy and safety of lumateperone 42 mg for the treatment of an MDE in patients with MDD with mixed features or bipolar depression with mixed features.

Methods: Eligible males and females (18-75 years) had DSM-5 diagnosed MDD or bipolar I or II disorder with mixed features and were experiencing an MDE (Montgomery-Asberg Depression Rating Scale [MADRS] Total score≥24 and a Clinical Global Impression Scale-Severity [CGI-S] score≥4). Patients, stratified by MDD or bipolar disorder, were randomized 1:1 to 6-weeks treatment with lumateperone 42 mg or placebo. The primary and key secondary efficacy endpoints were change from baseline to Day 43 in MADRS Total score and CGI-S score, respectively, analyzed using a mixed-effects model for repeated measures. Three populations with mixed features were assessed: the combined MDD/bipolar population, the individual MDD population, and the individual bipolar depression population. Additional efficacy measures were response (≥50% MADRS Total score decrease from baseline) and remission (MADRS Total score ≤10). Safety assessments included adverse events (AEs), laboratory parameters, vital signs, and extrapyramidal symptoms.

Results: In this study, 385 patients received treatment (placebo, n = 193; lumateperone, n = 192) and 344 (89.4%) completed the study. Patients in the combined MDD/bipolar population with mixed features treated with lumateperone 42 mg had significantly greater MADRS Total score improvement compared with placebo as indicated by mean change from baseline to Day 43 (placebo, n = 191; lumateperone, n = 192; least squares mean difference vs placebo [LSMD], −5.7; 95% confidence interval [CI] −7.60 to −3.84; effect size [ES], −0.64; P < .0001). Improvements with lumateperone were also significant in individual patient populations with MDD with mixed features (placebo, n = 92; lumateperone, n = 92; LSMD, −5.9; 95% CI −8.61 to −3.29; ES, −0.67; P < .0001) or bipolar depression with mixed features (placebo, n = 99; lumateperone, n = 100; LSMD, −5.7; 95% CI −8.29 to −3.05; ES, −0.64; P < .0001). Significant improvements compared with placebo were also observed for CGI-S, the key secondary endpoint, in the combined MDD/bipolar population (LSMD, −0.6; 95% CI −0.81 to −0.39; ES, −0.59; P < .0001), individual MDD population (LSMD, −0.6; 95% CI −0.89 to −0.27; ES, −0.57; P < .001), and individual bipolar depression population (LSMD, −0.6; 95% CI −0.91 to −0.31; ES, −0.61; P < .0001). MADRS response rate was significantly greater at Day 43 with lumateperone compared with placebo in the combined MDD/bipolar population (placebo, 40.8%; lumateperone, 59.4%; P < .001). Remission rate at Day 43 was also significantly higher with lumateperone vs placebo in the combined MDD/bipolar population (placebo, 19.9%; lumateperone, 38.5%; P < .0001).

Lumateperone treatment was generally safe and well tolerated and consistent with prior studies. The most common treatment-emergent AEs with lumateperone (≥5% and twice placebo) were somnolence, dizziness, and nausea. No serious AEs were reported with lumateperone.

Conclusions: Lumateperone 42 mg demonstrated robust efficacy over placebo in patients with MDD or bipolar depression with mixed features. Lumateperone was generally safe and well tolerated with no new safety concerns. These results suggest lumateperone 42 mg is a promising new treatment for MDEs in MDD with mixed features or bipolar depression with mixed features.

Keywords: Lumateperone, Major Depressive Disorder, Bipolar Disorder, Mixed Features

Disclosure: Intra-Cellular Therapies, Inc.: Employee, Stock/Equity (Self).

P232. Identification and Prospective Replication of a Resting-State EEG Biomarker for Predicting the Response to 10Hz Left DLPFC Repetitive Transcranial Magnetic Stimulation in Patients With Major Depression

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Background: Repetitive transcranial magnetic stimulation (rTMS) is an effective noninvasive treatment for treatment-resistant major depressive disorder (MDD), but its response rates remain low. Current practice relies on trial-and-error for the selection of patients to receive rTMS. Here, we sought to develop and prospectively replicate a machine-learning (ML) based biomarker of individual response to rTMS treatment, utilizing 19-channel resting-state electroencephalography (rsEEG) data in MDD.

Methods: A training set of 101 MDD patients who completed a 10 Hz left dorsolateral prefrontal cortex rTMS treatment course in an open-label clinical trial was used for model development. Features extracted from the pre-treatment rsEEG were used to build an ML model to assign a biomarker predictive of change in Hamilton Depression Rating Scale (HAMD17) scores from baseline across 8 weeks. The model was evaluated with cross-validation on the training set and prospectively tested on an unseen holdout set ($N = 47$) from the same clinical trial, which had been locked and was inaccessible to the trial analysts during biomarker development. Mixed Models for Repeated Measures (MMRM) were used to evaluate the effect of rTMS on HAMD17 scores in subjects with vs. without the rsEEG biomarker. Replication was assessed in the holdout population as observing an effect size of the biomarker of at least $d = 0.3$. Statistical significance was evaluated with one-sided p-values in the holdout set given directional hypotheses.

Results: A regularized linear classification model successfully identified an rsEEG biomarker that was significantly associated with a greater HAMD17 score change in the training set of the open label study (cross-validated week 8 $d = 0.58$, $p = 0.003$). The rsEEG biomarker was then prospectively applied to the locked holdout set, where successful replication of the biomarker-based treatment prediction was observed (week 8 $d = 0.66$, $p = 0.03$). In the total sample ($N = 148$), 47% of patients were classified as being in the biomarker positive group.

Conclusions: The study reveals the potential for predicting individual responses to rTMS treatment with pretreatment rsEEG using ML. The successful prospective replication on the holdout set illustrates the promise of precision psychiatry approaches.

Keywords: Repetitive Transcranial Magnetic Stimulation (rTMS), Electroencephalography (EEG), Machine Learning, Predictive Biomarker

Disclosure: Alto Neuroscience Inc.: Founder (Self).

P233. A Phase I Study to Evaluate the Safety, Tolerability, Pharmacokinetic, and Pharmacodynamic Profiles of DLX-001, a Novel Neuroplastogen

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Background: Psychedelic compounds have demonstrated preliminary efficacy across a range of neuropsychiatric conditions, with effects that are believed to be mediated by their rapid and enduring impact on brain structural neuroplasticity. However, the dissociative experiences and hallucinations produced by these compounds will likely limit their widespread clinical use. In an attempt to discover and develop a non-hallucinogenic, non-dissociative plastogenic compound that could be used to treat neuropsychiatric conditions with high unmet need, such as MDD, we synthesized a novel isotryptamine, DLX-001, and are examining its safety, tolerability, pharmacokinetic (PK), and pharmacodynamic (PD) profiles in a combined single- and multiple-ascending dose study in healthy volunteers (ECTN 2023-503390-38-00).

Methods: This 3-part inpatient study is being conducted in male and female healthy volunteers (HVs) aged 18 to 55. Part A is a randomized, double-blind, placebo-controlled SAD study to investigate the safety, tolerability, PK, and PD of single-ascending oral doses of DLX-001 in up to 7 cohorts (8 participants per cohort). Part B is a standard, open-label, crossover design study to investigate the effect of food on the PK of DLX-001. In Part B, HVs will receive a single oral dose of DLX-001 on 2 separate occasions, one in a fed state and the other in a fasted state. The 2 treatments (fasted and fed) will be separated by a 7-day washout period. Part C is a randomized, double-blind, placebo-controlled MAD study in which participants will receive multiple oral doses of either DLX-001 or placebo once a day for up to 7 days.

Results: Part A (SAD) is currently underway, with Part B (Food Effect) and Part C (MAD) commencing shortly. In SAD cohorts completed to date (2 mg, 6 mg, 20 mg), no serious adverse events (SAEs) or discontinuations due to study drug have been reported, and no clinically-significant vital sign, laboratory, ECG, or qEEG changes have been observed. To assess for the presence of dissociative effects, the Clinician-Administered Dissociative States Scale (CADSS) is being administered serially. Similarly, C-SSRS (Columbia Suicide Rating Scale) and BPRS (Brief Psychiatric Rating Scale) scores are being assessed in each cohort. From a pharmacokinetic perspective, Cmax and AUClast have been consistent with modeled predictions. Recruitment in the remaining SAD, Food Effect, and MAD cohorts will continue through the end of 2023, and results from the completed cohorts will be shared in the final poster presentation.

Conclusions: DLX-001 is a novel isotryptamine neuroplastogen that is undergoing first-in-human testing. As reported elsewhere, the preclinical pharmacology of DLX-001 predicts that it will increase structural neuroplasticity and have rapid and enduring antidepressant effects in humans without producing the dissociative or hallucinatory effects observed with traditional psychedelic agents. Given that promotion of neuroplasticity plays a critical role in the efficacy and onset of action of rapid-acting antidepressants, DLX-001 can potentially address significant unmet needs within MDD and other neuropsychiatric conditions.

Keywords: Neuroplasticity, Depression, Treatment-Refractory Depression, Phase 1

Disclosures: Delix Therapeutics: Employee (Self). Cerevel Therapeutics: Employee (Spouse/Partner). Sage Therapeutics: Stock / Equity (Self).

P234. Lumateperone in the Treatment of Patients With Major Depressive Disorder and Bipolar Disorder With Anxious Distress and Mixed Features

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Background: Anxious distress and mixed features are Diagnostic and Statistical Manual of Mental Disorders 5 (DSM-5) episode specifiers for major depressive episodes (MDE) associated with Major Depressive disorder (MDD) and bipolar disorder. Both are

common and compared to patients with depressive episodes without the specifiers represent subgroups with more pernicious forms of depressive illness (symptom severity, more comorbidities, increased suicide risk) and poor treatment response.

