



ABSTRACTS COLLECTION



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P1. Longitudinal Characterization of Sex Hormone-Related Cerebellar Gray and White Matter Volumes Measured From Pre-Puberty, Across the Pubertal Transition, and Into Late Adolescence

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Background: The cerebellum has long been classically associated with motor coordination, but there is now an important body of emergent literature documenting not only the rich interconnections of “the little brain” with the cerebral cortex (1), but also its prominent role in high-order functions, including emotion regulation and executive control (2). These behavioral and cognitive processes undergo significant maturation across development, and atypical cerebellar structure and function are implicated in several neuropsychiatric conditions that typically emerge around puberty, such as schizophrenia and major depression (3, 4). Because puberty marks a period of increased sex hormone exposure (5, 6) as well as marked changes in behavior, emotion, and cognition (7, 8), some studies in humans have documented both the emergence of sex differences (9) and sex-hormone related changes (10) in the cerebellum. However, a number of reported findings have not focused on puberty per se and have been complicated by confounding variables (such as age and menstrual cycle status), cross-sectional designs, or temporally sparse sampling of longitudinal data during a period when neurodevelopmental changes may be occurring rapidly. Here, we employed data from the ongoing “NIMH Intramural Longitudinal Study of the Endocrine and Neurobiological Events Accompanying Puberty” to characterize developmental trajectories of cerebellar gray matter and white matter volumes and the ratio between these measures in healthy boys and girls with temporally high density from pre-puberty to age 18. We also explored the impacts of circulating estradiol and testosterone

measured contemporaneously to identify associated patterns of change.

Methods: Typically developing children between the ages of eight (when they were ascertained by clinicians to be pre-pubertal) and 18 years of age were studied every nine months with neuroimaging and hormonal measurements. Fasting morning blood samples to measure estradiol and testosterone serum concentrations and three 3T structural MRI scans (GE MR750 scanner, 1mm isotropic voxels; TE = 1.8ms; TR = 10.5ms) were collected across 584 cumulative longitudinal visits from 130 typically developing children (55 girls). To control for menstrual cycle-related hormonal effects, structural scans from menarchal girls were collected during the follicular phase (days 4–11 of the menstrual cycle) as confirmed by serum progesterone levels < 2 ng/ml. Structural images from each visit were processed through the Freesurfer v7.4 pipeline to yield measures of total brain volume, whole cerebellar volume, cerebellar gray matter volume, and cerebellar white matter volume. Longitudinal mixed-effects spline models (using R’s *gamm4* package) were used to identify sex differences, main effects of age, sex-by-age interactions, and associations of estradiol and testosterone with cerebellar measures across age, controlling for total brain volume.

Results: Consistent with previous findings, there were significant sex differences and sex-by-age interactions for total brain volume and absolute whole cerebellum volume (p ’s < 0.001). When controlling for total brain volume, we found a main effect of sex for cerebellar gray matter volume ($p = 0.0004$), as well as main effects of age (p ’s < 0.001), and sex-by-age interactions (p ’s < 0.03) for bilateral cerebellar white matter, gray matter, and white/gray ratio. Results from mixed-effects spline-modeling revealed several significant developmental relationships between sex hormones and cerebellar volumes. Specifically, for testosterone in boys, significant age-by-testosterone interactions were observed for cerebellar white matter ($p = 0.001$), gray matter ($p = 0.04$), and white/gray matter ratio ($p = 0.007$). For estradiol in girls, significant age-by-estradiol interactions were found only for cerebellar gray matter ($p < 0.001$).

Conclusions: Our longitudinal findings, measured with high temporal sampling, document estradiol- and testosterone-associated structural changes in the cerebellum from

pre-puberty, through the pubertal transition, and beyond. The observation that testosterone interacted with age-related changes for both cerebellar white and gray matter, while estradiol's impact beyond that of age was observed only for the gray matter, suggest that the influence of these gonadal hormones may be tissue-specific in this region. These changes in cerebellar volume related to variations in gonadal hormones during puberty may have important implications for understanding the emergence of sex differences and the increases in vulnerability to neuropsychiatric disorders that are well documented during this period. Future directions include identifying inflection points of change in the longitudinal trajectories and incorporating other important pubertal hormones into the model.

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Keywords: Puberty, 17- β -estradiol, testosterone, cerebellum, Brain development

Disclosure: Nothing to disclose.

P2. Functional Network Topographic Correlates of Positive and Negative Urgency in Male and Female Children From the ABCD Study

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Background: Acquiring the ability to inhibit behavior is a fundamental component of development. Deficits in impulse control occur in a diverse range of psychiatric conditions in youth. The tendency to exhibit impulsive actions during intense emotion, known as urgency, may differ depending on whether the affective context involves positive or negative emotion. Prior work has implicated the salience network (SN) in detecting and processing information related to emotional context and interoceptive awareness. The SN is also posited to mediate switches between executive control and external goal-directed functions of the frontoparietal network (FPN) and diverse functions of the default mode network (DMN), including autobiographical, affective-processing, social cognition, and self-referential processes. There is growing evidence for positive urgency (PU) and negative urgency (NU) as key features of clinically significant behaviors in children and adolescents, and while some prior research has investigated the neural substrates underlying impulse control, relatively few studies have taken into account individual variation in network topography. This study examined associations between surface areas of cortical functional networks and PU and NU in children from the baseline time point of the Adolescent Brain Cognitive Development (ABCD) Study.

Methods: Data from 5970 children (ages 9.0-10.9 years; 51.7% female, 48.3% male) from the ABCD Study were analyzed. Emotion-relevant impulsivity was measured on the self-report

Urgency, Premeditation (lack of), Perseverance (lack of), Sensation Seeking-Positive Urgency (UPPS-P) Impulsive Behavior Scale, using the NU and PU subscales. For functional network surface areas, we generated dense whole-brain correlation matrices using resting state functional magnetic resonance imaging (rs-fMRI) data from the ABCD dataset. For each participant, individual-specific topography of fourteen canonical networks in the cortex and subcortical nuclei were identified using a supervised community detection algorithm (template matching). To control for variation in total cortical surface area between participants, each functional network's area was expressed as a proportion of the individual's total cortical surface area. NU and PU scores, as well as SN, DMN, and FPN proportional areas, were compared between sex groups. As previous work has found sex-related differences in NU and PU, we conducted separate primary and exploratory analyses for male and female groups. Using generalized linear models, we examined relationships between UPPS emotion-relevant impulsivity (NU and PU) and surface areas of resting state networks of interest (SN, DMN, and FPN), covarying for pubertal development and parent education level (which correlated with NU and PU), with separate models for male and female children. In exploratory analyses, we also examined relationships between UPPS-P NU and PU scores and SN/FPN and SN/DMN surface area ratios, due to observed differences in DMN and FPN areas between male and female groups in our sample.

Results: NU and PU were higher in male children than female children. Pubertal development was more advanced in female than in male participants. The proportion of cortical surface occupied by the DMN was greater in female children, whereas the FPN proportion was greater in males; SN proportion did not differ between sexes. Among male children, there were no significant relationships between SN, DMN, or FPN surface areas and NU, or between SN, DMN, or FPN surface areas and PU. However, in female children, SN surface area was associated with NU ($\beta = 5.308$, $p = .016$, $\text{padj} = .048$) and with PU ($\beta = 7.167$, $p = .003$, $\text{padj} = .009$). DMN and FPN surface areas did not have significant relationships with either NU or PU in female children. In exploratory analyses, SN/FPN was significantly related with NU in male ($\beta = 0.288$, $p = .01$) and female ($\beta = 0.258$, $p = .006$) children. SN/FPN also was also significantly related with PU in male ($\beta = 0.318$, $p = .005$) and female ($\beta = 0.316$, $p = .006$) children. In female children, SN/DMN was significantly related to NU ($\beta = 0.377$, $p = .018$) and PU ($\beta = 0.464$, $p = .003$), whereas in male children SN/DMN did not have significant relationships with NU or PU.

Conclusions: The cortical surface area allocated to the SN was associated with NU and PU in female children, but not male children. Due to differences in relative proportions of DMN and FPN areas between sexes, SN/DMN and SN/FPN ratios may provide additional insight into the relationship between allocation of cortical resources among these networks and observed sex differences in emotion-relevant impulse control. Earlier pubertal onset in female youth may influence observed differences between sexes in NU and PU, but understanding its role in functional network development requires further research. Future analyses from longitudinal observation of functional network topography will yield a more comprehensive understanding of developmental trajectories of neural mechanisms underlying the acquisition of impulse control.

Keywords: Impulsive behavior, Urgency, Salience network, Resting-state fMRI, Children

Disclosure: Nothing to disclose.

P3. Sex-Specific Impact of Peripubertal Stressors on the Activity of the Thalamic Reuniens Nucleus in Rats

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Background: Stress is a socio-environmental risk factor for psychiatric disorders, with the age of exposure potentially determining the outcome. Early life adversity significantly impacts the development of neuropathological states, with males and females having different vulnerability periods. Male rats are sensitive to stress during peripuberty (PeriP; Postnatal day, PD31-40) and females to postpuberty (PostP; PD41-50), inducing adult hyperdopaminergic states and cognitive deficits. Male susceptibility is linked to increased basolateral amygdala activity, not seen in females. The precise neurobiological mechanisms contributing to sex differences in stress response are understudied. Here, we investigate if the nucleus reuniens of the thalamus (RE) and thalamic reticular nucleus (TRN) are involved in this disparity, as they are crucial for dopaminergic activity and cognitive regulation. TRN has extensive expression of parvalbumin interneurons (PV) and their perineuronal nets (PNN), an extracellular matrix implicated in the feedforward inhibition of thalamic relay neurons. We examined the impact of stress exposure during PeriP and PostP on adult RE activity, TRN gamma oscillatory activity, 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. 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 1-2 weeks and 5-6 weeks of stress (1-2 weeks: $n = 6-7$; $U = 14.84$, $p < 0.05$, Kruskal-Wallis; 5-6 weeks: $n = 9-13$; Sex and Stress interaction, $F_{1,36} = 16.74$, $p < 0.05$, Two-way ANOVA). PeriP stress did not affect the number of active RE neurons in males and females after 1-2 weeks and 5-6 weeks (1-2 weeks, $n = 6-8$, $p > 0.05$; 5-6 weeks, $n = 8$ all groups, $p > 0.05$; Kruskal-Wallis). Sex and age differences were observed in both experiments with males having more spontaneously active RE neurons at baseline than females at PD75-64, PD75-82, and PD85-92 (Sex, $F_{1,56} = 45.61$, $p < 0.05$; Age, $F_{3,56} = 17.92$, $p < 0.05$; Two-Way ANOVA). At PD47-54, male and female rats had a similar number of RE neurons per track with females at this age having more neurons per track than females from PD75-64, PD75-82, and PD85-92. The firing rate and % of spikes in burst were not affected by PostP stress at any time-point ($p > 0.05$; Kruskal-Wallis). PeriP stress increased the % of spikes in bursts in male and female rats after 1-2 weeks ($U = 16.49$, $p < 0.05$, Kruskal-Wallis) and only in female rats after 5-6 weeks ($U = 13.64$, $p < 0.05$, Kruskal-Wallis). The firing rate was not affected by PeriP stress after 1-2 weeks, but it was increased in stressed females after 5-6 weeks ($U = 13.49$, $p < 0.05$, Kruskal-Wallis). For local field potential, PeriP stress (Male/females; PD31, $n = 6$; PD41: $n = 6-7$; PD51: $n = 6-8$, PD75: $n = 6-10$) and PostP stress (Male/females; PD41, $n = 6-7$; PD51: $n = 6-7$; PD61: $n = 6-8$, PD85: $n = 7-8$) did not affect low and high gamma power (mV2) at all time points. For the PeriP experiment, an age and sex effect were observed in naïve rats with females at PD31 having a higher low and high gamma power in the TRN than females at PD51; and females at PD31 having a higher low gamma power than males at the same age (Low gamma, sex-age interaction, $F_{3,44} = 6.6$, $p < 0.05$; High gamma, $F_{3,44} = 4.70$, $p < 0.05$; Two-way ANOVA). For the PostP experiment, an age effect was found with male naïve rats at PD61 having a higher high gamma power than

naïve females at the same age and naïve males at PD41 and 51 in the TRN (Age, $F_{3,44} = 3.68$, $p < 0.05$; Two-way ANOVA). For PV/PNN staining, PostP stress decreased the number of PV interneurons in the TRN at PD61 in females (male, $n = 2-4$; female, $n = 5-9$ naïve; sex-stress interaction, $F_{1,16} = 7.53$, $p < 0.05$, Two-way ANOVA). Females were found to have more PV interneurons than males (sex, $F_{1,16} = 5.94$, $p < 0.05$, 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 mainly by RE. PostP stress increased the RE activity short- and long-term 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 may lead to disruption in the inhibitory drive to RE, driving increased RE activity. TRN gamma oscillations were not affected by either PeriP or PostP stress which suggests that these transmissions do not underlie male and female vulnerability to stress. Therefore, our data suggest that TRN and RE seem to be involved in the sex-dependent neurobiological disrupted circuit underlying early-life stress vulnerability, especially in females.

Keywords: Adolescence, nucleus reuniens, Early life stress

Disclosure: Nothing to disclose.

P4. Graph Theoretical Analysis of Reward Network Properties in Adolescent Depression and Cannabis Use

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Background: Cannabis is the most used illicit substance among teenagers. Over the past decade, adolescent cannabis use rates have accelerated while perception of harm related to cannabis use has declined. Convergent data suggest that adolescent exposure to exogenous cannabinoids, particularly the psychoactive agent Δ^9 -tetrahydrocannabinol (THC), results in long-lasting alterations of the reward system and depressive-like behaviors in rodents. Further, human neuroimaging studies have showed structural and functional alterations across the reward circuitry in cannabis-using adolescents. However, evidence for reward dysfunction in the context of concurrent adolescent depression and cannabis use remains elusive. Building upon prior research and addressing this knowledge gap, we aimed to investigate properties of reward networks related to adolescent cannabis use and depression through a graph theoretical analysis of resting-state functional magnetic resonance imaging (fMRI) data.

Methods: Participants consisted of adolescents with diverse clinical symptomatology recruited from the New York metropolitan area. Each adolescent and their accompanying parent separately underwent semi-structured interviews with a trained clinician. Depression was assessed dimensionally with the clinician-rated Children Depression Rating Scale-Revised (CDRS-R) and the self-reported Beck Depression Inventory (BDI). Cannabis use was determined from clinician interviews, self-reports, and urine toxicology screens prior to neuroimaging scans. Each adolescent's cannabis use pattern was coded to be one of the following: never used, tried once, low use, moderate use, heavy use. Those who have never used or only tried once were considered non-users. MRI was performed on a 3T Siemens Skyra using protocols similar to those of the Human Connectome Project (HCP) Lifespan studies, including anatomical T1w MPRAGE and T2w SPACE (0.9mm isotropic), as well as resting-state fMRI (2.3mm isotropic, TR=1s, 600 volumes, 5x multiband). Participants additionally completed a reward fMRI task to localize reward networks. Preprocessing followed the HCP Pipelines, including ICA-FIX

denoising and MSMAll surface alignment. Resting state data was additionally processed with CompCor denoising, 24 movement parameter regression, and bandpass filtering (0.1–0.01Hz), as well as parcellated via the Cole-Anticevic Brain-wide Network Partition. Reward network masks, constructed from 10% of nodes most activated by Reward Anticipation, Reward Attainment, and Reward Prediction Error contrasts as well as any corresponding contralateral nodes, were applied to parcellated resting state data. The Brain Connectivity Toolbox in MATLAB was utilized to estimate three subject-level weighted graph theoretical metrics—Strength Centrality (CStr), Eigenvector Centrality (CEig), and Local Efficiency (ELoc)—within each reward network. Non-parametric group-level analyses were conducted in FSL PALM with 10,000 permutations and corrected for familywise error rate (FWE) and over multiple contrasts. Regressors in group analyses were demeaned. All results were thresholded at $p\text{FWE} < 0.05$.

Results: For this interim analysis, we included available data from 122 adolescents (age: 15.0 ± 2.2 years; 64.5% female). All findings were adjusted for depression severity (CDRS-R); findings that remained significant when additionally adjusting for age and sex are marked with an asterisk. Relative to non-users, cannabis users ($n = 23$) exhibited various alterations across reward networks. Specifically, cannabis users showed: weaker Reward Anticipation CEig of the right thalamus ($t = -3.84$, $p\text{FWE} = 0.018$); stronger Reward Attainment CStr of the right dorsolateral prefrontal cortex (dlPFC; $t = 3.63$, $p\text{FWE} = 0.035$) and left inferior parietal cortex (IPF; $t = 3.68$, $p\text{FWE} = 0.032$); stronger Reward Attainment ELoc of the right dlPFC ($t = 3.70$, $p\text{FWE} = 0.026$) and right IPF ($t = 3.60$, $p\text{FWE} = 0.035$); stronger Reward Prediction Error CStr of the right IPF ($t = 3.48$, $p\text{FWE} = 0.042$) and left dlPFC ($t = 3.50$, $p\text{FWE} = 0.041$); and stronger Reward Prediction Error ELoc of both the right ($t = 4.51$, $p\text{FWE} = 0.0015^*$) and left ($t = 3.70$, $p\text{FWE} = 0.021$) medial superior temporal cortex (MST). When assessed dimensionally, heavier cannabis use was associated with weaker Reward Anticipation CEig of the right thalamus ($t = -4.27$, $p\text{FWE} = 0.0052^*$) but with stronger Reward Prediction Error ELoc of the right MST ($t = 3.72$, $p\text{FWE} = 0.022$). Results were similar when depression severity was indexed by BDI. Interestingly, there was a significant interaction effect between cannabis use pattern and depression severity (CDRS-R) on Reward Prediction Error ELoc of the left thalamus ($t = 3.48$, $p\text{FWE} = 0.0496^*$).

Conclusions: Our findings indicate differential resting-state network properties across reward nodes in adolescent cannabis use spanning a spectrum of depression severity as well as cannabis use frequency. Notably, analyses across a range of reward networks and graph theoretical measures consistently implicated the dlPFC, IPF, MST, and thalamus, suggesting these regions may be particularly vulnerable to the effects of cannabis use in youth. Taken together, our results further support the possible compounding effect of cannabis use and depression on the developing reward system. As the study is on-going, future work will include additional analyses and data from an expanded sample of cannabis users.

Keywords: Adolescence, Depression, Cannabis use, Resting and task fMRI, Reward network

Disclosure: Nothing to disclose.

P5. Altered Maturation of Posterior Brain Regions With Shared Transcriptomic Patterning Predicts Subsequent Psychopathology in Youth

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Background: Maturation of the cerebral cortex during adolescence is temporally associated with emergence of a range of psychopathology. However, inferring causal relationships between cortical maturation and psychopathology risk has been complicated by numerous factors. These include an incomplete understanding of normative age-related cortical thinning; inadequate sample size for detecting meaningful, longitudinal associations of brain structure and psychopathology; and lack of triangulation with underlying biological mechanisms. We sought to overcome these obstacles using longitudinal data from the ongoing Adolescent Brain Cognitive Development (ABCD) Study, which provides an unprecedented opportunity to study brain development and emergent psychopathology prospectively, and on a population level.

Methods: ABCD is a 10-year, longitudinal study of adolescent brain development that enrolled 11,875 boys and girls at age 9 or 10 across 21 U.S. sites. Cortical thickness was extracted from individual brain volumes using Freesurfer v7.1, following extensive quality control measures (Elyounssi et al., BioRxiv, 2023). Analyses were conducted using linear mixed models that included biological (age, age², sex, puberty stage), technical (intracranial volume, surface hole number), and study design-related (site, scanner, subject and family ID) factors and controlled for multiple comparisons across 68 cortical regions with FDR. Using linear mixed models we leveraged baseline (BL), Year 2 (Y2), and Year 4 (Y4) clinical and structural MRI data to address three related questions. First, we determined whether linear or quadratic effects of age best accounted for longitudinal changes in cortical thickness among the 2,200 participants who had complete, high quality MRI data across three time points (BL, Y2, and Y4) as of Data Release 5.1. Next, in the larger sample ($n = 10,209$, including data from any timepoint) we compared three competing models that temporally related cortical thickness to a broad index of dimensional psychopathology (Child Behavior Checklist total t-score), using thickness as a predictor of prior, concurrent, or future CBCL scores. Finally, we determined whether regional loading for each of three temporospatially patterned gene co-expression programs, derived from Allen Human Brain Atlas transcriptomic data (Dear et al., Nat Neurosci, 2024), co-segregated with regions where differences in cortical thickness were associated with psychopathology.

Results: Using the sample with data from each time point (ages 9–10, 11–12, 13–14), we observed significantly stronger quadratic effects of age on thickness, compared to linear effects, across most of the cortical mantle (45 of 68 regions, $p < .05$, FDR). Posterior and medial regions, especially medial occipital cortex, showed decelerations of thinning with age, while anterior and lateral regions showed accelerated thinning with age. In the larger cohort, 11 regions demonstrated significant ($p < .05$, FDR) associations between thickness and future CBCL scores (i.e., BL and Y2 cortical thickness predicted Y2 and Y4 symptoms, respectively), but there were no significant relationships between thickness and concurrent or prior CBCL scores. Each of these significant regions was posterior to the central sulcus, and most (including medial occipital regions, which had the strongest predictive relationships, $d = 0.05$) had also demonstrated decelerations in thinning with age. Among the three previously described gene networks defined by their spatial and temporal co-expression in postmortem brain, only the “C1” network, which is mainly expressed in posterior and medial regions (and mainly during perinatal life) demonstrated significant spatial overlap with regions that predicted future psychopathology ($R = .65$, $p = .0003$).

Conclusions: These results provide an updated neurodevelopmental framework for understanding the relationships among cortical maturation, emergent psychopathology, and transcriptomic mechanisms. They augment previous findings of posterior-anterior and medial-lateral gradients of age-related thinning by

revealing the non-linearity of these patterns, and their associated early and late infection points. Causal relationships are supported by the observation that variation in late thinning associated with future, rather than prior or current psychopathology, and that predictive regions track with previously identified spatially and temporally patterned gene co-expression networks. Importantly, these relationships were detectable in some of the earliest-maturing cortical regions, suggesting the possibility that MRI data from early adolescence could be harnessed for early risk detection and intervention. Confirmation and extension of these findings will become possible in future years as the ABCD cohort matures into late adolescence.

Keywords: ABCD study, Cortical maturation, Structural MRI, Dimensional child psychopathology, Brain transcriptomics

Disclosure: Nothing to disclose.

P6. Subtypes in Late Childhood and Their Relation to Psychopathology and Substance-Use Initiation During Early Adolescence: An ABCD Study

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Background: The prevalence of severe psychiatric symptoms in children has steadily increased over the past decade, with 75% of mental illnesses emerging before age 25. To mitigate their detrimental effects on adolescent development and their long-term consequences into adulthood, effective early detection, prevention, and intervention strategies are crucial. To develop such strategies, understanding the complex functional mechanisms that contribute to psychiatric vulnerability during late childhood and adolescence—and how these mechanisms and related behaviors evolve over time—is critical. This research utilizes data from the Adolescent Brain Cognitive Development (ABCD) Study to explore whether distinct subtypes with unique functional profiles and developmental trajectories can be identified. Identifying these subtypes could provide valuable insights into the heterogeneous mechanisms and pathways driving mental health risks and development.