Lumateperone (lumateperone tosylate, ITI-007) is a mechanistically novel compound that is FDA-approved for the treatment of schizophrenia and for depressive episodes associated with bipolar I or bipolar II disorder as monotherapy and as adjunctive therapy with lithium or valproate. In a recent Phase 3, randomized, double-blind, placebo-controlled trial (Study 403; NCT04285515) lumateperone 42 mg demonstrated robust efficacy over placebo and a favorable safety profile in patients with MDD with mixed features or bipolar depression with mixed features. This post hoc analysis of Study 403 data investigated the efficacy of lumateperone 42 mg in the prespecified patient population with MDD or bipolar depression with mixed features who also met the DSM-5 criteria for anxious distress.

Methods: Eligible patients included males and females (18-75 years) meeting DSM-5 criteria for an MDE with mixed features and diagnosed MDD, bipolar I, or bipolar II disorder, with Montgomery-Åsberg Depression Rating Scale [MADRS] Total score ≥24 and a Clinical Global Impression Scale-Severity [CGI-S] score ≥4 at screening and baseline. Patients were stratified by MDD or bipolar disorder diagnosis and randomized 1:1 to 6-weeks treatment with lumateperone 42 mg or placebo, administered once daily in the evening. This analysis evaluated patients with mixed features and DSM-5 anxious distress in the overall population (combined MDD/bipolar depression) and separately in the MDD and bipolar depression individual populations.

Assessments included change from baseline in MADRS Total score, CGI-S score, and MADRS inner tension item score. Analyses were carried out using a mixed-effects model for repeated measures in the modified intent-to-treat (mITT) population.

Results: Of 383 patients in the combined MDD/bipolar depression mITT population with mixed features, 244 (63.7% of mITT; placebo, 121; lumateperone, 123) had anxious distress. As expected, anxious distress was common both in patients with MDD (73.9% of MDD mITT; placebo, 69; lumateperone, 67) and in patients with bipolar depression (54.3% of bipolar depression mITT; placebo, 52; lumateperone, 56).

Compared with placebo treated patients, the lumateperone group had significantly better improvement in change from baseline for MADRS Total score at Day 43 in all 3 populations with anxious distress: combined MDD/bipolar depression population (least squares mean difference vs placebo [LSMD], −6.1; 95% confidence interval [CI] −8.52 to −3.71; effect size [ES], −0.67; P < .0001), MDD individual population (LSMD, −6.8; 95% CI −9.82 to −3.77; ES, −0.79; P < .0001), and bipolar depression individual population (LSMD, −5.5; 95% CI −9.34 to −1.62; ES, −0.59; P < .01). In all 3 populations, significantly greater (P < .05) reductions in change from baseline of MADRS Total score occurred by Day 15 and persisted throughout the study in lumateperone treated patients.

Similarly, compared with placebo treated patients, change from baseline for CGI-S score at Day 43 showed significantly greater improvement for lumateperone treated patients for the combined MDD/bipolar population (LSMD, −0.5; 95% CI −0.78 to −0.26; ES, −0.54; P < .0001) with anxious distress, MDD individual population (LSMD, −0.6; 95% CI −0.98 to −0.30; ES, −0.66; P < .001) with anxious distress, and bipolar depression individual population (LSMD, −0.4; 95% CI −0.82 to −0.05; ES, −0.48; P < .05) with anxious distress.

Lumateperone also showed significantly greater improvement in change from baseline for the inner tension MADRS single-item score at Day 43 compared with that of placebo for the combined MDD/bipolar depression population (LSMD, −0.6; 95% CI −0.94 to −0.30; ES, −0.52; P < .001) with anxious distress, MDD individual population (LSMD, −0.9; 95% CI −1.26 to −0.47; ES, −0.77; P < .0001) with anxious distress, and bipolar depression individual population (LSMD, −0.5; 95% CI −0.93 to −0.00; ES, −0.42; P < .05) with anxious distress.

Conclusions: Our results demonstrate that lumateperone 42 mg is efficacious in improving symptoms of major depression with mixed features and anxious distress, including global disease severity and inner tension, in patients whether their lifetime mood disorder diagnosis was MDD or bipolar disorder.

Keywords: Lumateperone, Major Depressive Disorder, Bipolar Disorder, Mixed Features, Anxious Distress

Disclosure: Intra-Cellular Therapies, Inc.: Employee, Stock/Equity (Self).

P235. Metabolic Markers Changes in Response to Superolateral Medial Forebrain Bundle Deep Brain Stimulation for Treatment-Resistant Depression

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Background: Depression is a debilitating condition affecting millions. Approximately 30-35% of patients with depression fail to achieve remission despite multiple therapeutic interventions, causing what is called treatment-refractory depression (TRD). The burden of TRD exponentially increases the longer it persists, with a higher risk of impaired functional and social functioning, vast losses in quality of life, and a significant risk of somatic morbidity and suicidality. Stimulation-based technologies, designed to electrically or chemically modulate abnormal neural activity, are emerging as potential therapeutic options for refractory MDD patients, including deep brain stimulation (DBS). However, there are still no objective biomarkers that can be used to predict clinical response and guide patients' selection. Previous data from our group uncovered that TRD patients have higher lactate-to-pyruvate ratio and GDF-15 and FGF-21 plasma levels compared to non-psychiatry subjects and MDD patients, suggesting a relationship between mitochondrial dysfunction and treatment response. In this study, we evaluate if metabolic/mitochondrial markers (lactate-to-pyruvate ratio, GDF-15, and FGF-21) are biomarkers of treatment response in patients with refractory depression who have undergone medial forebrain bundle (slMFB) DBS.

Methods: Our sample included 12 TRD patients recruited for the Deep Brain Stimulation (DBS) Therapy for Treatment-Resistant Depression - Clinical Trial (NCT02046330). Patients were considered eligible for the study if they met the following inclusion criteria: (a) Major depression, severe, unipolar, diagnosed by SCID-I; (b) Hamilton Depression Rating Scale (HDRS) score > 21 on the first set of items; (c) MADRS score > 21; (d) Global Assessment of Function score of < 45; (e) a recurrent (≥ 4 episodes) or chronic (episode duration ≥ 2 y) course and a minimum of 5 y since the onset of the first depressive episode; (f) age 22–70 y; (g) refractory to > 6 weeks of multiple medication regimens; (h) refractory to > 20 sessions of psychotherapy; (i) refractory to a trial of electroconvulsive therapy (≥ 6 bilateral treatments). Surgical procedure and electrode positions were performed using the Leksell frame (Elekta, Sweden) and following our standard protocol (34, 35). The primary outcome measure was the antidepressant response on the MADRS, where a 50% score reduction was interpreted as a positive response. Plasma samples were collected at baseline and 12 months after surgery. Plasma

lactate, pyruvate, GDF-15, and FGF-21 levels were measured using commercial kits.

Statistical analyses were performed using Statistical Package for the Social Sciences, v.23.0 (SPSS Inc., USA). Data distribution normality was assessed using the Shapiro-Wilk test and histogram visualization. Chi-squared was applied to comparisons between the categorical variables. Data with nonparametric distributions were analyzed by the Mann-Whitney U test. The level of statistical significance was set at $p < 0.05$. Due to the exploratory nature of the study, we did not correct for multiple testing.

Results: Our results showed that the mean MADRS score reduction was 58% at 12 months. Seven out of these 12 patients were considered responders (58.3%) with >50% reduction in MADRS scores over time. Our results revealed no changes in the lactate-to-pyruvate ratio ($p = 0.249$), GDF-15 ($p = 0.811$), and FGF-21 ($p = 0.480$) levels over 52 weeks of treatment. On the other hand, we observed that patients that responded to treatment (>50% reduction in MADRS at 1-year follow-up) showed a lower lactate-to-pyruvate ratio than patients that did not respond. Follow-up analyses using paired samples t-tests revealed that in non-responders' patients after 12 months post-surgery, the lactate-to-pyruvate ratio ($p = .0085$) was significantly lower.

Conclusions: Energy impairment due to mitochondrial dysfunction has been implicated in depression and TRD pathophysiology. Our results showed that non-responders have a common metabolic signature in their plasma pre- and post-treatment. This suggests that an increased pre-operative lactate-to-pyruvate ratio could be used as a potential metabolic signature that distinguishes responders and non-responders. However, in this preliminary study, we cannot rule out the possibility of a type I error because of the small sample size. Moreover, the study did not control lifestyle factors like physical activity and alcohol use. Therefore, our results should be seen as exploratory and require replication and validation.

Keywords: Treatment-Resistant Depression, Adaptive Deep Brain Stimulation, Lactate-to-Pyruvate Ratio, FGF-21, GDF-15

Disclosure: Nothing to disclose.

P236. Efficacy and Safety of Esketamine Nasal Spray Versus Psychoactive Control for Rapidly Reducing Depressive Symptoms in Adolescents With Major Depressive Disorder at Imminent Suicide Risk: Results of a Double-Blind, Randomized Study

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Background: Increasing rates of major depressive disorder (MDD) and suicidal behavior in adolescents pose a major public health concern in the United States (US) and Europe. Esketamine nasal spray, co-administered with oral antidepressant therapy, is approved by the US Food and Drug Administration for treating depressive symptoms in adults with MDD with acute suicidal ideation or behavior and by the European Medicines Agency for adults with a moderate-to-severe episode of MDD, as acute short-term treatment, for the rapid reduction of depressive symptoms, which according to clinical judgement constitute a psychiatric emergency. Reported herein is a study conducted to compare the efficacy and safety of esketamine nasal spray to that of psychoactive comparator (oral midazolam), in combination with standard-of-care (SOC) treatment, in reducing symptoms of MDD, including suicidal ideation, in adolescents with MDD and at imminent risk for suicide. This study is the first pharmacological treatment trial conducted in this imminently suicidal, vulnerable patient population.

Methods: This double-blind (DB), double-dummy, phase 2b study randomized (1:1:1:2) adolescents (12 to < 18 years old) to esketamine nasal spray (28, 56, or 84 mg) or oral midazolam twice-weekly for 4 weeks (NCT03185819). All participants also received comprehensive SOC treatment, including initial hospitalization, an oral antidepressant, and psychotherapy.