Methods: We analyzed complete baseline data from 9,586 children (48% female, mean age = 9.9 years) from the ABCD Study. Using exploratory factor analysis (EFA), we extracted latent factors from 42 variables spanning psychological, cognitive, and health variables across multiple domains of functioning. Latent profile analysis (LPA) was then used to uncover data-driven subtypes based on the extracted latent factors. To ensure replicability and generalizability, we performed random split-half validation, determining the optimal subtype solution in the training data ($N = 4,793$), applying it to the testing data ($N = 4,793$), and extending it to the full sample. To characterize and clinically validate the identified subtypes, we used Welch's ANOVA and chi-square tests to compare subtype profiles on measures not included in the subtype formation, examining associations with psychopathology, substance use, and socio-environmental factors at ages 9-10 and across multiple yearly follow-ups.

Results: The EFA revealed twelve latent factors ($RMSEA = 0.034$, $TLI = 0.924$, $CFI = 0.967$) that covered three functional domains: approach behavior, negative emotionality and executive function. The LPA generated four replicable subtypes with distinct functional profiles. The first identified subtype, a 'High Negative Emotionality' type ($N = 923$, 38% female), was characterized by elevated general psychiatric symptoms, externalizing behaviors, poor planning abilities, higher prevalence of DSM diagnoses, low pro-social involvement, and stricter parental drinking rules. Across follow-ups during early adolescence, individuals demonstrated the

highest tobacco and marijuana experimentation and exhibited a significantly greater prevalence of all DSM diagnoses assessed. The second identified type, a 'High Approach' subtype ($N = 1,967$, 42% female), was marked by heightened sensation seeking, urgency, and reward motivation, with the highest peer-network health and stringent parental substance and drinking rules. Across follow-ups, individuals demonstrated the lowest levels of alcohol use but slightly elevated tobacco and marijuana experimentation, along with a moderately higher proportion of diagnosed Externalizing, Obsessive-Compulsive, and Neurodevelopmental disorders. The third recovered subtype, 'Low Approach' ($N = 2,369$, 53% female), exhibited low motivation for positive behaviors, poorer peer-relationship quality, lenient parental substance use rules and positive alcohol expectancies. Across follow-ups during early adolescence, this group showed significantly higher alcohol use and experimentation, along with a moderately increased proportion of diagnosed Externalizing and Neurodevelopmental disorders. The fourth identified subtype, the 'Average' type ($N = 4,327$, 50% female), demonstrated average functioning across all measured factors, with validation metrics indicating the lowest levels of alcohol and substance use experimentation and overall DSM diagnoses across follow-ups.

Conclusions: The identified data-driven subtypes reveal substantial heterogeneity in functional profiles during late childhood that were linked to distinct developmental trajectories into early adolescence. These results may inform the identification of early risk factors and guide the development of targeted prevention and intervention strategies. Future studies will determine the mental health risks associated with these trajectories in late adolescence and early adulthood.

Keywords: Subtyping, Early Identification of Risk, ABCD Study, Developmental Psychopathology, Substance Use Initiation

Disclosure: Nothing to disclose.

P7. Peripubertal Antagonism of Corticotropin-Releasing Factor Receptor 1 Results in Sustained, Sex-Specific Changes in Behavioral Plasticity and the Transcriptomic Profile of the Amygdala

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Background: Peripuberty represents a critical phase in neurodevelopment, characterized by substantial changes that have enduring impacts on both brain function and behavior. Research has shown that inhibiting type 1 corticotropin-releasing factor receptors (CRFR1) in neonatal and peripubertal rats can mitigate the harmful consequences of early-life stress. This intervention improves neural plasticity, behavior, and stress hormone regulation, with benefits persisting long after the cessation of the treatment. Interestingly, the antagonism of CRFR1 does not only ameliorate the effects of stress but also influences neural and behavioral development under non-stressful conditions. This suggests that CRFR1 plays a fundamental role in shaping the brain's developmental trajectory, affecting neural processes and behavior in a lasting manner.

Methods: To investigate this further, we acutely administered the CRFR1 antagonist R121919 (CRFR1a; 10mg/kg, SQ) or saline (vehicle control), without an accompanying stressor, to peripubertal male and female Sprague Dawley rats across a four-day period. One hour following each treatment, rats were tested for locomotion, social behavior, mechanical allodynia, or prepulse inhibition (PPI) of the acoustic startle reflex ($n = 8/\text{group}$). In adulthood, two months after the last CRFR1a dose, the behavioral

tests were repeated without CRFR1a treatment ($n = 8/\text{group}$), and amygdala samples collected and evaluated by RNA sequencing ($n = 3/\text{group}$). Additionally, cannabinoid type 1 receptor (CB1R) expression in the amygdala, associated with impaired sensorimotor gating, altered HPA responses, and anxiety, was quantified ($n = 8/\text{group}$).

Results: Systemic blockade of CRFR1 immediately reduced % PPI in peripubertal males (77 dB: $F(1, 14) = 10.174$, $p = 0.007$, $\eta^2 = 0.439$; 81 dB: $F(1, 14) = 8.080$, $p = 0.014$, $\eta^2 = 0.383$, versus Saline), but not females ($p > 0.05$). CRFR1a exposed male rats continued to experience PPI deficits while in females, the PPI disruptions only manifested in adulthood (Males (81 dB): $F(1, 14) = 5.510$, $p = 0.034$, $\eta^2 = 0.282$; Females (73 dB): $F(1, 14) = 7.028$, $p = 0.019$, $\eta^2 = 0.334$, versus Saline). Additionally, when CRFR1a exposed females reached adulthood, altered locomotion and social behavioral symptoms also emerged (distance traveled: $F(1, 14) = 10.675$, $p = 0.006$, $\eta^2 = 0.443$; visits to a novel social conspecific: $F(1, 14) = 4.173$, $p = 0.048$, $\eta^2 = 0.252$; visits to a novel object: $p > 0.05$, versus Saline). In the adult male amygdala, peripubertal CRFR1a induced alterations in pathways related to neural plasticity and stress. In males, 219 genes were significantly DE by CRFR1a ($q < 0.05$ versus Saline), and 31 genes were significantly DE by CRFR1a exposure in females ($q < 0.05$ versus Saline). Threshold-free RRHO analysis showed substantial overlap in gene expression profiles across males and females as a function of CRFR1a exposure. Moreover, peripubertal CRFR1a exposure reduced CB1R protein expression in females ($F(1, 14) = 7.981$, $p = 0.022$, $\eta^2 = 0.499$).

Conclusions: Understanding the sustained impacts of acute neuropharmacological exposure on brain function and behavior, even without further doses, is crucial for developing effective adolescent psychiatric treatment protocols. This knowledge can inform clinicians about the potential long-term effects of brief treatments during adolescence, helping to optimize therapeutic strategies and minimize adverse outcomes.

Keywords: Adolescence, Type 1 corticotropin releasing factor receptor, Neuroplasticity, Sex Differences

Disclosure: Novartis: Employee (Spouse/Partner)

P8. A Longitudinal, Prospective Study of Head Impacts Induced Structural Brain Changes in Male High School Football Players

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Background: Repetitive, subconcussive impacts can negatively affect the brain and cognitive function during sensitive developmental stages. Preventing the neurocognitive consequences of concussions in high school football players is an important public health priority. Diffusion tensor imaging (DTI) is an MRI technique that quantitatively characterizes the brain's microstructures. Prior DTI studies have demonstrated that sub-concussive head impacts can result in sub-clinical brain injury. While mild traumatic brain injuries are extensively studied in adults, few studies have been conducted in children or adolescents, whose brains are thought to be more vulnerable to head injury. Advances in technology now allow detailed analysis of the frequency and intensity of repetitive head impacts in contact sports. Helmet-based sensors characterize the kinematic features of concussive impacts and typical head-impact exposure profiles for athletes. This longitudinal study aims to assess the effects of repeated mild head impacts on brain structure, function, cognition, and behavior in high school football players.

Methods: We conducted a four-year longitudinal study involving 53 adolescent football players from Crescenta Valley High School (CVHS) and Los Angeles club sports, monitored with the Sideline Response System (SRS) Helmet Sensor during practices and games to document head accelerative and concussive events. Thirty matched controls, including non-contact athletes and sedentary individuals, were also included. Participants underwent neuropsychological testing and MRI scans, including DTI, at baseline, annually, and after suspected concussions. We correlated MRI measures with head impacts, concussion severity, and cognitive performance, comparing contact athletes to controls. MRI scans and neuropsychological assessments were acquired pre-season, post-concussion, and annually to evaluate cognitive and behavioral impacts of mild traumatic brain injury (TBI). Videography verified head impact events. Data were analyzed using a general linear model at each voxel of spatially normalized FA or ADC maps, controlling for age at the time of the scan. Multiple comparisons were controlled using the False Discovery Rate (FDR), and significant findings were displayed on a template brain.

Results: The number of head impacts over time significantly correlated with lower fractional anisotropy (FA) and widespread increases in apparent diffusion coefficient (ADC) in deep white matter fibers such as the internal capsule and inferior longitudinal fasciculus, as well as in long white matter fibers like the superior longitudinal fasciculus in football players. Additionally, football players showed reduced FA in the deep white matter fibers of the internal and external capsule, along with increased FA and reduced ADC in cortical and subcortical gray matter regions over the study period. Compared to controls, FA reduced significantly in deep white matter fibers and ADC increased in subcortical gray matter regions, such as the thalamus, in football players over time.

Conclusions: Repetitive head injuries without clinically evident concussions resulted in white matter changes that correlated with multiple helmet impact measures, persisting over four years. This lack of white matter recovery could potentially contribute to cumulative white matter changes with subsequent head injury exposures. Reduced FA and increased ADC in white matter structures are likely due to axonal injury and subsequent hypomyelination. Increased FA and ADC in gray matter might be attributed to neuronal injury and impaired dendritic arborization.

Keywords: brain imaging, Diffusion Tensor Imaging (DTI), concussion

Disclosure: Nothing to disclose.

P9. Adolescent Social Media Use Disrupts Sleep

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Background: Mental health in youth has been declining rapidly in the past decade, highlighting the need for actionable targets for intervention and prevention. Nearly all US adolescents use at least one social media platform, and approximately 30% report engaging with social media "almost constantly". Our limited understanding of the pathways by which social media use may increase mental health problems hinders our ability to effectively mitigate risk where it is highest. Recent work identifies the critical role sleep plays in emotional regulation and mental health. The goal of the present study was to test the hypothesis that the negative effects of social media use on mental health are mediated through sleep disruption.

Methods: Recruitment is ongoing. So far 32 adolescents (mean age: 14.7 ± 1.7 , 20F, 12M) completed a 14-day assessment that included wrist actigraphy for accurate and non-invasive measurement of sleep and wake habits, smart-phone app based ecological momentary assessments of daily mood and affect, and an in-lab clinical interviews and symptom ratings. Daily Social Media Use (SMU) durations were recorded utilizing the Apple Screen Time app (mean: 125 min/day, 0-358 mins/day). Daily screen time duration on smart phones were tracked with the Avicenna/Ethica smart phone app.

Results: Participants spent an average of 126 mins interacting with social media every day, with youth in higher stages of pubertal development spending more time on their phones ($r = .70$, $p = .008$). SMU duration predicted night-to-night variability in sleep duration ($r = .79$, $p < .001$) and sleep efficiency ($r = .58$, $p = .03$), such that participants who spent more time interacting with social media, in turn, had disrupted and less regular sleep. Severity of internalizing symptoms was significantly associated with objective metrics of sleep disruption ($r = .66$, $p = .007$) corresponding to heightened night-to-night variability in sleep fragmentation in youth with more severe mood symptoms. While our preliminary analyses do not reveal any relations between SMU duration and clinical symptoms, screen time correlated with the severity of mood symptoms ($r = .68$, $p = .01$), reflecting increased daily phone use in adolescents with heightened depression and anxiety symptoms

Conclusions: Our preliminary analyses are in line with previous literature that reveals strong links between objective and subjective measures of sleep quality and mood symptoms in adolescents. We also observed that prolonged social media use strongly and directly predicts sleep disruption but not mental health problems. This reflects that the social media environment is complex and dynamic, with features that are likely both beneficial and harmful for adolescent mental health. Our findings reveal that not only does SMU disrupt sleep among adolescents, but also that those with heightened clinical symptoms are more vulnerable to sleep disruption, highlighting the need for fine-grained, multi-dimensional and objective measurement of SMU. Data collection is ongoing and future analytical plans include elucidating the effects of day-to-day differences in SMU on night-to-night variability in sleep.

Keywords: Social Media Use, Adolescence, Sleep disturbances, ecological momentary assessment

Disclosure: Nothing to disclose.

P10. Do Psychotic Symptoms Mediate the Relationship Between Chronotype and Academic Performance 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 depression and psychosis. Previous studies have explored the relationship between academic performance, chronotype, and depression but not psychosis 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. The relationship between academic performance, chronotype, and psychosis has not been extensively examined in large, diverse samples like the Adolescent Brain Cognitive Development (ABCD) study. We hypothesized that in the ABCD cohort, that later chronotypes and greater social jetlag would predict poorer academic performance and would be mediated by symptoms of psychosis.

Methods: Year 2 (ages 11-14) and Year 3 (ages 12-15) longitudinal data from the ABCD cohort ($n = 5822$ adolescents) was used to evaluate whether academic performance as a function of chronotype was mediated by psychosis symptoms. 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. A two timepoint (Years 2 and 3) mediation model was employed to examine this hypothesized relationship, while controlling for relevant covariates (age, household income, sex, ethnicity).

Results: Using Year 2 and Year 3 longitudinal data from the ABCD cohort, we found that while worse academic performance was predicted by later chronotype and associated with psychosis symptoms, these psychosis symptoms did not mediate the relationship between academic performance and chronotype at a statistically significant level. Mediation analysis will be repeated within the next 3 months once the second half of the Year 3 ABCD data is released that should effectively double the sample size.

Conclusions: Our study is the first to look at whether academic performance predicted by chronotype is mediated by psychosis in the ABCD sample. Our early findings will require further clarification by repeating this mediation upon release of the second half of Year 3 ABCD data. In addition, we hope to add a third timepoint (Year 4) as a future next step in understanding whether psychosis symptoms mediate the relationship between academic performance and chronotype.

Keywords: chronotype, academic performance, adolescence, ABCD, psychosis

Disclosure: Nothing to disclose.

P11. Severity of Childhood Adversity and Blunted Afternoon Cortisol Levels in Adults

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Background: Advances in stress biology support the mediational role of stress-induced dysregulations in the hypothalamic-pituitary-adrenal axis (HPA) on chronic disease outcomes, but the literature has been mixed on whether adversities result in overactive or blunted HPA activity. Heterogeneity in the latter may be due to several factors, including age of onset of adversities, severity of adversities, and circadian variation in the secretion of cortisol, among other things. In this prospective study, we collected salivary cortisol levels at different time points to examine the association between severity of childhood adverse exposures and cortisol secretion.

Methods: This cross-sectional analysis used baseline data from a two-year prospective study of the effects of social adversities in 93 adults between the ages of 18 and 45 years, living in the Washington, DC metropolitan region. Each participant received serial collections of salivary cortisol samples at 8:00 AM, noon and 4:00 PM. All participants completed psychological assessments, including Adverse Childhood Event (ACE) questionnaires,

Childhood Life Exposure Scale (CLES) and semi-formal psychiatric diagnostic interview. The exposure is the total number of childhood adverse events, and the outcome of interest is salivary cortisol levels in $\mu\text{g/dl}$. The research protocol was approved by the Howard University Institutional Review Board.

Results: Adjusting for differences in age, sex and other demographic variables, mean total ACE score was significantly associated with 0.047 (95%CI, 0.074 – 0.014 units reduction ($P = 0.005$)) in 4 PM cortisol levels ($P = 0.005$). Similarly, adjusting for same demographic variables, mean total CLES scores were significantly associated with 0.082 (95%CI, 0.146–0.018) units' reduction in 4PM cortisol levels ($P = 0.013$). For the 12 PM cortisol collections, total CLES scores were significantly associated with 0.143 (95%CI, 0.242 – 0.044) units' reduction in cortisol levels ($P = 0.005$), whereas mean total ACE scores were modestly associated with 0.051 units reduction in cortisol levels ($P = 0.094$). There were no significant associations between total childhood adverse events and 8 AM cortisol levels.

Conclusions: These results provide evidence for blunting of afternoon cortisol levels in accordance with severity of adversities experienced during childhood in a population of mostly young adults. Absence of significant associations between levels of adverse exposures and morning cortisol levels, and variable strengths of associations using different scales of childhood adversities may explain some of the heterogeneity in previous studies.

Keywords: Childhood Adversity, Cortisol, Cortisol response to stress

Disclosure: Nothing to disclose.

P12. Aggressive Neighborhood Policing is Associated With Worse Reading Among Black Youth in New York City

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Background: From unjustified traffic stops to the use of deadly force, the police disproportionately criminalize and harm Black people, increasing risk for physical and mental health conditions across the lifespan. Accumulating evidence demonstrates that residing in an aggressively policed community is also associated with adverse outcomes, such as higher risk of preterm birth among Black birthing people and worse English Language Arts academic performance among Black boys. Given these findings and the known deleterious effects of threat on learning, the current study seeks to examine whether residential proximity to aggressive policing is associated with worsened measures of reading acquisition along lines of race.

Methods: This study included 3139 youth from the Healthy Brain Network, a community-referred sample across five sites in New York City. Recruitment aimed to include a high proportion of participants with socioemotional and neurodevelopmental concerns, a demographic that is overrepresented in the carceral system. The ethnoracial composition of the sample was as follows: 1576 white, 458 Black, 327 Hispanic/Latinx, 542 multiracial, and 236 other. The mean age was 9 years. Data on all police stops and arrests in NYC from 2013 through 2022 were downloaded from the New York Police Department website and geocoded to census tracts. We calculated the total number of police stops that occurred in each participant's census tract in the year prior to their HBN evaluation, providing a spatiotemporally specific exposure variable. A rough measure of neighborhood violence was obtained by the same approach using data on arrests for assaults. Covarying for prior year neighborhood violent crime allows the

stops variable to be interpreted as a measure of aggressive policing. Outcomes included two measures of reading acquisition from the Wechsler Individual Achievement Test-III (WIAT-3): word reading (measure of orthographic knowledge) and pseudoword decoding (measure of phonological decoding). Robust logistic regression models were fit featuring the interaction between participant race and prior year neighborhood police stops on "Below Average" reading performance classification based on standard cutoffs: standard score of 90 (i.e., 25th percentile). Other potential covariates included age, sex assigned at birth, household socioeconomic status, child psychiatric diagnosis, and study site. The significance of the interaction was ascertained using a quasi-deviance test; a p-value below 0.05 prompted examination of the effects of police stops on reading within ethnoracial groups.

Results: Nearly 3 million arrests and > 300,000 police stops occurred in NYC during the study period. The interaction between race and police stops (controlling for neighborhood violent crime) significantly contributed to prediction of WIAT-3 word reading ($p < .01$), but not pseudoword decoding ($p > .05$). Specifically, living in a neighborhood with more prior-year police stops was associated with a higher likelihood of Below Average word reading performance for Black youth ($p < .001$) and not for other youth (p 's > .05). This association was robust to multiple model reconfigurations including: changing the policing variable from total stops to total frisks or total encounters involving police force; including only encounters involving Black civilians; controlling for psychiatric and neurodevelopmental comorbidities. Furthermore, when the policing variable was the number of stops and arrests in the year after the assessment, the interaction was not significant ($p > .05$), establishing temporal plausibility and supporting causal inference. Exploratory analyses showed similar results with spelling ($p < .01$).

Conclusions: Proximity to aggressive policing, operationalized as police stops in excess of neighborhood violence, was associated with worse reading and spelling among Black youth in a neurodiverse sample of New York City youth. Accumulating evidence documents that racist, aggressive policing not only directly harms people but also exerts deleterious spillover effects on entire communities. Our study extends this line of research, underscoring the neurodevelopmental harm of policing. Given the cascading effects of early academic difficulties, diminishing aggressive policing would likely benefit the wellbeing of Black people across the lifespan.

Keywords: neurodevelopment, public health, neighborhood resources, racial discrimination, reading

Disclosure: Nothing to disclose.

P13. Olfactory Bulb Volume as a Marker of PTSD Resilience and Vulnerability in Childhood Trauma-Exposed Individuals

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Background: There is growing evidence that olfactory brain structures play important roles in stress biology. Studies in animal models reveal that surgical removal of the olfactory bulbs (OB) lead to increased startle response and autonomic dysregulation in response to stress stimuli and a previous study in a small sample of HIV-infected individuals ($N = 16$) revealed that assaultive trauma during childhood was associated with reduced OB volumes in adulthood. In a larger sample of non-HIV infected individuals, we investigated if resilience and vulnerability to post-traumatic stress disorder (PTSD) following childhood assaultive trauma was associated with olfactory bulb morphometry.

Methods: Forty-five African American men and women, ages 18–45 years, residing in Washington, DC metropolis were enrolled

to receive psychiatric diagnostic interviews, psychometric assessments for childhood trauma exposures, salivary cortisol assay and MRI acquisition using a standard protocol on a 3.0 Tesla Signa HDx scanner (General Electric Medical Systems, Milwaukee, Wisconsin, USA). A T2-weighted fast spin-echo sequence of the OB was performed using previously published approaches. Statistical analyses were conducted in Stata 16 (StataCorp., College Station, TX, USA). All research activities were approved by the Howard University Institutional Review Board.

Results: Twenty-six of the 45 participants reported assaultive trauma (i.e., physical or sexual abuse) during childhood, 15 of whom developed PTSD and 11 of whom did not; the remaining 18 participants did not experience childhood trauma or significant adversities during childhood and did not meet diagnostic criteria for PTSD. PTSD diagnosis is associated with significant reduction in left OB volumes ($\beta = -12.3$, $SE = 4.7$, $P = 0.012$) and right OB ($\beta = -12.4$, $SE = 4.4$, $P = 0.007$). Within the childhood trauma-exposed group, those without PTSD diagnosis had greater left OB volumes ($\beta = 14.6$, $SE = 6.2$, $P = 0.027$) and right OB volumes ($\beta = 13.2$, $SE = 6.0$, $P = 0.038$). Also, the trauma-exposed group resilient to PTSD had slightly, but not significantly greater OB volumes than non-trauma exposed controls. Both right and left OB volumes were correlated with higher resilience ratings in several items of the Connor-Davidson Resilience Scale (CD-RISC).

Conclusions: This study confirms previous report in HIV populations that PTSD related to childhood assaultive trauma exposure is associated with reduced OB volumes and provides new evidence that OB morphometry may reflect biological resilience to development of PTSD in those exposed to severe childhood adversity.