Results: 147 adolescents were randomized, and 146 were included in the safety analysis dataset. At baseline, mean Children's Depression Rating Scale-Revised (CDRS-R) total score was 76.3 and mean MADRS total score was 38.8, with 94% moderately-to-extremely suicidal per Clinical Global Impression of Severity of Suicidality-Revised (CGI-SS-R), and 80% having a lifetime suicide attempt, 54% within the past month.

The full efficacy analysis dataset included 82 adolescents in the esketamine groups (28 mg, $n = 28$; 56 mg, $n = 31$; 84 mg, $n = 23$) and 63 in the midazolam group. According to a prespecified sequential multiple-testing procedure for the primary efficacy endpoint, pooled esketamine doses (56 and 84 mg) showed superiority over midazolam in reducing CDRS-R at 24 hours post-initial dose ($p = 0.037$, ANCOVA). The between-group difference (of LS means) in the individual dose groups was clinically meaningful (84 mg: 5.7, $p = 0.123$; 56 mg: -5.9, $p = 0.072$). Response rate (≥50% improvement in CDRS R total score from baseline) and remission rate (CDRS-R total score ≤28) at 24 hours post-initial dose were greater for all esketamine dose groups (28 mg: 53.6%/17.9%; 56 mg: 61.3%/16.1%; 84 mg: 56.5%/21.7%) versus midazolam (49.2%/7.9%).

Numerically greater improvement in depressive symptoms was also seen at 24 hours post-initial dose based on change in Montgomery Åsberg Depression Rating Scale total score in each esketamine dose group (mean [SD], 28 mg: -18.0 [11.98], 56 mg: -19.8 [9.66], 84 mg: -19.7 [11.94]) versus midazolam (-16.8 [12.13]) (LS mean between-group difference [95% CI] was -1.4 [-5.97; 3.24], -3.5 [-7.97; 0.92] and -3.3 [-8.32; 1.72] for the esketamine 28 mg, 56 mg, and 84 mg groups, respectively), ANCOVA).

At the end of the DB treatment period (day 25, 4 hours post-dose), all 4 treatment groups showed continued improvement on CDRS-R total score (midazolam -43.0; esketamine 43.6 to -48.8); the between-group difference (LS mean [95% CI]) was -5.1 [10.12, 0.15], -0.7 [-5.82, 4.46], and -5.8 [-11.76, 0.06] for the esketamine 28 mg, 56 mg, and 84 mg groups, respectively. Continued improvement of depressive symptoms on day 25, 4 hours post-dose, was also observed in all 4 treatment groups based on MADRS total score (midazolam: -28.0; esketamine -27.5 to -31.5); the between-group difference (LS mean [95% CI]) was -3.5 [-7.3, 0.31], 0.5 [-3.39, 4.39], and -2.7 [-7.28, 1.79] for the esketamine 28 mg, 56 mg, and 84 mg groups, respectively.

Severity of suicidality improved in all 4 groups; the between-group difference in CGI-SS-R was not statistically significant.

During the DB phase, the most common adverse events (AE; ≥30%) reported for esketamine were dizziness, nausea, dissociation, headache, dysgeusia, and somnolence; 21 participants (midazolam: 9 [14.3%], esketamine 28 mg: 4 [13.8%], esketamine 56 mg: 7 [22.6%], esketamine 84 mg: 1 [4.3%]) experienced a serious AE(s). No participant discontinued from an esketamine group due to an AE. Mania (i.e., Young Mania Rating Scale total score >12) was not seen in any participant. In the esketamine groups, dissociative symptoms, measured by the Clinician Administered Dissociative States Scale total score, peaked at 40 minutes and generally resolved by 1.5 hours post-dose, as did small, transient increases on the Brief Psychiatric Rating Scale (positive symptoms subscale). Fewer participants in an esketamine group (28 mg: 5/29 [17.2%], 56 mg: 6/31 [19.4%], 84 mg: 6/23 [26.1%]) had Modified Observer's Assessment of Alertness/Sedation score ≤3 (i.e., moderate or greater sedation) vs. midazolam (37/63 [58.7%]).

Conclusions: Esketamine with comprehensive SOC rapidly improved depressive symptoms among adolescents at imminent risk for suicide.

Keywords: Mood Disorders, Esketamine Nasal Spray, Adolescents

Disclosure: Janssen, Lundbeck, Sage, Alkermes, Shire, Myriad: Contracted Research (Self). Janssen, Sage, Alkermes, Myriad: Consultant (Self),

P237. Long-Term Safety and Efficacy of Esketamine Nasal Spray in Patients With Treatment-Resistant Depression: Results of the Final Data From the SUSTAIN-3 Study

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Background: Patients with treatment-resistant depression (TRD) have higher rates of relapse and pronounced deficits in daily functioning and health-related quality of life compared to patients with major depressive disorder who are not treatment-resistant, underscoring the need for treatment choices with sustained efficacy and long-term tolerability. Esketamine nasal spray, in conjunction with an oral antidepressant, has been approved for TRD by the US Food and Drug Administration, European Medicines Agency, and other health authorities in >70 countries based on proven efficacy and safety from phase 2 and phase 3 studies in TRD patients (history of nonresponse to ≥2 antidepressants in the current episode) treated for 4 weeks to 1 year. Reported herein is a study conducted to assess the long-term safety and efficacy of individualized, intermittently-dosed esketamine nasal spray, combined with an oral antidepressant, in patients with TRD.

Methods: Adults, both sexes, with TRD who participated in ≥1 of 6 phase 3 "parent" studies could continue esketamine treatment, combined with an oral antidepressant, by enrolling in phase 3, open-label, long-term extension study, SUSTAIN-3 (NCT02782104). Based on their status at parent study end, eligible participants entered a 4-week induction phase followed by an optimization/maintenance phase of variable duration, or directly entered the optimization/maintenance phase of SUSTAIN-3. Intranasal esketamine dosing was flexible, twice-weekly during induction and individualized to depression severity during optimization/maintenance.

Results: The study was conducted from June 2016 to December 2022 in 27 countries. 1148 participants were enrolled, 458 at induction and 690 at optimization/maintenance. The mean (SD, range) age of all participants was 49.6 (12.28, 19-83) years, with the majority (650 [56.6%]) aged 45−64 years. Of the 1,148 enrolled participants, 468 (40.8%) were withdrawn; the most frequent reasons for discontinuation were adverse event (AE; 74 [6.4%]), withdrawal by participant (63 [5.5%]), lack of efficacy (61 [5.3%]), lost to follow-up (23 [2.0%]), and other reasons (215 [18.7%]: relocation, improvement in symptoms/remission/feels better, early termination by site, job/school schedule conflicts, non-compliance, etc.).

Total exposure to esketamine in SUSTAIN-3 was 3,777.0 cumulative patient-years. Mean (SD, range) total duration of exposure was 42.9 (24.22, 0-79) months; 728 (63.4%) participants had ≥36 months exposure. The final dose was 84 mg for most participants during induction (78.2%) and during maintenance (64.4%). During maintenance, the most frequent dosing interval was weekly (approximately 40-50% of participants for most of their dosing), followed by every other week (approximately 35-45%), with every 4-week dosing less common (up to approximately 25%).

Common treatment-emergent AEs (≥20%) were headache, dizziness, nausea, dissociation, nasopharyngitis, somnolence, dysgeusia, and back pain. Incidence of increased blood pressure and related events did not rise over time. No events of treatment-emergent interstitial/ulcerative cystitis or respiratory depression were reported. No new concerns or trends related to cognition or events suggestive of abuse potential were identified. Nine participants died: COVID-19 related (3 cases), pneumonia (2 cases), completed suicide, myocardial infarction, multiple injuries, unknown cause (one each). The rates of completed suicide (0.026 vs. 0.47 per 100 patient-years, respectively) and all-cause mortality (0.24 vs. 0.79–4.6 per 100 patient-years) were lower in SUSTAIN-3, as compared to rates reported in the literature.

Mean Montgomery-Åsberg Depression Rating Scale (MADRS) total score decreased during induction, and this reduction persisted during optimization/maintenance (mean [SD] change from baseline to endpoint of the induction phase: -12.8 [9.73]; mean [SD] change from the optimization/maintenance baseline to the endpoint: +0.2 [9.93]), with 35.6% of participants in remission (MADRS ≤12) at induction endpoint, and 54.1%, 51.1%, 46.8%, 47.7%, and 46.9% at weeks 56, 104, 160, 208, and 256, respectively.

Improvement of depressive symptoms was also seen based on participants' responses to the Patient Health Questionnaire (PHQ-9): Mean PHQ-9 total score decreased during induction, and this reduction was maintained during optimization/maintenance (mean [SD] change from baseline to endpoint of the induction phase: -5.8 [5.84]; mean [SD] change from the optimization/maintenance baseline to the endpoint: +0.6 [6.22]), with 19.8% of participants in remission (PHQ ≤5) at induction endpoint, and 33.7%, 34.1%, 30.5%, 32.7%, and 31.2% at weeks 56, 104, 160, 208, and 256, respectively.

Conclusions: No new safety signal was identified during long-term treatment (up to 6.6 years) using intermittently-dosed esketamine in conjunction with daily antidepressant and improvement in depression ratings generally persisted among participants who remained in maintenance treatment.

Keywords: Esketamine, Treatment-Resistant-Depression, Major Depressive Disorder, Long-Term

Disclosures: Janssen Research and Development, LLC: Employee (Self). Johnson and Johnson: Stock / Equity (Self).