Keywords: post-traumatic stress disorder, olfactory bulb, MRI, trauma, resilience

Disclosure: Nothing to disclose.

P14. Sex Differences in the Risk of Alcohol Use Disorder Conferred by Genetics, Parental History, and Childhood Trauma via Negative Emotionality Factors

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Background: Alcohol use disorder (AUD) is driven by both genetic and environmental factors. Genome wide association studies have identified multiple loci associated with AUD and problematic alcohol use (PAU). However, individual locus contributes only a small portion of the overall risk of AUD. Polygenic scores (PGS) index the overall risk conferred by these loci. Family history (FH) of PAU is a strong risk factor for AUD, and individuals with positive family history are at higher risk of AUD due to their inherited genetic risk and early life factors, such as childhood adversity. The effects of genetics, family history, and childhood trauma on the risk of AUD may be mediated by dysregulation in negative emotionality, a core neurofunctional domain underlying AUD. To elucidate the etiological pathways conferred by these factors, we used path analysis to investigate the relationship between parental history of PAU and lifetime risk of AUD via contributions of genetics, childhood trauma, and negative emotionality factors. Additionally, we sought to investigate whether these etiological pathways differ between males and females.

Methods: The analytical sample consisted of $N = 1,382$ individuals recruited from the National Institute on Alcohol Abuse and Alcoholism Natural History Protocol (42.5% female, 43.6% Caucasian White, 60.0% with lifetime AUD diagnosis). Lifetime AUD status was

determined using the Structured Clinical Interview for the DSM-IV or DSM-5. Parental FH (FH-Mother and FH-Father) was captured using the family tree questionnaire. Effect sizes and p-values of genes associated with PAU were obtained from a genome-wide meta-analysis of PAU ($n = 865,041$) and were used as our discovery dataset to compute PGS for PAU in the present sample. Childhood trauma was assessed using the childhood trauma questionnaire (CTQ). Negative emotionality factors were derived using factor analyses using measures of depression, anxiety, neuroticism, aggression, anger, and hostility. Associations between parental FH, PGS, childhood trauma, negative emotionality factors, and lifetime AUD were modeled using structural equation models with age, sex, and race as covariates. Structural invariance testing was used to test for sex differences, controlling for age and race.

Results: Two negative emotionality factors were identified: internalizing and externalizing ($CFI = 0.99$, $TLI = 0.98$, $RMSEA = 0.05$). Individuals with a lifetime AUD diagnosis exhibited greater PGS for PAU, and greater internalizing and externalizing scores ($p's < 0.001$). Additionally, individuals with a lifetime AUD diagnosis, compared to those without, were more likely to report a positive FH-Mother (21.6% vs. 5.0%; $p < .0001$) and a positive FH-Father (44.9% vs. 17.3%; $p < 0.001$). Individuals with either a positive FH-Mother or FH-Father exhibited greater PGS for PAU ($p's < 0.05$) and endorsed higher levels of childhood trauma ($p's < 0.001$). In the full model, the relationships between FH-Father and FH-Mother on lifetime AUD risk were mediated by internalizing scores (36.5% and 33.5% of total effect, respectively). However, the sources of this mediation effect differed between sexes. Among males, CTQ accounted for 36.7% of the internalizing mediation effect between FH-Father and lifetime AUD risk but accounted for 91.5% of the internalizing mediation effect between FH-Mother and lifetime AUD risk. For females, however, the relationship between FH-Father and lifetime AUD risk was fully mediated by CTQ, and internalizing scores accounted for 62.4% of this mediation effect. The relationship between FH-Mother and lifetime AUD risk was more complex, with internalizing scores explaining 26.1% of the total effect and CTQ explaining 31.9% of the total effect. Overall, for males, 63.7% of the risk for lifetime AUD conferred by parental history was accounted for by positive FH-Father, while for females, only 18.7% of the risk was accounted for by positive FH-Father.

Conclusions: Parental risk of PAU conferred risk of lifetime AUD diagnosis through both genetics (PGS) and non-genetic (CTQ) paths. The internalizing factor from the negative emotionality domain of AUD partially mediated these effects. Sex differences were detected in these etiological pathways. The effect of a positive FH-Father on risk of AUD played a larger role among males than among females. These results provide evidence for the complex interplay between genes and environment in the etiology of AUD. Future work will focus on the incentive salience and executive function neurofunctional domains of AUD, and explore psychiatric comorbidities frequently observed with AUD.

Keywords: Polygenic scores, alcohol use disorder, Childhood trauma, negative emotionality, family history of alcohol use disorder

Disclosure: Nothing to disclose.

P15. Early Life Stress, Psychiatric Disorders and Mitochondrial DNA Copy Number

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Background: Increasing evidence indicates that mitochondria respond to psychosocial and environmental stressors. There have

been mixed findings regarding psychopathology and mitochondrial DNA copy number (mtDNAcn). This study assessed mtDNAcn in peripheral blood mononuclear cells (PBMCs) in medically healthy young adults, with and without ELS.

Methods: Participants (N = 181; 69% female) ages 18-40 were recruited from community. N = 108 participants had ELS (moderate-severe maltreatment, with or without parental loss), and among them, n = 59 (54.6%) had a current psychiatric disorder and n = 49 (45.4%) had no current psychiatric disorder. Control participants (n = 73) had no maltreatment, loss, or psychiatric disorders. Standardized interviews and self-reports assessed demographics, adversity, and psychiatric history. PBMCs were isolated, DNA was extracted, quantified by Qubit fluorometer, and prepared for sequencing using the Illumina TruSeq Nano library preparation kit. Ultra-low coverage whole genome sequencing (ULC-WGS) libraries were multiplex sequenced in the Molecular Genomics Core (MGC) using NextSeq 2000 P2 flowcells and mtDNAcn was determined using Burrows-Wheeler Aligner and the fastMitoCalc bioinformatics tool. Values of mtDNAcn > 3.29 standard deviations from the mean were winsorized. A three-way ANOVA compared rates of mtDNAcn among ELS participants with a psychiatric disorder, ELS participants without a psychiatric disorder, and control participants.

Results: Participants with ELS and psychiatric disorders demonstrated significantly greater mtDNAcn compared to participants with ELS and no psychiatric disorders ($p = .006$). This remained significant when adjusting for age and sex ($p < .05$). There was no significant difference between mtDNAcn in control participants and those with ELS and psychiatric disorders and those with ELS and no psychiatric disorders ($p > .05$).

Conclusions: In this sample, participants with ELS and psychiatric disorders demonstrated higher numbers of mtDNAcn compared to those with ELS and no psychiatric disorder. These preliminary findings add to recent evidence indicating that mtDNA is impacted by childhood adversity and psychiatric disorders, and further suggest that psychiatric disorders may be necessary for stress effects on mtDNAcn. Next steps include additional assessments of relationships with mitochondrial structure and function, metabolic hormones, and type and timing of adversity.

Keywords: Early life stress (ELS), mtDNA, Mitochondria

Disclosure: Nothing to disclose.

P16. Mast Cells: A New Link Between Early Life Exposures and Psychiatric Susceptibility

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Background: Background: Epidemiological and preclinical studies increasingly demonstrate a link between early-life exposure to environmental pollutants such as flame retardants and the development of multisystem pathologies. These disorders range from neuropsychiatric and neurodevelopmental conditions like anxiety and autism to peripheral issues including intestinal barrier dysfunction, allergies, heightened autoimmune disorders, and metabolic abnormalities. Despite these observations, the mechanisms by which perinatal exposures drive such diverse health issues remain poorly understood. Our data suggest that mast cells, the primary effector cells of the immune system, may play a fundamental role. With their wide tissue distribution—including the gut, brain, and meninges—and their ability to quickly respond to a diverse range of stimuli, from toxins and pathogens to hormones and neurotransmitters, mast cells selectively release and synthesize a host of bioactive products from cytokines and

proteases to neurotransmitters. They play important roles in a wide range of physiological processes, from recruitment and activation of innate and adaptive immune cells to tissue development and repair, as well as modulation of neuronal and glial functions. Therefore, any alteration in mast cell functions can result in multisystemic symptomatology. Here, we used mice to investigate whether perinatal exposure via maternal transfer to Firemaster 550 (FM550), a flame retardant mixture abundantly found in the environment and human tissues (including maternal milk and blood), has long-lasting effects on mast cell functions and behavior.

Methods: Adult dams were given treats loaded with either vehicle or two human relevant doses of FM550 (low dose: 0.12 mg/day, mid dose: 0.6 mg/day) throughout pregnancy and lactation. Pups were left undisturbed until adulthood, at which point each litter (per treatment: 4 litters total, average 14M/16F) was divided into five different experiments (to minimize litter effect in each experiment): 1. Allergy-relevant IgE antibody + antigen challenge; 2. Infection-relevant LPS challenge; 3. Body composition measures and bone marrow collection; 4. Baseline behavior in the elevated plus maze and social interaction tests; and 5. Behavior after exposure to IgE two months following transplants of mast cells derived from wild-type non-exposed animals. Statistical analysis were done for each sex separately, and 1 (early life treatment) or 2-way ANOVAs (early life treatment x adult treatment) were used depending on the experiment. Fisher LSD was used to compare groups in significant ANOVAs. Alpha criterion was set to 0.05 for statistical significance.

Results: The effects of perinatal exposure to FM550 were sex-dependent. Compared to vehicle controls, males to a greater extent than females exposed to both doses of FM550 showed exacerbated hypothermia to IgE-antigen (veh vs low and med FM550 $p < 0.05$) and LPS challenges (veh vs low and med FM550 $p < 0.05$), two mast cell-dependent responses. Interestingly, perinatal exposure to low but not high FM550 increased body fat % in males (veh vs low FM550 $p < 0.0001$), while high but not low FM550 decreased body fat % in females (veh vs high FM550 $p < 0.05$). While behavior in the EPM or social interaction were not significantly affected by low or mid-dose FM550 exposure, preliminary data suggests that social investigation time was significantly reduced after IgE injection in FM550 but not vehicle exposed males and females and that, remarkably, transplants with healthy mast cells seemed to revert these effects.

Conclusions: Together, these results indicate that perinatal exposure to FM550 permanently alters mast cell functions, increasing adult susceptibility to exaggerated physiological responses to allergens and infection-like stimuli. Notably, FM550-exposed animals also demonstrate heightened sensitivity to reduced social investigation behavior following a single IgE antibody injection, and that transplants with healthy mast cells seem to be sufficient to prevent this. Given that IgE binds to Fc epsilon receptors on mast cells, enhancing their proliferation and cytokine production, our findings suggest that increased mast cell sensitization—even in the absence of antigens—may contribute to abnormal social behaviors. This supports the growing evidence linking allergy disorders with social deficits, as observed in conditions such as autism. Ongoing studies are assessing whether the effects of FM550 are present at the hematopoietic progenitor level by growing bone marrow-derived mast cells in vitro.

Keywords: Early life adversity, immune cells, environmental contaminants, Social Behavior, inflammation

Disclosure: Nothing to disclose.

P17. Cell Type-Specific Roles of H3 Serotonylation in Postnatal Neurodevelopment

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Background: The serotonergic (5HTergic) system and its homeostasis in early life are critical to establishing the proper architecture of the developing brain. Early life stress (ELS) can alter these precise developmental trajectories and increase the lifetime risk for depression. Studies from our lab have established a novel ELS paradigm using a combination of maternal separation with limited nesting, that leads to a priming effect on stress susceptibility following subsequent exposures to stress in adulthood. Historically, the actions of 5HT were thought to be mediated exclusively through ligand-receptor interactions. However, our lab recently established a novel role for 5HT in the epigenetic regulation of gene expression. Specifically, 5HT forms covalent bonds with histone H3 tail where it stabilizes the adjacent H3 lysine 4 trimethylation post-translational modification (PTM). This combinatorial mark, termed H3 serotonylation (H3 Ser.) is critical for permissive gene expression in the neonatal developing brain both in vitro and in vivo. H3 Ser. has been functionally linked to responses to stress in adulthood. However, functional roles for H3 Ser. during postnatal neurodevelopment and impacts of aberrant environmental stimuli during early life have largely been unexplored.

Methods: We employed immunosorbent assays to examine 5HT levels across postnatal neurodevelopment in the medial prefrontal cortex (mPFC) – a region receiving dense 5HTergic innervation – with and without ELS. After examining differences in regional and developmental abundance of 5HT, we sought to investigate changes in H3 Ser. genomic localization across normal and perturbed neurodevelopment. To do this we leveraged fluorescent activated nuclei sorting (FANS)-using antibodies against the neuron-specific antigen, NeuN, to isolate neuronal- vs. glial-enriched mPFC cell populations in both male and female mice at each of our developmental time points of interest. I coupled this approach with C and R (Cleavage Under Targets and Release Using Nuclease)-sequencing for H3 Ser. on both cell populations from mPFC to directly assess epigenomic H3 Ser. enrichment patterns during normal postnatal development and following perturbation by ELS (3-4 biological replicates/group (three animals pooled/ replicate). To first decipher the developmental trajectories of H3 Ser. in both control and ELS animals, I performed differential enrichment analysis on FANS mPFC tissues from all time points assessed, anchored to the earliest development time point in the study (P10), using DiffBind (significance cut off: $FDR < 0.1$ and $FC > |1.5|$). Bioinformatic analysis was used to further dissect these results. Finally, immunohistochemistry was used to determine the cell-type specific roles of H3 Ser. in cell-type differentiation.

Results: We found mPFC 5HT levels fluctuate during postnatal neurodevelopment of mice and ELS decreases levels of 5HT acutely in adolescence ($p = 0.0188$), an effect not seen in adulthood ($p = 0.9761$; $n = 3-4$ /group). FANS-coupled C and R revealed that normal developmental differences – as well as those perturbed by ELS – were more pronounced in males vs. females in both neurons and glia. Strikingly, in glia, ELS increased the number of differential loci by 150-fold in adolescence with 7,927 differential enriched sites, representing the most pronounced differential enrichment observed across all conditions. These differential binding sites were largely gained in comparison to juvenile time points (P10) and were found to be distinct from age-matched controls ($p > 0.99$). These differentially enriched loci are enriched for genes involved in the regulation of chromatin remodeling, transcription, and post-translational protein modifications and are largely located at promoter regions. Cell-type deconvolution analysis revealed, that of the 7,927 differential sites induced by ELS in NeuN- fractions at P21, 26.2% occurred within cell type-specific loci, with microglia, astrocytes, mature

oligodendrocytes, oligodendrocyte progenitor cells, and endothelial cells all represented. Notably, upstream transcription factor analyses of ELS-induced differential peaks in glia at adolescence (P21) predicted OLIG2, (oligodendrocyte transcription factor 2) – a master regulator for oligodendrocyte lineage specification, which regulates key stages of early OL development – as a top associated transcription factor. Interestingly, in males, H3 Ser. displays increased Olig2 promoter binding in response to ELS at adolescence (P21) compared to age-matched controls. Immunohistochemistry (IHC) also revealed that Olig2+ cells show a trend toward increased H3 Ser. intensity ($n = 2$ /group, $p = 0.04$) and the number of Olig2+ cells ($p = 0.06$). We also find an increased number of oligodendrocyte precursor cells with no change in the number of mature oligodendrocytes ($p = 0.04$ and 0.6444 respectively; $n = 4$ /group). This is the first evidence to examine H3 Ser.'s role in oligodendrogenesis.

Conclusions: These findings provide novel insight into how H3 serotonylation regulates neurodevelopment and the mechanisms by which disruptions to this PTM cause aberrant pathophysiological states. We demonstrate normal; neurodevelopmental patterns of H3 serotonylation enrichment are reorganized by early life stress in a cell type-specific manner. This data provides novel evidence for the roles of H3 serotonylation in the regulation of oligodendrogenesis demonstrating novel roles for serotonin.

Keywords: Serotonin, Early life stress, Epigenetics

Disclosure: Nothing to disclose.

P18. Chronic Adolescent Stress Attenuates Morphine-Induced Antinociception and Morphine-Induced Central Amygdala Activity in Adult Male and Female Rats

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Background: Stress exposure during developmental periods has been shown to increase the risk for an array of psychological and physiological pathologies in adulthood, including substance use disorder and chronic pain. The aim of the current study was to characterize the effects of chronic adolescent psychosocial stress on morphine-induced antinociception and neuronal activation in adult male and female rats.

Methods: Male and female Wistar rats were exposed to chronic adolescent stress (CAS) and tested for antinociceptive response to morphine ($n = 16-17$ /group) or morphine-induced expression of cFos in the central nucleus of the amygdala (CeA) and periaqueductal gray (PAG) in adulthood ($n = 9-12$ /group). Antinociceptive response to increasing doses of morphine was assessed with the warm water tail-withdrawal test, from which % maximum possible effect (%MPE) values were calculated using the following formula: $\% MPE = [(Test-Saline)/(20-Saline)] \times 100$, where "Test" is withdrawal latency after morphine, "Saline" is withdrawal latency after saline, and "20" is the maximum cutoff latency. Morphine-induced (10 mg/kg) cFos expression was assessed with cFos immunohistochemistry (IHC) as a proxy for neuronal activation. Baseline tail withdrawal data was analyzed using t-test, and dose response curves were analyzed using Mann-Whitney tests. Log-linear regression of each rat's morphine dose-effect curve was then used to determine morphine ED50s, which were analyzed using t-test. CeA and PAG cFos levels were assessed using Mann-Whitney test and two-way ANOVA, respectively.

Results: No differences in tail withdrawal latency were observed between non-stressed (NS) and CAS groups ($p = 0.36$). However, morphine's antinociceptive effects were attenuated in CAS

subjects, evidenced by lower %MPEs at the log0.5 dose ($p = 0.02$) and lower ED50s ($p = 0.01$) compared to NS subjects. Morphine increased CeA neuronal activation compared to vehicle in NS group only ($p < 0.01$), with no difference detected between vehicle- and morphine-treated CAS groups ($p = 0.12$). This reduction was specific to the CeA, as no effect of CAS ($p = 0.51$) nor an interaction between morphine and CAS ($p = 0.91$) were detected in the PAG. Effects of CAS on reduced morphine-induced antinociception and CeA activity in adulthood did not differ between male and female rats.

Conclusions: Collectively, these data suggest that a history of adolescent stress can influence adult nociception and the CeA may be a critical area for future mechanistic consideration.

Keywords: opioid, central amygdala, Early life stress (ELS), antinociception, Adolescence

Disclosure: Nothing to disclose.

P19. Ketogenic Diet Prevents Social Dysfunctions and Improves Stress Resilience in a Preclinical Model of Prenatal Stress Exposure

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Background: Early life stress exposure can lead to long-lasting behavioural and neurobiological consequences, thus increasing the susceptibility to the onset of mental disorders including depression. Since current pharmacological treatments show limited efficacy in almost 30% of patients, alternative therapeutic strategies, including dietary interventions, are currently being explored. Recent research suggests that adopting a high-fat low-carb diet, known as a ketogenic diet (KD), which prompts the body to use fats as its main energy source, could potentially help individuals struggling with mental disorders. Emerging data suggest that KD show neuroprotective and anti-inflammatory effects, improving mitochondrial functions, monoaminergic circuits, and hypothalamus–pituitary–adrenal axis and it may also suppress oxidative stress.

In this study, we aimed at investigating the efficacy of KD in preventing and/or treating the behavioural and functional abnormalities emerging as a consequence of early-life stress exposure and the potential mechanisms underlying the effects of KD. In parallel, we aim to discern potential sex differences since very little is still known about the role of sex in the overall effects of KD.

Methods: To induce a stressful condition, pregnant Sprague Dawley rats were exposed to a paradigm of prenatal stress (PNS), thus restrained for 45 min 3 times a day during the last week of gestation. Since weaning (P21), both control (CT) and PNS offspring were fed with control diet (CD) or KD for 4 weeks. During late adolescence (P42–45), CT and PNS male and female offspring fed with either CD or KD ($n = 15$ animals/group) were tested to investigate sociability and anhedonia. Body weight gain and food consumption were analysed with two-way repeated measured ANOVA followed by multiple comparisons when appropriate. Behavioural data were analysed with two-way ANOVA followed by multiple comparisons when appropriate. Cluster analysis of vulnerability/resilience traits was conducted by using Gaussian distributions. All procedures included in this study comply with the ARRIVE guidelines, are in conformity with the rules and principles of the EU Directive 2010/63/EU and have been approved by the Institutional Animal Care and Use Committee.

Results: As expected, we observed a reduction of body weight gain (Males: $F(1, 62) = 151$, $p < 0.0001$; Females: $F(1, 62) = 126.2$,

$p < 0.0001$) and food consumption (Males: $F(1, 18) = 1409$, $p < 0.0001$; Females: $F(1, 18) = 661.2$, $p < 0.0001$) in both CT and PNS male and female offspring fed with KD compared to CD-fed animals. In line with our previous studies, PNS animals regardless of sex showed a reduction in the time spent interacting with an unfamiliar animal ($p < 0.001$), indicating an impairment of social behaviours. Interestingly, such long-lasting dysfunction was not observed when the animals were fed with KD from weaning ($p < 0.01$), suggesting a potential for KD to prevent sociability deficits (Interaction effect males: $F(1, 59) = 4.115$, $p < 0.05$; Interaction effect females: $F(1, 58) = 7.166$, $p < 0.01$). Moreover, looking at anhedonic-like phenotypes, although PNS exposure did not impair grooming behaviours tested in the splash test, KD led to increased total grooming time, indicative of self-care behaviours (Males: $F(1, 61) = 31.29$, $p < 0.0001$; Females: $F(1, 60) = 44.56$, $p < 0.0001$). In line with our recently published data, we found that around 50% of both male and female animals treated with CD were vulnerable to PNS exposure. Interestingly, KD treatment reduced this percentage to 22% and 12% in male and female PNS offspring respectively, suggesting its potential in preventing stress-induced emotional dysfunctions.

Conclusions: Altogether, our data provide novel evidence on the beneficial effect of KD against behavioural disturbances in an early life stress model. Ongoing molecular analyses will allow to unravel the underlying molecular mechanisms, with a focus on two brain regions involved in the effects of stress exposure and emotions as the prefrontal cortex and ventral hippocampus.

Keywords: Ketogenic diet, Early life stress, neurobehavioral disorders

Disclosure: Nothing to disclose.