P238. Subjective and Pharmacodynamic Effects of the Novel 5-HT2A Receptor Agonist GM-2505 in Healthy Volunteers Show High Translatability From Rodent Data and Hold Promise for Future Development in Patients With Depression

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Background: GM-2505 is a novel 5-HT2A receptor agonist and 5-HT releaser designed to have a half-life intermediate between DMT and psilocybin. A wealth of clinical data demonstrates dose dependent subjective effects of 5-HT2A agonists, however limited data are available on the dose-dependence of 5-HT2A agonist-mediated effects on resting state EEG (rsEEG). Here we describe the PK/PD relationship for GM-2505 in healthy human participants generated in the first-in-human Phase 1 clinical trial. We compare

these data to analogous translatable biomarkers of target engagement collected in rodents.

Methods: GM-2505 was evaluated in an adaptive, randomized, double-blind, placebo-controlled first in human study to evaluate the safety, pharmacokinetics, and pharmacodynamics of single ascending intravenous doses of GM-2505 in healthy adult volunteers. Study participants received single iv infusions of GM-2505 (0.34, 1, 3.3, 10, 15, or 20 mg; n = 6/cohort) or placebo (n = 2/cohort). The effects of GM-2505 and placebo on treatment-emergent adverse events (TEAEs), vital signs and 12-lead ECGs were recorded for each cohort. Blood samples were collected for PK measurements. Pharmacodynamic assessments included resting-state electroencephalogram (rsEEG) and subjective effects assessed using questionnaires including the 5-Dimensional Altered States of Consciousness Rating Scale (5D-ASC) and the Mystical Experience Questionnaire-30 (MEQ-30).

The ability of GM-2505 (0.032-10 mg/kg, sc) to induce head twitches and wet dog shakes was measured in male SD rats for 20 min immediately after dosing. The effects of GM-2505 (0.3-3 mg/kg, sc) on quantitative EEG (qEEG) were measured using a within-subjects design in a cohort of 8 freely moving male SD rats implanted with 2 cortical electrodes connected to a DSI transmitter. GM-2505 was evaluated for its ability to reverse anhedonia caused by the chronic mild stress paradigm in adult male WKY rats. GM-2505 (0.3 and 1 mg/kg, sc) was dosed once weekly 24 h before measurement of sucrose intake. [3H]Cimbi-36 binding in SD rats dosed with GM-2505 (1-10mg/kg; n = 4/group) was used to measure in vivo 5-HT2A receptor occupancy.

Results: GM-2505 was safe and well tolerated at the doses tested. GM-2505 achieved dose proportional increases in exposure after iv infusion. The median t½ across all cohorts was 45 min.

GM-2505 administration resulted in dose-dependent increases in TEAEs. Expected TEAEs consistent with administration of a psychedelic and included altered states of consciousness, altered visual depth perception, abnormal thinking, euphoric mood, feeling drunk, feeling of body temperature changes, relaxation, sensory processing disorder (including intense visual effects with color changes), sensory overload, and time perception altered. Expected transient increases in systolic and diastolic blood pressure generally were observed with doses of 10-20 mg.

GM-2505 produced dose-dependent subjective effects as measured by 5-DASC and MEQ-30. Robust subjective effects were observed at plasma concentrations ≥ 22 ng/mL with even greater effects being observed at exposures of 44 ng/mL. Subjective effects were accompanied by dose-dependent effects on low-frequency EEG power, especially in alpha EEG band (8-13 Hz). Decreases in alpha power were observed at doses achieving Cmax plasma exposures ≥ 6 ng/mL. Increases in EEG gamma power were only observed at doses achieving Cmax ≥ 22 ng/mL.

In rats, peak head twitch and wet dog shake response was achieved at 1 mg/kg, sc which was associated with a Cmax exposure of 41 ng/mL. At this dose, GM-2505 decreased low frequency EEG power: delta power was reduced by ~50% and theta power by ~35%. At the 3 mg/kg dose there was a greater reduction in theta power (61%) and an increase in gamma power (49%) was observed. Both doses of GM-2505 (0.3-1 mg/kg; $p < 0.0001$) reversed stress-induced anhedonia, in rats. This dose range of GM-2505 displaced [3H]Cimbi-36 binding in rat frontal cortex with an EC50 of 11 ng/mL.

Conclusions: The PK profile of GM-2505 was consistent with this molecule being a shorter acting 5-HT2A agonist than psilocybin. There was a strong relationship between plasma exposure, subjective effects and rsEEG. The subjective effects of GM-2505 were very robust and consistent in magnitude with what has been reported in the literature for LSD, DMT and psilocybin. There was very good alignment between clinical plasma exposures associated with these robust pharmacodynamic effects and that associated with peak head twitch response in rats. The dose-dependence of the effects of GM-2505 on rsEEG have not previously been reported for 5-HT2A agonists. In the chronic mild stress paradigm, GM-2505 showed efficacy at exposures achieving 50% 5-HT2A receptor occupancy consistent with clinical report on psilocybin. Seeing strong translation of preclinical pharmacodynamic effects to the clinic setting, coupled with the measurement of clinical 5-HT2A receptor occupancy, will provide confidence in the potential translation of GM-2505's preclinical antidepressant effects to human subjects.

Keywords: Psychedelics, Major Depressive Disorder (MDD), Rapid-Acting Antidepressant, Translational Biomarker Approaches to Drug Development

Disclosure: Gilgamesh Pharmaceuticals: Employee (Self).

P239. A Customized Adherence Enhancement Program for Adolescents and Young Adults With Suboptimal Adherence and Bipolar Disorder

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Background: While medication non-adherence is common in bipolar disorder (BD), few studies have specifically assessed non-adherent adolescents and young adults (AYAs) with BD. This ongoing 6-month prospective randomized-controlled trial (RCT) pilot, which has closed enrollment, tested a novel behavioral intervention adapted for AYA (CAE-AYA) vs. enhanced treatment as usual (ETAU) to examine changes in medication adherence.

Methods: The 2-site RCT enrolled AYAs age 13-21 with a DSM-5 diagnosis of BD, type 1 or II with suboptimal adherence defined as self-reporting missing ≥ 20% of prescribed evidence-based BD medications, i.e., mood stabilizer (e.g., lithium, valproic acid, or carbamazepine) or second-generation antipsychotics. Individuals randomized to CAE-AYA received 5 remotely delivered sessions that addressed barriers to medication adherence relevant to that individual while those randomized to ETAU received written materials specific to BD among AYAs and follow-up telephone calls controlled for contact frequency. Participants were followed for 6 months with assessments conducted at Screening, Baseline, and Weeks 8, 12 and 24. Adherence, the primary outcome, was measured via: 1) self-reported Tablets Routine Questionnaire (TRQ) in the past 7 days and 2) electronic monitoring (SimpleMed pillbox). Participants were given SimpleMed pillboxes at screening and these data were available at baseline and follow-up time-points. Symptoms were measured with the Hamilton Depression Rating Scale (HAM-D), the Young Mania Rating Scale (YMRS), and the Clinical Global Impression Scale (CGI). The study was approved by local Institutional Review Boards (IRBs) at each site. This analysis focused on baseline and 12 week interim outcomes.

Results: Mean age of the sample (N = 36) was 19.1 years (SD = 2.0); 66.7 % (N = 24) female, 25.0 % (n = 9) non-white. The majority of participants had Type I BD (86%). Mean percentage of missed BD medications of the total sample based on TRQ was 34.9% (SD = 28.9) at screening and 30.6% (SD = 33.0) at baseline. Both the CAE and ETAU groups improved on TRQ from screening to baseline. Mean percentage of missed medication using SimpleMed at baseline was 42.1 (SD = 37.0). At baseline, the mean HAM-D (mean = 7.1, SD = 4.7) and YMRS scores (mean = 6.0, SD = 7.3) were consistent with relatively mild BD manic and depressive symptom severity. There were 22 individuals assessed at 12-weeks (11 in the CAE arm and 11 in the ETAU arm) in this interim analysis. Change from baseline to 12 weeks on TRQ in the 22 individuals who had both baseline and 12-week data showed a

modest improvement of 0.4% (SD = 40.3) for the CAE arm and worsening of 5.2% (SD = 39.9) in the ETAU arm, not a statistically significant difference between groups. Change from baseline to 12 weeks on SimpleMed in the 22 individuals who had both baseline and 12-week data was a worsening of 9.0% (34.7) in the CAE arm and a worsening of 19.7% (28.8) in ETAU arm, not a statistically significant difference between groups.

Conclusions: While adherence monitoring can temporarily improve adherence behaviors among AYA with BD, longer-term use/engagement with pill monitoring devices may be challenging. Numeric differences in adherence favoring CAE were not statistically significant in this interim analysis and assessment of longer-term (6-month) outcomes are needed in order to better understand the potential impact of the intervention among poorly adherent AYA with BD.

Keywords: Bipolar Disorder, Adolescence, Treatment Adherence, Clinical Trial

Disclosures: Alkermes, Otsuka, Janssen, Lundbeck, Teva, Neurelis: Consultant (Self). Springer Press, Johns Hopkins University Press, Oxford Press, UpToDate: Other Financial or Material Support (Self). American Physician's Institute (CMEtoGo), Psychopharmacology Institute, American Epilepsy Society, Clinical Care Options, American Academy of Child and Adolescent Psychiatry, Neurocrine: Other Financial or Material Support (Self).

P240. Navacaprant (NMRA-140), A Novel and Highly Selective Kappa Opioid Receptor Antagonist, in Patients With Major Depressive Disorder: A Randomized Placebo-Controlled Phase 2 Trial

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Background: Major depressive disorder (MDD) is a leading cause of disability, morbidity, and mortality, with approximately 264 million people worldwide and 21 million adults in the U.S. reporting symptoms of depression. Despite available treatments, a significant unmet need remains, as many patients do not adequately respond to first-line pharmacotherapies and often experience side effects. Current antidepressants also do not adequately treat anhedonia, a core clinical feature of MDD that affects approximately 70% of patients and is associated with more severe depressive symptoms. New targeted therapies are needed to treat MDD, including symptoms of anhedonia, while also improving tolerability over current antidepressants. Kappa opioid receptors (KORs) are novel targets for anhedonia that are abundantly expressed in brain circuits regulating reward, motivation, stress, and anxiety. KOR activation is a strong negative modulator of multiple neurotransmitters, including dopamine, and results in dysphoria. Conversely, KOR antagonists are believed to restore the regulation of multiple neurotransmitters including dopamine in reward processing pathways, which play an important role regulating mood, cognition, reward, and behavior. Navacaprant (NMRA-140, BTRX-335140) is a novel, potent, and highly selective KOR antagonist with no agonist activity at mu, kappa, or delta opioid receptors. Navacaprant is in development as monotherapy to treat the symptoms of MDD.

Methods: This 8-week, randomized, double-blind, placebo-controlled Phase 2 clinical trial evaluated the efficacy and safety of navacaprant vs placebo in adults with MDD and symptoms of anhedonia and anxiety. Participants that met the blinded-rule list with a 17-item Hamilton Rating Scale for Depression (HAMD-17) score of 14-30, a Hamilton Anxiety Rating Scale (HAM-A) score of ≥8, and a Snaith-Hamilton Pleasure Scale (SHAPS) score of ≥26 were randomized 1:1 to navacaprant (80 mg) or matched placebo taken orally once-daily. The primary efficacy endpoint was the change from baseline (CFB) to Week 8 in HAMD-17 score. Secondary endpoints included CFB to Week 4 in HAMD-17 score and CFB to Weeks 4 and 8 in SHAPS score. The primary efficacy analysis was conducted using mixed models repeated measures (MMRM). Since ≥10% of subjects had missing data, a prespecified Last Observation Carried Forward (LOCF) analysis of covariates (ANCOVA) was used for the primary and secondary endpoints and prespecified subgroups. To evaluate the efficacy of navacaprant in patients with moderate-to-severe MDD, who comprise the most common population in clinical trials, a prespecified subgroup analysis was performed on the primary efficacy endpoint by stratifying results according to baseline HAMD-17 score of ≥22. Safety was assessed during the 8 weeks of treatment and a 4-week safety follow-up.

Results: 204 patients from 31 US sites were randomized (102 in each group) and included in the safety population. In the efficacy population (n = 88 navacaprant, n = 83 placebo), which included patients with baseline HAMD-17 scores as low as 14, statistically significant separation of navacaprant from placebo on HAMD-17 score was detected at Week 4 (least squares mean difference, LSMD [SE], MMRM -2.7 [0.90], P = 0.003) but not Week 8 (-1.7 [1.08], P = 0.121) which was the primary endpoint. In the prespecified LOCF analysis, a statistically significant improvement was detected for navacaprant 80 mg over placebo for both Week 4 (LSMD [SE], LOCF -2.9 [0.88], P = 0.002) and Week 8 (-2.2 [0.98], P = 0.024). For symptoms of anhedonia, navacaprant showed a significant improvement over placebo at both Week 4 and Week 8 (SHAPS: LSMD [SE], LOCF -2.8 [0.96], P = 0.004; and -3.4 [1.10], P = 0.002, respectively).

In the prespecified subgroup analysis of patients with moderate-to-severe MDD (baseline HAMD-17 ≥22, n = 53 navacaprant, n = 47 placebo), a statistically significant difference at Week 4 and Week 8 favoring navacaprant was detected for depression (HAMD-17: LSMD [SE], LOCF -3.0 [1.20], P = 0.015; and -2.8 [1.33], P = 0.037, respectively). For anhedonia, a trend was observed at Week 4 (SHAPS: LSMD [SE], LOCF -2.4 [1.31], P = 0.071) and a statistically significant difference was observed at Week 8 (LSMD [SE], LOCF -4.8 [1.35], P = 0.001).

Navacaprant was generally safe and well tolerated. The incidence of treatment-emergent adverse events (TEAEs) was lower in the navacaprant group compared with placebo (35.3% vs 44.1%). Most TEAEs were mild to moderate, with the most common TEAEs being headache (4.9% both groups), nausea (4.9% navacaprant, 1.0% placebo), and COVID-19 (3.9% navacaprant, 2.9% placebo). Discontinuations due to TEAEs were less frequent in the navacaprant group (1.0%) than in the placebo group (11.8%). There was no observed weight gain and no spontaneous reports of sexual dysfunction reported in those receiving navacaprant. No serious TEAEs were reported in the navacaprant group vs 1.0% in the placebo group (1 suicide attempt). No evidence of suicidal behavior was reported in the navacaprant group.

Conclusions: Navacaprant resulted in statistically and clinically significant reductions in symptoms of depression and anhedonia compared with placebo following 8 weeks of treatment in patients with moderate-to-severe MDD. Navacaprant was generally safe and well tolerated, with low rates of discontinuation due to TEAEs. These findings support the further development of navacaprant as an antidepressant monotherapy.

Keywords: Major Depressive Disorder, Anhedonia, Navacaprant, Antidepressant, Kappa Opioid Receptor Antagonist

Disclosure: Almatica Pharma, Biohaven, BioXcel Therapeutics, Boehringer-Ingelheim, Brii Biosciences, Clexio Biosiences, COMPASS Pathways, Delix Therapeutics, Douglas Pharmaceuticals, Eleusis, EMA Wellness, Engrail Therapeutics, Levo Therapeutics, Liva Nova, Merck, Perception Neurosciences, Praxis Precision

Medicines, Neumora Therapeutics, Neurocrine, Relmada Therapeutics, Sage Therapeutics, Seelos Therapeutics, Signant Health, Sunovion, XW Pharma: Consultant (Self). Boehringer-Ingelheim, Neurocrine, Sage Therapeutics: Grant (Self).

P241. Poster Withdrawn

P242. The Pact-Md Randomized Clinical Trial: Prevention of Alzheimer's Dementia With Cognitive Remediation Plus Transcranial Direct Current Stimulation in Mild Cognitive Impairment and Depression

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Background: Prevention of cognitive decline and dementia in high risk conditions such as remitted Major Depressive Disorder (rMDD) or Mild Cognitive Impairment (MCI) is needed.

Methods: PACt-MD was a double-blind randomized trial comparing cognitive remediation (CR) plus transcranial Direct Current Stimulation (tDCS) vs. sham-CR+sham-tDCS delivered 5 days/week for 8 weeks followed by 5-day semi-annual boosters and at-home daily, in participants with rMDD or MCI. Assessments occurred at baseline, week 8 and yearly.

The hypotheses were that compared to sham+sham, CR+tDCS would: slow cognitive decline; reduce progression to MCI or dementia; and acutely improve cognition.

The primary outcome was a composite score of at least four of six cognitive domains: executive function, language, processing speed, verbal memory, visuospatial memory, and working memory.

Results: 375 participants were randomized (Active: N = 188, Mean Age = 72.1 + /- 6.3; Sham: N = 187, Mean Age = 72.3 + /- 6.4) and received at least one intervention session. The median follow-up period from the start of the intervention was 47.9 [1.9 – 85.0] months.

Likelihood-ratio test (LRT) for an overall treatment effect gave p = 0.032 with a year-5 adjusted z-score difference (Active - Sham) of 0.15 (95%CI [0.005, 0.29]). Week-8 adjusted z-score difference (Active - Sham) was 0.06 (95%CI [-0.005, 0.12]).

The Hazard Ratio (Active vs. Sham) was 0.66 (95%CI [0.40, 1.08], p = 0.10) for progression.

A preplanned secondary analysis showed an overall treatment effect for executive function (LRT p = 0.046) and verbal memory (LRT p = 0.021) with weaker evidence for the other domains (p's>0.06). Year-5 adjusted z-score difference (Active - Sham) was 0.05 (95%CI [-0.15, 0.25]) for executive function and 0.30 (95%CI [0.07, 0.53]) for verbal memory. Week-8 adjusted z-score difference (Active - Sham) was 0.16 (95%CI [0.03, 0.29]) for executive function and -0.02 (95%CI [-0.17, 0.12]) for verbal memory.

Another preplanned secondary analysis showed a randomization diagnosis sub-group effect (LRT p = 0.012) with the rMDD group showing slower cognitive decline in response to the active treatment than the MCI group.

Conclusions: This study provides evidence for CR+tDCS efficacy in slowing cognitive decline in a group of older adults at risk of dementia, with pronounced effects in executive function and verbal memory, and in rMDD.

Keywords: Major Depression Disorder, Mild Cognitive Impairment Due to AD, Cognitive Remediation, Transcranial Direct Stimulation, Prevention

Disclosure: Nothing to disclose.

P243. Clinical and Neurophysiological Effects of Alpha Transcranial Alternating Current Stimulation for the Treatment of Depression: A Randomized, Controlled Clinical Trial

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Background: Major depressive disorder (MDD) can be conceptualized as a disorder of connectivity. Specifically, alpha power and connectivity have been hypothesized to stabilize the brain in a depressed state. Transcranial alternating current stimulation (tACS) in the alpha frequency recently yielded promising results in alleviating symptoms in people with MDD. A proposed mechanism of action is that cortical oscillations are entrained during tACS stimulation and that subsequent stimulation sessions induce a rebound phenomenon, resetting oscillators in the prefrontal cortex, thus leading to a reemergence of regular, endogenous oscillatory activity. Here, we directly test this hypothesis in a clinical trial with daily measurements of brain activity before and after stimulation.

Methods: This triple blind, randomized, controlled, clinical trial applied 10Hz-tACS for 5 consecutive days in 20 participants with major depressive disorder. Participants received bifrontal 10Hz-tACS (4mA peak-to-peak) for 40 minutes while watching a relaxing video. High-density 128-channel EEG was recorded on each stimulation day before and after tACS, and at the 2-week follow-up. Clinical assessments were performed on Day 1, Day 5 of stimulation, and at the follow-up visit. The primary outcome is the change in left frontal alpha power. Secondary outcomes include the HDRS-17 and other clinical scores as well as the correlation between clinical scores and left frontal alpha power. The study is registered at the National Clinical Trial Register (NCT03994081).