P20. Early Life Adversity Increases Basolateral Amygdala Outputs to Cortical and Limbic Regions, With Dissociable Influences of Adolescent Neural Activity

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Background: Early postnatal life is a dynamic period of brain development and traumatic experiences during this time are associated with increased risk for affective disorders. As many regions within the brain continue to develop and mature postnatally, external stimuli impact this development and regulate the degree of connectivity to other brain areas. Since subcortical limbic regions undergo substantial synapse formation and pruning during early development while higher-order areas such as the prefrontal cortex (PFC) mature throughout adolescence, early postnatal life and adolescence are two sensitive periods when limbic and prefrontal regions form functional connections in response to external stimuli and neuronal activity. The basolateral amygdala (BLA) has unique integration with aversion- and reward-related circuitry, and serves as the primary input nucleus of the amygdala. Monosynaptic BLA connectivity with the PFC increases throughout adolescence in healthy rats, facilitating the integration of emotional information with cognitive appraisals of the environment. Development of BLA connectivity with output limbic regions such as the BNST is less understood, however BLA-BNST connectivity plays a crucial role in the expression of many anxiety-related behaviors that are impacted by ELA. Challenges to the early amygdala that alter local activity may induce activity changes within efferent projections, which may result in changes to behavior when the circuit is recruited during adulthood. Here, we investigated the impacts of ELA on BLA innervation to the PFC and the BNST, and whether early adolescent activity within the BLA influences the development of outputs to these structures.

Methods: Male and female pups ($n = 6-9$) were reared either in a control environment or with ELA in the form of maternal separation from postnatal day (p) 2-20. Pups were weaned from their mother at p21. All experimental procedures were conducted with approval from the Institutional Animal Care and Use Committee at Northeastern University and the University of Illinois Chicago. On p26 subjects underwent a stereotaxic surgical injection of the inhibitory DREADD AAV-CAMKIIa-HM4DGi-mCherry to the BLA. Daily from p33-39, rats were weighed and given an intraperitoneal injection of the DREADD agonist clozapine-N-oxide dihydrochloride (CNO, 2mg/kg, Bio-Techne Tocris, Minneapolis, MN) or vehicle saline (SAL). Animals were sacrificed at p55-60 for analysis of axonal boutons originating from the BLA. Subjects with a total bolus volume that filled $< 50\%$ of the BLA were excluded from analysis. Sections from the corresponding PFC and BNST were processed using immunohistochemistry to identify mCherry expression. Z-stacks were analyzed for axonal bouton count and intensity. GraphPad Prism 10.0 Software was used for all data analyses and graphical representation. Sex $\times$ rearing $\times$ treatment 3-way ANOVAs were conducted, and when main effects of sex or interactions with sex were observed, 2-way ANOVAs with factors of treatment and rearing were conducted for each sex with Post-hoc Tukey's multiple comparison's tests when appropriate.

Results: In the PFC, three-way ANOVA revealed a sex $\times$ rearing interaction on bouton count in the PL ($F_{1,57} = 3.245$; $p = 0.0770$; partial $\eta^2 = 0.054$) and the IL ($F_{1,57} = 3.617$; $p = 0.0623$; partial $\eta^2 = 0.06$). Due to the presence of a moderate effect size for the sex $\times$ rearing interaction, each sex was analyzed separately, with two-way ANOVAs assessing effects of rearing condition and treatment (CNO or SAL). In males, MS increased PL bouton count ($F_{1,25} = 4.661$; $p = 0.0407$; partial $\eta^2 = 0.157$) and IL bouton count ($F_{1,25} = 5.098$; $p = 0.0329$; partial $\eta^2 = 0.169$). However, no effects were observed in females. Three-way ANOVA also revealed a main effect of rearing on bouton intensity in the PL ($F_{1,57} = 10.87$; $p = 0.0017$; partial $\eta^2 = 0.160$) and IL ($F_{1,57} = 15.58$; $p = 0.0002$; partial $\eta^2 = 0.215$), and a sex $\times$ rearing interaction in bouton intensity within the PL ($F_{1,57} = 15.00$; $p = 0.0003$; partial $\eta^2 = 0.208$) and IL ($F_{1,57} = 13.19$; $p = 0.0006$; partial $\eta^2 = 0.160$). Due to the presence of a sex $\times$ rearing interaction, each sex was analyzed separately, revealing another male-specific main effect of rearing on PL bouton intensity ($F_{1,25} = 36.09$; $p < 0.0001$; partial $\eta^2 = 0.591$), and IL bouton intensity ($F_{1,25} = 29.53$; $p < 0.0001$; partial $\eta^2 = 0.541$). Treatment with CNO had no effects on either measure (count or fluorescent intensity). Data from the BNST is more preliminary with males and females combined, as only a subset of tissue was available for analysis. Two-way ANOVA revealed a significant rearing $\times$ treatment interaction on bouton count in the BNST ($F_{1,32} = 4.212$; $p < 0.0001$; partial $\eta^2 = 0.115$), with saline-treated MS animals displaying higher bouton density in the BNST ($p < 0.05$). No difference was observed between CNO-treated MS animals and controls.

Conclusions: These findings indicate that ELA via maternal separation induces hyperinnervation from the BLA to both PFC and BNST by early adulthood, and that BLA inhibition during early-mid adolescence is sufficient to prevent hyperinnervation to the BNST but not the PFC. Ongoing work will aim to understand how the development of BLA outputs are influenced by reciprocal inputs from the PFC as well as how these altered patterns of connectivity influence distinct behavioral domains throughout development.

Keywords: basolateral amygdala, Medial Prefrontal Cortex (mPFC), Rodents, maternal separation, DREADDs

Disclosure: Nothing to disclose.

P21. Impact of Early Life Environmental Adversity Effects on Chemosignals That Shape Social Interactions

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Background: Mammalian offspring produce social chemosignals that serve as significant cues for eliciting parental caregiving. Maternal preference for offspring olfactory chemosignals promote survival and parent-offspring social and somatosensory interactions that shape developmental trajectories. In humans and animals, olfactory chemosignals can arise from various bodily sources including the placenta, amniotic fluid, breast milk, saliva, breath, and skin excretions. One measure of these chemosignals is through analyses of volatile organic compounds (VOCs) which constitute the "volatilome" and have been demonstrated to convey critical information about health, identity and emotional state. More recent exploration of the volatilome has focused on its reflection of environmental exposures. In the present study, we investigated the effect of an adverse postnatal rearing environment consisting of low bedding material on VOCs emitted by rat pups. This manipulation is a pre-clinical model of early life adversity known to induce more fragmented maternal care primarily attributed to maternal stress caused by the low-resource environment.

Methods: Long Evans rat dams and litters were placed in an environment with abundant bedding (3 inches; Control group; $n = 19$ litters) or low bedding (.5 inch; LB group; $n = 20$ litters) starting on postnatal day (P) 0. VOC emissions from one male and one female pup per litter were measured at P6 and P12 for ten minutes by placing pups in glass flow-through chambers connected to an Aerodyne Vocus 2R Proton Transfer Reaction Time-of-Flight Mass Spectrometer (Vocus 2R PTR-ToF-MS). This method allows for real-time measures of emitted VOCs from awake, behaving pups. Because VOC emissions can be affected by temperature, pup core body temperature was measured immediately before and after the test on P12 using BioTherm RFID tags implanted subcutaneously in each pup's flank. In addition, pup urine, known to contain olfactory signals that regulate the amount of licking and grooming a pup receives during postnatal mother-infant interactions, was collected on P13 and was measured with the Vocus 2R PTR-ToF-MS using a similar flow-through apparatus with samples warmed in an oven set to 37 degrees to simulate pup body temperature conditions.

Mixed model analysis was used to assess effects of pup sex and the postnatal environment on CO₂ emission and total VOC emission on P6 and P12 with pup body weight and temperature included as covariates and litter ID included as a random factor. The DeSeq2 bioinformatics package was used to evaluate differential emission of VOCs (Control vs. LB) by pup sex on P6 and P12. Differential emission of VOCs by postnatal bedding condition was evaluated at P6 and P12 by comparing the LB group versus the Control group in males and females separately.

Results: Pup CO₂ emissions were positively correlated with total VOC emissions and pup body weight on P6 and P12 ($p < .05$). Pup body temperature, total VOC emission, and pup body weight were also positively correlated on P12 ($p < .05$). LB reared pups emitted more CO₂ than pups reared with abundant bedding on P6 ($p < .05$) but not P12 ($p = .09$). Best-match formula confidently assigned from the accurately measured monoisotopic mass revealed that the most abundant molecules emitted by pups on P6 and P12 was C₃H₆OH⁺, followed by C₂H₄O₂H⁺ and C₃H₈O₂H⁺, consistent with acetone, acetic acid and propionic acid, respectively. The number of differentially emitted VOCs by male vs female pups increased from seven VOCs on P6 to 38 VOCs on P12 (FDR $< .05$). On P6, male pup emission rates for 13 VOCs and female pup emission rates for 12 VOCs were affected by bedding condition, with 6 VOCs differentially emitted in both sexes (FDR $< .05$). LB had a greater effect on differential VOC emission rates at P12 compared to P6. On P12, 29 VOCs were differentially emitted by male pups and 46 VOCs were

differentially emitted by female pups exposed to LB. Of these differentially emitted VOCs on P12, 14 were differentially emitted for both males and females exposed to LB.

Conclusions: These data describe the total VOCs emitted by awake, behaving neonatal pups that may serve as social chemosignals to facilitate maternal care. The most abundant VOC emitted by pups was C₃H₆OH+ (acetone), which is known to increase emission in breath during stress. Furthermore, LB-associated increases in CO₂ emissions on P6 may reflect an elevated metabolic rate induced by the adverse environment. The number of VOCs that were differentially emitted by pup sex and by environmental condition increased from P6 to P12, suggesting continued development of pup chemosignal profile that may be attributed to hormonal, maternal, and environmental factors. Together these data show that early life exposure to adverse environmental conditions can impact the VOC emission profile of pups with potential to impact dam-pup interactions.

Keywords: Early life adversity, Maternal behavior, Olfaction, Social communication

Disclosure: Nothing to disclose.

P22. Prenatal Adversity-Induced Maladaptive Plasticity in Neuronal Enhancers and Associated Gene Expression in Ventral Hippocampal Ensembles Governs Future Anxiety Behavior in Stressful Environment

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Background: Adversity during early (pre- and postnatal) life is a significant risk factor for the development of psychiatric disorders, characterized by maladaptive behaviors such as high arousal, vigilance, and exaggerated fear-like reactions in anxiety disorders. Adversity-induced maladaptive behaviors are thought to be driven by epigenetic and associated gene expression changes in behaviorally relevant neuronal circuits. Although prior studies revealed the powerful and lifelong effect of early life adversity on the neuronal epigenome, little is known about how these epigenetic changes come about and lead to abnormal behaviors, including anxiety, in the offspring. The goal of our study was to identify prenatal adversity induced epigenetic signatures in the ventral dentate gyrus (vDG), a hippocampal region linked to contextual anxiety, that persist to adulthood and, via transcriptional and neuronal changes, promote anxiety behavior.

Methods: Prenatal adversity was represented by a maternal proinflammatory environment that results in anxiety-like behavior in the elevated plus maze (EPM) and increased stress responsiveness (escape directed behavior) in the forced swim test in the adult male, and less prominently, female offspring. Adversity-induced changes in the synaptic transmission and intrinsic excitability of ventral hippocampus dentate granule cells (vDGCs) from male mice was assessed by whole-cell patch-clamp slice electrophysiology, and DNA methylation and chromatin modifications were assessed by whole genome reduced representational bisulfite sequencing and CUT and TAG histone profiling, respectively, from isolated vDGCs. Transcriptional profiling of vDGCs, sorted to activated (FOS+) and non-activated (FOS-) populations following a 10 min EPM test was by single nucleus RNA-Seq. Neuronal activity in vDGCs during EPM performance was monitored by fiber photometry.