Results: Repeated measures ANOVA with HDRS-17 score as dependent variable resulted in a significant effect of time, $F(2,34) = 29.16$, $p < .001$, $\eta^2 = 0.334$, but did not yield a significant interaction between stimulation condition and time, p = .6, indicating an overall improvement of depression severity regardless of group allocation. However, numerically the tACS group improved more (-8.9 HDRS points) than the sham group (-6.8 HDRS points) in the 2-week follow-up. In agreement with our hypothesis, change in individual left frontal alpha power and HDRS score strongly correlated in the tACS group, $r(13) = 0.705$, p = .003, but not in the sham group, p = .961.

Conclusions: With this pilot RCT, we show a correlation between improvement in depression severity and the reduction of alpha oscillation power in the left prefrontal cortex in the 10Hz-tACS group. This points towards a successful target engagement by our treatment intervention and is in agreement with previous studies of tACS targeting alpha oscillations in people with depression. Numerically, the tACS group improved 2.1 HDRS points more than the sham group. However, this difference did not reach statistical significance, suggesting that our study was underpowered to detect a group effect. In the next analysis steps, we will track daily changes of alpha oscillations in this unique

dataset and investigate the modulation of functional networks in depression by 10Hz-tACS.

Keywords: Major Depressive Disorder (MDD), Noninvasive Brain Stimulation, Neuronal Oscillations, Randomized-Controlled Trial

Disclosure: Nothing to disclose.

P244. Evaluation of AXS-05 (Dextromethorphan-Bupropion) in Major Depressive Disorder Using the Interest-Activity Domain

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Background: Individuals with major depressive disorder (MDD) who have significant symptoms of interest loss and reduced activity respond poorly to serotonergic antidepressants, a finding that has been replicated in large studies (Psychol Med. 2012;42:967-80). Individuals with diminished levels of interest and activity may therefore need alternative antidepressants.

AXS-05 [dextromethorphan-bupropion (Auvelity® extended-release tablet)] is a novel, oral, NMDA receptor antagonist with multimodal activity approved by the FDA for the treatment of MDD in adults. 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 is an aminoketone and CYP450 2D6 inhibitor, which serves primarily to increase the bioavailability of dextromethorphan.

Data examining the effects of AXS-05 on interest and activity symptoms and the impact of the severity of impairment in the interest-activity domain at baseline on the efficacy of AXS-05 have not been previously reported. Our objective was to explore the effects of baseline interest-activity symptoms on depression outcomes and to evaluate the efficacy of AXS-05 in improving interest-activity domain scores.

Methods: AXS-05 (dextromethorphan 45 mg- 105 mg bupropion) was evaluated in two double-blind, randomized, controlled, 6-week trials in individuals with moderate to severe MDD. The GEMINI trial (N = 327; J Clin Psychiatry. 2022;83:21m14345) was placebo-controlled and the ASCEND trial (N = 80; Am J Psychiatry. 2022;179:490-499) used bupropion as the control. In both studies, the Montgomery-Asberg Depression Rating Scale (MADRS) was the primary efficacy variable, and the 16-item Quick Inventory of Depressive Symptomatology (QIDS) was utilized as a secondary efficacy measure. The data from these two studies were pooled and the interest-activity symptom score was calculated using the methods of Uher et al. (J Clin Psychiatry 2020;81:20m13229) as the sum of the MADRS items for concentration, lassitude, and inability to feel and the QIDS items for concentration, interest, and energy. The QIDS items were doubled to give equal weight to the MADRS items.

In this post-hoc analysis, we tested the hypothesis that the efficacy of AXS-05 would be maintained regardless of individuals' baseline severity of interest-activity score. To evaluate the effects of baseline interest-activity impairment on depression outcomes between AXS-05 and control, a mixed model for repeated measures (MMRM) was fit with MADRS change from baseline as the outcome. Fixed effects consisted of the three-way interaction of treatment group, week, and baseline interest-activity score and all lower-order terms as well as baseline MADRS score. Additionally, we assessed the efficacy of AXS-05 compared to control in improving interest-activity symptoms by examining the marginal mean change from baseline estimated from an MMRM with fixed effects of baseline interest-activity scores, treatment group, week, and the interaction of treatment group and time; we also evaluated response rates, defined as having at least a 50% improvement in this domain.

Results: Averaged across treatment weeks, higher baseline impairment in interest-activity was associated with less improvement in MADRS total score in the control group (b = 0.27; 95% CI, 0.02-0.52; p = 0.037). In contrast, there was no significant association between baseline interest-activity score with MADRS total score in the AXS-05 group (b = -0.04; 95% CI, -0.24 to 0.31; p = 0.787). Similarly, there was no association between baseline interest-activity score and MADRS change from baseline in the AXS-05 group at any treatment week (all p values > 0.184).

The estimated marginal mean improvement in the interest-activity domain score for AXS-05 compared to control was statistically significant at every timepoint including Week 1 (p = 0.001). At Week 6, the improvement from baseline in the interest-activity domain was -11.76 (95% CI = -12.97, -10.55) compared to -8.72 (95% CI = -9.89, -7.54) in the AXS-05 and control groups, respectively (p < 0.001). Response rates in the interest-activity domain were higher with AXS-05 compared to control starting at Week 1 (p = 0.007). At Week 6, 58% of subjects in the AXS-05 group achieved ≥ 50% improvement on the interest-activity domain compared to 37% in the control group (p < 0.001).

The most commonly reported adverse reactions (≥5% and twice the rate of placebo) with AXS-05 were: dizziness, headache, diarrhea, somnolence, dry mouth, sexual dysfunction, and hyperhidrosis.

Conclusions: Individuals with impaired interest/activity have previously shown poor response to serotonergic antidepressants. AXS-05 exhibited comparable reductions in depressive symptoms regardless of severity of baseline interest-activity and AXS-05 significantly improved the interest-activity symptom dimension compared to control. These results suggest that AXS-05 may be a particularly effective treatment option in individuals with MDD who have significant impairment in the interest-activity domain.

Keywords: Major Depression Disorder, N-methyl-D-aspartate, Anhedonia, AXS-05

Disclosure: Axsome Therapeutics: Employee, Stock/Equity (Self).

P245. ANC-501-A Novel V1b Receptor Antagonist: Results of a Personalized Phase 2 Trial in MDD

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Background: ANC-501 is a novel specific V1b receptor antagonist that is being developed as a personalized treatment specifically for patients with HPA axis disruption. The current trial, ANC5010005 is an exploratory open label, Phase II fixed-dose trial exploring the safety, tolerability and efficacy of ANC-501 as an adjunctive treatment, specifically in MDD patients with elevations in baseline cortisol measurements.

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. ANC-501 is a novel investigational new drug with specific antagonistic activity of the vasopressin receptor 1b (V1b receptor). In nonclinical and early studies, ANC-501 appeared to be a promising candidate for clinical development with a novel mode of action that may benefit MDD patients. In a previous

6-week phase 2 trial in MDD, ANC-501 demonstrated numerical improvement in both 10 mg (-9.0 [-13.9, -4.1]) and 50 PO -9.0 [-13.4, -4.5]) compared with Placebo (6.4[10.7, -2.2]) (LSM [95% CI]). Consistent with the mechanism of action of ANC-501, patients with elevated urinary cortisol (defined as >22.7 nmol/L) showed markedly better separation from placebo 10 mg (-11.4 ± 10.97), 50 mg (-9.9 ± 8.22) vs Placebo (-3.3 ± 3.40).

Methods: Male and female adult MDD outpatients 18 and over were recruited through physician referral along with traditional and online advertising. MADRS score at the time of dosing was ≥26. Patients were also screened for 12 overnight urinary concentrations of ≥ 8.2ng/mL. MDD diagnosis and severity were confirmed by structed clinical interview (SCID) both by the PI and independently confirmed by external independent raters. All patients were on stable doses of antidepressant medication for 6 weeks prior to starting and throughout treatment with ANC-501. received 50 mg of ANC-501 PO for 8 weeks in addition to their ongoing SSRI or SNRI antidepressant medication. The primary efficacy end point was change in baseline Montgomery-Åsberg Depression Rating Scale total score (MADRS) from baseline to the end of dosing at week eight. Secondary endpoints include MADRS Response (50% improvement from baseline) and Remission (MADRS ≤ 10 at end of dosing), clinical global impression of severity (CGI-S), Hamilton Anxiety Rating Scale (HAM-A) and Patient Global Impression of Change (PGI-C). Additional exploratory endpoints included Ecological Momentary Assessment, ePRO measures and safety including treatment emergent adverse events and spot pharmacokinetic assessments.

Results: 13 patients (9F, 4M) mean age 45.4 (range 24-63) were dosed for 8 weeks in the current study. Mean baseline MADRS was 34.8 (±3.67 s.d.) At week 8, mean change from baseline (CFB) in MADRS score was -18.78 (-20.12 -11.43; 95% CI). With 66% of that change (-12 points) occurring within the first week of treatment initiation. Mean CFB in CGI-S, HAM-A were all consistent with the primary endpoint. Importantly, 10/13 (77%) of patients rated their improvement as either very much improved (n = 6) or much improved (n = 4). 10/13 Patients had a treatment-emergent adverse event (TEAE) most of which were mild and self-limited. There were no Serious Adverse Events, deaths or worsening of suicidal ideation reported.

Conclusions: In the current study of a proactively selected population of MDD with patients with cortisol elevations, ANC-501 50mg PO QD for 8-weeks adjunctively administered along with standard antidepressant treatment was well tolerated and demonstrated a strong signal for antidepressant activity. This study supports continued development of ANC-501 as an adjunctive treatment in MDD specifically for patients with HPA axis disruptions.

Keywords: Depression, Cortisol Response to Stress, Phase II Clinical Trial, Vasopressin 1b Antagonist, Personalized Medicine

Disclosure: EmbarkNeuro: Employee (Self).

P246. Magnetoencephalography Changes During a Working-Memory Task in Treatment-Resistant Depression Patients Following a Subanesthetic Dose of Intravenous Ketamine

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Background: Individuals with unipolar and bipolar treatment-resistant depression (TRD) frequently present with cognitive deficits that persist during and between mood episodes. These cognitive deficits, including problems in working memory, contribute to a decline in both psychosocial abilities and quality of life. Working memory, a cognitive function associated with the temporary storage and manipulation of information, can be measured with an n-back task and is crucial for work, school, and problem-solving skills. There is a lack of effective pharmacological interventions to treat cognitive dysfunction in patients with depression. Ketamine, a high-affinity antagonist of the N-methyl-D-aspartate type glutamate receptor, has been studied for its rapid-acting antidepressant effects. Preliminary evidence suggests ketamine could improve cognition in patients with TRD through its synaptogenic effects on the prefrontal cortex. Magnetoencephalography (MEG) studies demonstrate that ketamine increases gamma power in parts of the occipital, temporal, and frontal cortices. Furthermore, pregenual anterior cingulate cortex activity during a working-memory task was correlated to response to ketamine in patients with TRD using MEG. This study aims to investigate the effect of ketamine on working memory performance (reaction time and accuracy on the n-back task) and on gamma power (30-58 Hz) in patients with unipolar and bipolar TRD.

Methods: This post-hoc analysis combined two cross-over double-blind, randomized, placebo-controlled trials performed at the National Institute of Mental Health. Twenty subjects (14 female, age mean 42.35 and SD = 11.10), 13 with bipolar disorder and 7 with major depressive disorder, received a single intravenous infusion of subanesthetic ketamine (0.5 mg/kg) or saline placebo over 40 minutes approximately two weeks apart. Patients with bipolar disorder had ongoing treatment with either lithium or valproate. Patients with major depressive disorder were unmedicated. The subjects performed an n-back task during MEG scanning at baseline (1-3 days before the first infusion) and 6-9 hours following each infusion. Depression was assessed 60 minutes prior and 230 minutes after each infusion using the Montgomery-Åsberg Depression Rating Scale (MADRS). We used a mixed model regression to compare the effects of the drug on n-back performance after each infusion. All models included a random intercept per person and a fixed drug effect. Study, age, gender, and MADRS were also included as covariates in the models. Gamma power was projected during the maintenance period of the task (-500 to 0 ms peristimulus time) for each subject and memory load (0-, 1-, and 2-back) using the multiple sparse priors algorithm in SPM12 (https://www.fil.ion.ucl.ac.uk/spm/). Two-way repeated measures analysis of variance examined the effect of infusion (ketamine vs. placebo) and memory load on source-localized gamma power.

Results: Ketamine led to a significant improvement on MADRS scores ($p < 0.01$). Behaviorally, there were no statistically significant differences comparing ketamine and placebo reaction time [(0-back: $p = 0.66$), (1-back: $p = 0.053$), (2-back $p = 0.26$)] or accuracy [(0-back: $p = 0.61$), (1-back: $p = 0.76$), (2-back: $p = 0.46$)] 6-9 hours post-infusions. While there was no effect of memory load on source-localized gamma power, ketamine increased gamma power in the parieto-occipital junction ($t = 2.61$, $p < 0.01$) and decreased gamma power in the posterior superior temporal sulcus ($t = 2.5$, $p < 0.01$) and the inferior frontal gyrus ($t = 2.51$ $p < 0.01$) compared to placebo.

Conclusions: A single infusion of ketamine, despite improving depression, was not associated with improvements in working memory performance measured by the n-back task compared to placebo. However, ketamine led to gamma power changes in regions associated with attention and working memory. Further studies are needed in order to investigate the cognitive impact of ketamine in TRD with larger sample sizes, different cognitive tests, other aspects of cognition, assessments at different time points, and repeated ketamine infusions.

Keywords: Ketamine, Cognition, Treatment-Resistant Depression, Working Memory, Magnetoencephalography

Disclosure: Nothing to disclose.

P247. Using Soluble Interleukin-6 Receptor (sIL-6R) to Predict Symptom Severity in Treatment Resistant Bipolar Depression (TRBDD) - Effects of Adjunctive Celecoxib on its Expression

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Background: In view of the high rate of treatment resistance in patients with bipolar depression (BDD), there is increasing interest in the utility of biomarkers to guide treatment decisions and individualized therapy (Yamasaki et al., 2020). Psychiatric disorders, and BDD in particular, have been linked to inflammation by demonstrated elevations in a range of inflammatory biomarkers, such as sIL-6R (Munkholm et al., 2013; Tu et al., 2019). After we demonstrated the efficacy of combined treatment with the anti-inflammatory celecoxib (CXB) and escitalopram (ESC) over ESC with placebo in a published randomized clinical trial, we conducted the following secondary analyses to determine how anti-inflammatory treatment may affect sIL-6R expression in TRBDD.

Methods: The study included data derived from a cohort of TRBDD subjects that underwent an eight-week treatment course with either ESC + CBX (n = 23) and ESC + PBO (n = 15). Subjects that met DSM-IV criteria for BD I or BD II and were characterized as treatment resistant were included. Blood level sIL-6R levels were measured by ELISA. At Weeks 0, 1, 2, 4 and 8, subjects were given the following self-rating and clinician-administered scales: Hamilton Rating Scale for Depression (HAM-D), Hamilton Anxiety Rating Scale (HAM-A), Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI) and Perception of Stress Scale (PSS). Treatment response was classified as greater than 50% reduction in HAM-D Week 8 score from baseline but final score > 7, while no response consisted of less than 50% reduction in HAM-D Week 8 score from baseline. Pearson correlations were performed to assess the relationship between symptom severity in the form of rating scales and levels of sIL-6R at Baseline, Week 4, and Week 8. Analysis of covariance was used to contrast levels of sIL-6R between treatment groups at Weeks 4 and 8.

Results: There was an overall decrease in mean sIL-6R levels from Baseline to Week 8 in both patients treated with ESC + PBO (-0.135 ng/mL) (n = 15) and those treated with ESC + CBX (-0.165 ng/mL) (n = 23). After controlling for Baseline sIL-6R, sIL-6R levels continued to decrease from Week 4 to Week 8 in both treatment groups, although the extent of the decrease was not statistically different for the two groups (p = 0.591). For post-treatment Week 8 sIL-6R levels, no difference (p = 0.681) was observed between those treated with ESC + PBO (n = 15) compared to those treated with ESC + CBX (n = 23).

For the entire cohort of TRBDD patients, weak positive correlations were found between sIL-6R levels and HAM-D17 (.161), HAM-D21 (0.192), and HAM-A (0.162) at baseline. Conversely, weak negative correlations were seen between sIL-6R levels and HAM-D17 (-0.193), HAM-D21 (-0.205), and HAMA (-0.310) at Week 8. Although the HAM-D correlations did not reach significance, the correlation between sIL-6R level and HAMA at Week 8 was significant (p = 0.046).

In patients who did not respond to treatment by Week 8 (n = 14), weak negative correlations were seen between sIL-6R and HAM-D17 (-0.231), HAM-D21 (-0.264), HAM-A (-0.485), BDI (-0.298), and PSS (-0.250). However, weak to moderate positive correlations were observed between sIL-6R and HAM-D17 (0.129), HAM-D21 (0.112), BDI (0.501), and PSS (0.383) in treatment responders (n = 28) at Week 8. The correlation between sIL-6R and BDI post-treatment was significant (p = 0.007).

Conclusions: In line with our hypothesis that inflammation may underlie the pathophysiology of BDD, we observed a decline in blood levels of the pro-inflammatory sIL-6R over the course of treatment in the TRBDD cohort. However, contrary to our initial assumption, the treatment arm with the CXB add-on did not show as rapid a decline in sIL-6R as expected, and differences in sIL-6R levels were not seen between treatment arms. Based on the results of the rating scale correlations, the role sIL-6R in predicting symptom severity and treatment response is not clear; therefore, further research is required to determine its potential as a biomarker. One mitigating factor for the present study may be the relatively small sample size. Additionally, it is possible that a treatment duration of eight weeks may not be sufficient to produce a significant reduction in blood levels of inflammation. We recommend future research should explore the effects of several months of treatment with determination of inflammatory biomarkers over prolonged periods of observation.

Keywords: Inflammation, Cytokine, Bipolar Depression, Celecoxib, Escitalopram

Disclosure: Nothing to disclose.

P248. Modifying the Emotion Regulation Brain Network in Depression: Mechanistic Insights From a Clinical Trial of Cognitive-Behavioral Therapy for Insomnia

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Background: Converging epidemiological and experimental evidence demonstrates that unhealthy sleep contributes to disrupted mood, especially depression. Depression commonly co-occurs with insomnia disorder, and sleep difficulties are considered both a core symptom and predictive factor of psychiatric illness. Disturbed sleep more broadly is also known to disrupt brain networks that regulate emotion, particularly in the nodes of the amygdala and medial prefrontal cortex (mPFC). Amygdala reactivity and connectivity with the mPFC are involved in the generation and regulation of emotions and are impacted by a lack of sleep, suggesting that these brain regions may represent mechanistic markers of emotional dysfunction related to sleep difficulties. However, it is currently unknown whether insomnia treatment can affect brain function involved in emotion regulation, and whether improving sleep alleviates depression symptoms by altering the mechanisms of emotion regulation. Here we report preliminary findings from the single-arm phase of an ongoing mechanistic clinical trial, that primarily aims to demonstrate feasibility and establish that brain networks involved in emotion regulation are engaged and modified when participants show improvements in depression and sleep symptoms following Cognitive-Behavioral Therapy for Insomnia (CBT-I).

Methods: At the time of this report, data was available for 22 participants who took part in an ongoing single-arm mechanistic clinical trial. Our preliminary sample is composed of mostly females (75%), with ages ranging from 26-59 (mean = 39 + /-11.7), representing a racial makeup of 65% Caucasian, 25% Asian, and 10% reporting mixed races. Ethnically, our sample is composed of 85% non-Hispanic individuals. Two participants were excluded from the imaging analysis due to excessive head movements. Imaging, mood and insomnia symptoms, and emotion regulation skills were acquired before and after receiving six sessions of CBT-I. Depression was evaluated via the Beck Depression Inventory (BDI), insomnia using the Insomnia Severity Index (ISI), anxiety using the Beck

Anxiety Inventory (BAI), and emotion regulation using the Emotion Regulation Questionnaire. Participants were adults with elevated depression symptoms (BDI>=14) and experiencing clinically meaningful insomnia symptoms (ISI>=10). We performed preliminary analysis of fMRI data from the Emotional Faces Task and Emotion Regulation Task using established methods with our primary brain targets being (1) amygdala reactivity and (2) amygdala-mPFC connectivity. In short, amygdala reactivity to emotional stimuli and psychophysiological interactions between the amygdala and nodes of the mPFC (pregenual anterior cingulate, pACC; dorsomedial prefrontal cortices, dmPFC) were modeled, beta weights extracted, and entered into statistical analyses. Paired one-tailed t-tests were used to evaluate changes in brain function, clinical sleep, and mood following CBT-I treatment.

Results: Preliminary results from the emotional regulation brain network align with the primary aim. Specifically, relative to baseline, participants experienced reduced amygdala reactivity to emotional stimuli ($t = 2.18$, $p = 0.02$, $d = -0.63$, 95% CI [-1.3, 0.07]) after CBT-I treatment. Reduced emotional reactivity within the amygdala was accompanied by increases in amygdala functional connectivity to pACC ($t = -1.7$, $p = 0.059$, $d = 0.40$, 95% CI [-0.3, 1.1]) and to dmPFC ($t = -2.13$, $p = 0.02$, $d = 0.76$, 95% CI [0.05, 1.5]), consistent with a normalization of cortical control of limbic emotional reactivity following insomnia treatment. In addition, primary clinical sleep and emotion outcomes were similarly improved at the end of treatment. There was a significant reduction in the severity of insomnia ($t = -8.4$, $p < 0.0001$, $d = -1.9$, 95% CI [-2.7, 1.2]) and depression ($t = -6.5$, $p < 0.0001$, $d = -1.4$, 95% CI [-2.1, -0.69]) symptom severity. For secondary outcomes, there were simultaneous reductions in anxiety symptoms ($t = -4.2$, $p = 0.0002$, $d = -0.86$, 95% CI [-1.5, -0.2]) and improvements in the ability to use cognitive reappraisal emotion regulation strategies ($t = 1.7$, $p = 0.048$, $d = 0.34$, 95% CI [-0.3, 0.97]). Initial analyses revealed effects of sex, particularly for changes in amygdala reactivity (Hedge's $g = -1.32$, 95% CI [-2.5, -0.15]), insomnia symptoms (Hedges $g = -0.69$, 95% CI [-1.71, 0.31]), and cognitive reappraisal abilities (Hedges $g = 0.46$, 95% CI [-0.52, 1.4]). However, these results should be considered with caution given the low sample size and distribution of sex across the sample.

Conclusions: While still preliminary, these findings support the primary aims of the current phase of this mechanistic trial. We found that the emotional regulation brain network exhibited changes in the direction of improvement following CBT-I treatment. Specifically, both amygdala emotional reactivity and connectivity to the pACC and dmPFC were modified relative to baseline. The pattern of reduced amygdala reactivity and increased connectivity to the prefrontal cortex is congruent with normalized top-down cortical control of limbic emotional reactivity. These changes in the brain occurred in parallel to reductions in depression and insomnia symptoms. Taken together, these results support the current phase aims, suggesting that emotional regulation brain network function may be a mechanistic response biomarker of the impact of CBT-I on depression, a hypothesis that will be directly tested in the second phase of the trial.

Keywords: Insomnia, Depression, Functional MRI (fMRI), Mechanistic Clinical Trial, Emotion Regulation

Disclosure: Nothing to disclose.

P249. A Prospective, Multi-Center, Randomized, Controlled, Blinded Trial of Vagus Verve Stimulation for Difficult to Treat Hyper-Resistant Major Depression: The Recover Trial Baseline Unipolar Data

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Background: Numerous studies demonstrate Vagus Nerve Stimulation (VNS) antidepressant efficacy in treatment-resistant major depression (TRD). The US FDA approved VNS for TRD in 2005. In 2007, the US Centers for Medicare and Medicaid Services (CMS) issued a "non-coverage decision," which limited access to VNS for TRD. In 2019, CMS requested a "coverage with evidence trial" to study VNS efficacy in Medicare patients with TRD. The RECOVER Trial, a large, multi-center (84 US sites), randomized, double-blind, sham-controlled trial assesses VNS efficacy over 12 months. What follows is summary data on the 500 randomized patients with unipolar TRD enrolled in the trial (there is also a separately powered bipolar TRD arm to RECOVER).

Methods: RECOVER inclusion criteria: ≥18 years old, Major Depressive Disorder (MDD) or Major Depressive Episode (MDE) current in unipolar/bipolar disorder, chronic (≥2 years) or recurrent (≥4 MDEs), failure of four, medical-record verified antidepressant (AD) treatments in current MDE, and a score of ≥ 22 on the Montgomery Åsberg Depression Rating Scale (MADRS). Exclusion criteria: substance use disorder (past year), lifetime psychosis, acute suicidal ideation/intention, and severe personality disorders. For further quality assurance, a Subject Eligibility Committee (SEC) with psychiatric expertise in TRD (headed by CRC and CK) reviewed medical records provided by the sites to verify subject eligibility. All subjects were implanted and randomized to active treatment (device on) or sham (device off) for 12 months. RECOVER employs offsite blinded raters; the primary outcome is months in response (active vs sham; 50% reduction from MADRS baseline) during the 12-month sham-controlled period. RECOVER secondary outcomes include MDD remission and changes in quality of life, overall function/disability, and suicidal ideation.

Results: The RECOVER trial enrolled highly treatment-refractory MDD patients. To date 500 unipolar patients were randomized, with a median age of 58.0 years (range 19.0-80.0), moderate-severe baseline depressive symptomology (median MADRS of 34.5). Using the Clinical Global Impression (CGI), the sample was judged as moderately ill (CGI = 4; 19.2%) or severely ill CGI ≥ 5; 80.6%). With the Quality of Life Enjoyment and Satisfaction Questionnaire – Short Form (Q-LES-Q-SF), the sample reported a low baseline quality of life (median Q-LES-Q-SF of 34, max score = 70). The median age of MDE onset is 25.0, with a median of 18.7 years in MDE (median of 36% lifetime spent in MDE). The sample has high suicidal ideation (77% positive), with 40% having a history of one or more previous suicide attempts. Median failed lifetime AD treatments is 11.0, with a median of 10.0 failed AD medications. Most (64%) of the sample had received aggressive AD treatments in the current MDE: repetitive transcranial magnetic stimulation (rTMS; 50%), electroconvulsive therapy (ECT; 38%), and ketamine (24%); 35% had received 2 of these three treatments, 7% had received all three. Most (64%) subjects reported previous psychiatric hospitalization(s). To date, 300 patients (including withdrawals) have completed the trial's first year.

Conclusions: RECOVER is the largest double-blind, prospective, randomized, device-based, sham-controlled TRD trial ever conducted. This study is enrolling well-characterized, severely resistant MDD patients who are highly treatment resistant with sustained depression, who, in most cases, have failed numerous pharmacotherapies and more aggressive somatic treatments. It will provide better understanding of VNS responsivity (e.g., onset of response and baseline outcome predictors). Additionally, the study should provide a clearer understanding of the effects of VNS on quality of life, overall function/disability and suicidal ideation. Results of the unipolar arm of trial are expected in the middle of 2024.

Keywords: Treatment Resistant Depression, Vagus Nerve Stimulation, Major Depression Disorder

Disclosure: LivaNova: Consultant (Self).

P250. Repeated Intramuscular Ketamine Injections are Safe and Effective for the Management of Treatment-Resistant Depression and Suicidal Ideation in an Outpatient Clinical Population

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Background: The antidepressant effects of ketamine are well established. To date, intranasal and intravenous routes of administration have received particular attention. However, intramuscular (IM) delivery represents a relatively underexplored and potentially promising route of administration due to its high bioavailability and relatively low cost. This retrospective case series (N = 30) is the first to shed light on the effectiveness and safety of IM ketamine administered as an acute series in patients with treatment-resistant depression (TRD).

Methods: Thirty patients with TRD underwent an acute series of 7 to 9 IM injections of 0.5mg/kg ketamine on a schedule of three injections per week during a 21-day period. Outcome measures included the Patient health Questionnaire 9-item scale (PHQ-9) as well as the single-item suicidal ideation item from the PHQ-9 (PHQ-SI). Outcomes measures were collected prior to each treatment. A linear mixed model with fixed effects of time, random intercept, and random slope was used to analyze the data. Vital signs and adverse events were monitored over the course of the injection treatment series.

Results: IM ketamine was associated with a significant decrease in overall depression symptoms (mean within-subject change [SE] = 6.5 [1.2]; F = 29.3; df = 26.2; 95% CI = 4.1-9.0; $p < 0.001$, Cohen's d = 6.4). Likewise, a significant decrease in suicidal ideation (PHQ-SI) was observed (mean within-subject change [SE] = 0.7 [0.2]; F = 12.4; df = 27.7; 95% CI = 0.3-1.1; $p = 0.002$, Cohen's d = .9). Response (defined as >50% reduction in baseline depression) and remission (PHQ-9 total score</=5) rates were 35.4% and 9.7%, respectively. No adverse events occurred over the course of the injection series.

Conclusions: This small clinical case series provides preliminary support for the effectiveness and safety of IM ketamine in TRD.

Keywords: (R,S)-Ketamine, Depression, Suicide

Disclosure: Nothing to disclose.