Results: Here we report that prenatal adversity produced a reduction in basal synaptic inhibition and excitability in the overall vDGC population, indicating a broad disruption of ventral hippocampal function. We show that thousands of small genomic

regions with an intermediate level of CG methylation were the preferential epigenetic targets of prenatal adversity. These regions were enriched in annotated transcriptional enhancers and underwent adversity induced chromatin transitions to active enhancer or heterochromatin states and increases and decreases in methylation depending on their baseline methylation states. The adversity-related epigenetic changes were associated with transcriptional changes in corresponding synaptic genes, many of them implicated in neuropsychiatric diseases, in a subset of neurons that was preferentially activated to the coding ensemble during the EPM trial in adulthood. While control mice displayed calcium activity in vDGCs that discriminated between the EPM compartments (with more activity in the anxiogenic open arms than closed arms) by fiber photometry, this discrimination did not occur in prenatal adversity mice with greater anxiety-like behavior.

Conclusions: We identified genomic regions that undergo early life adversity-induced coordinated gain and loss in methylation and chromatin state transitions. These are associated with altered gene expression in a population of cells that is preferentially activated during future stressful environment in adulthood, leading to aberrant vDG activation and behavior and an overall dysregulation of vDG network function in adult mice that experienced early life adversity. Altogether, we propose that maladaptive plasticity in neuronal enhancers and associated gene expression in adversity encoding neuronal ensembles governs lifelong anxiety through a broad dysregulation of ventral hippocampal circuit function.

Keywords: prenatal adversity, anxiety, epigenetic, transcription, neuronal ensemble

Disclosure: Nothing to disclose.

P23. The Role of Childhood Trauma, Estrogen Receptor Beta, and Inflammation on Executive Function and Psychomotor Speed

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Background: Trauma exposure during childhood poses a risk for long-term cognitive function. Women living with HIV (WLH) endorse high rates of childhood trauma and may be more vulnerable to cognitive impairment due to the immune component of HIV. The impact of trauma on immune function, and estrogen receptor beta (ERβ) mediated regulation of inflammatory responses, are both linked to cognitive impairment with inflammation being the common factor. The severity of cognitive impairment in women often becomes more apparent during the menopausal transition and into the postmenopausal stage. Though circulating estradiol is reduced with the menopausal transition, it is unclear if the expression of ERβ is also impacted by the hormonal shift. The current study aimed to determine if the combination of childhood trauma history, peripherally derived estrogen receptor expression and inflammatory profile are associated with cognitive performance and whether HIV or menopausal status influence the relationship.

Methods: All participants (n=81, 24 without HIV (17 premenopausal, 7 postmenopausal), 57 WLH (22 premenopausal, 35 postmenopausal)) were women > 30 years of age, recruited from the MACS-WIHS combined Cohort Study (MWCCS) in Atlanta, GA. Participants provided informed consent and menopausal status was determined using the Stages of Reproductive Aging Workshop (STRAW + 10) criteria. Inclusion criteria included being a women age ≥18 years, either living with HIV (WLH) or

demographically matched without HIV. A clinical interview conducted by a clinician trained to administer trauma assessments was completed. The Childhood Trauma Questionnaire (CTQ) was used to assess childhood trauma history. Total CTQ was summed and group variables were created for “none-to-low” and “moderate-to-severe” trauma exposure for each CTQ subscale as well as the total CTQ. Participants completed a comprehensive neuropsychological test battery and a blood draw where peripheral blood mononuclear cells (PBMCs) were extracted. PBMCs were cultured, supernatant was collected to quantify the secreted cytokine concentrations of IL-6, IL-8, IL-10, IL-4, and TNF α via MesoScale Discovery multiplex assays and inflammatory profile scores were created from the summed log transformed data. RNA from the PBMCs was used to quantify gene expression of ER β by RT-qPCR. ANCOVAs were used to assess the effects of childhood trauma (low vs. high) on cognitive performance as well as the effects and interactions of HIV and menopausal status on cognitive performance. Linear regression models were used to assess the association of estrogen receptor beta gene expression, total inflammatory score and CTQ total score with cognitive domains. Covariates were chosen based on known associations with either inflammation, or cognitive performance, and then screened using multivariate linear regression. Power analysis using G*Power version 3.1.9.7 for linear multiple regression indicated 89.8% power and an effect size of 0.15 with our sample size of 81 participants. All other statistical analyses were performed using SPSS version 28 with $\alpha = .05$.

Results: The women who participated in this study did not differ significantly by age, years of education, or total childhood trauma exposure (p 's $> .05$). Two-way ANCOVAs for CTQ subscales by HIV and menopausal status showed a main effect of HIV status on CTQ emotional abuse and CTQ physical neglect such that WLH reported lower emotional abuse ($p = .043$), and physical neglect ($p = .029$). Two-way ANCOVAs for t-scored cognitive domain performance by HIV and menopausal status with covariates for CTQ total score and hyperlipidemia did not identify main effects of HIV status or menopausal status, nor interactions (p 's $> .05$). In the stepwise multiple regression model, CTQ alone was not associated with cognitive performance in any domain (p 's $> .05$). However, the multivariate multiple regression model of the relationship between ER β gene expression, total inflammatory score, and CTQ total score on cognitive performance showed that CTQ total score was negatively associated with performance in the domains of executive function ($B = -.205$, $p = .010$) and psychomotor speed ($B = -.174$, $p = .004$). Neither ER β gene expression or total inflammatory score were associated with cognitive performance in any domain in this model (p 's $> .05$).

Conclusions: The current data suggest greater childhood trauma is associated with worse cognitive performance when the variability of ER β gene expression and inflammation are taken into account. In this context, childhood trauma is more impactful to cognition than HIV or menopausal status.

Keywords: Childhood trauma, Cognitive performance, inflammation, HIV, estrogen receptor beta

Disclosure: Nothing to disclose.

P24. Association Between Number of Adverse Childhood Experiences and Cannabis Use is Moderated by Race

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Background: Adverse Childhood Experiences (ACEs) are harmful or traumatic events such as emotional, sexual, or physical abuse,

neglect, domestic violence, or having a family member who is incarcerated prior to the subject being 18 years old. Early exposure to ACEs is significantly associated with an increased risk of morbidity and mortality. Additionally, ACEs have been linked to various mental health issues, such as depression and substance abuse. While exposure to ACEs has been connected to increased cannabis use, how this relationship is influenced by race remains unclear. This study aims to examine the association between the number of ACEs and a history of cannabis use, as well as to explore how this association varies across different racial groups. Identifying factors, such as race that may moderate the risk of mental health problems is crucial for developing targeted prevention strategies for individuals with ACEs.

Methods: We completed a cross-sectional analysis of the 2020 Behavioral Risk Factor Surveillance System (BRFSS) data, involving 46,684 adult respondents. Exposure to ACEs was categorized as no ACEs, 1 ACE, 2-3 ACEs, and > 4 ACEs: we created categories of ACE scores based on a review of the literature. ACEs included questions assessing exposure to eight types of adverse childhood experiences: three types of abuse (physical, emotional, and sexual) and five types of household challenges (substance misuse by a household member, incarceration, mental illness, parental divorce, or witnessing intimate partner violence) before the age of 18 years. Respondent's use of cannabis in the last 30 days prior to the interview (yes/no) was also assessed. Weighted multivariable logistic regression models were used to examine the association between number of ACEs and cannabis use, stratified by race—namely Non-Hispanic White (White), Non-Hispanic Black (Black), and Hispanic. These three racial/ethnic groups were utilized based on the categorization by BRFSS dataset. Each model was adjusted for age, tobacco smoking status, income, education, marital status, and body mass index. The list of covariates was based on all characteristics that have been reported previously as confounders in the association between cannabis use and adverse childhood experiences.

Results: In this sample of adults, 35.87%, 22.64%, 20.87%, and 20.62% reported experiencing no ACEs, 1 ACE, 2-3 ACEs, and > 4 ACEs, respectively. Most respondents were White (70.77%), 13.41% were Blacks, 6.20% were Hispanics, and 9.62% belonged to other racial groups. Cannabis use was reported by 22% of survey respondents. A bivariate analysis showed that among respondents experiencing no ACEs, 1 ACE, 2-3 ACEs, and > 4 ACEs, cannabis use was reported by 3.85%, 8.13%, 11.37%, and 20.17%, respectively (p -value: < 0.0001). Cannabis use was reported by 8.83% of White, 12.05% of Blacks, and 13.41% of Hispanics (p -value: < 0.0001). No ACEs (36.65%), 1 ACE (21.94%), 2-3 ACEs (36.65%), and > 4 ACEs (20.37) was reported by White respondents; no ACEs (34.58%), 1 ACE (27.51%), 2-3 ACEs (19.70%), and > 4 ACEs (18.21%) was reported by Black respondents; no ACEs (29.93%), 1 ACE (21.02%), 2-3 ACEs (22.15%), and > 4 ACEs (26.90) was reported by White respondents (p -value: < 0.0001). Respondents who experienced 1 ACE were more likely to report cannabis use compared with those who had no ACE across all race/ethnicity groups: White (aOR=1.84; 95% CI: 1.46, 2.32), Blacks (aOR=1.68; 95% CI: 1.02, 2.76), and Hispanics (aOR=2.27; 95% CI: 1.15, 4.48). Respondents who experienced 2-3 ACEs were more likely to report cannabis use compared with those who had no ACEs across all race/ethnicity groups: White (aOR=2.68; 95% CI: 2.16, 3.33), Blacks (aOR=1.98; 95% CI: 1.19, 3.30), and Hispanics (aOR=2.52; 95% CI: 1.20, 5.28). Respondents who experienced four or more ACEs were more likely to report cannabis use compared with those who had no ACEs across all race/ethnicity groups: White (aOR=3.76; 95% CI: 3.03, 4.68), Blacks (aOR=3.25; 95% CI: 2.51, 7.17), and Hispanics (aOR=7.24; 95% CI: 3.83, 13.65).

Conclusions: Individuals who experienced ACEs irrespective of the number of ACEs were more likely to report cannabis use in each race/ethnic group, but effect sizes were higher with higher number of ACEs across all racial/ethnic groups, with highest effect

size seen among respondents who experienced > 4 ACEs who identified as Hispanics. This potential variation by race has the potential utility for selective prevention while also warranting further research into identification of factors responsible for such disparities.

Keywords: Racial/ethnic differences, Adverse life experiences, cannabis use

Disclosure: Nothing to disclose.

P25. Early Life Adversity and the Paraventricular Nucleus of Thalamus Network Connectivity Interact in the Neurobiology of Adolescent Mental Health

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Background: Emotional and memory circuit maturation is shaped by sensory signals in the environment during early life. There is growing evidence that the paraventricular nucleus of the thalamus (PVT) acts as a central hub involved in storing memories of adverse early life experiences, such that PVT activity influences behavior in adulthood. However, PVT functional connectivity has not yet been evaluated in the adolescent human brain, and the impact of early life adversity (ELA) on the development of PVT connectivity with circuitry involved in both emotive and memory processing is largely unknown.

Methods: This study analyzed data from two cohorts. The first cohort was from the Conte Center at UCI and consisted of a longitudinally monitored sample of mother-child dyads ($n = 152$, children are 78 female, 74 male, range 9-17 years). In this cohort, unpredictability of early life environment was quantified in this cohort using the Questionnaire of Unpredictability in Childhood (QUIC) and whole-brain fMRI images were acquired at three separate timepoints during childhood and early adolescence. The second sample was from the Adolescent Brain Cognitive Development (ABCD) cohort and consisted of data from the baseline visits from the two largest sites ($n = 674$, children are 347 female, 307 male, range 8.9-11 years). ELA in ABCD was quantified using Adverse Childhood Experiences (ACEs). Image quality, site and nesting in family structure were controlled for in ABCD analysis. Structural and resting-state functional 3T MRI data were pre-processed with the CONN toolbox. Functional connectivity (FC) between these regions and the PVT were calculated and entered into seven independent mixed effects models testing for a main effect of ELA adjusted for age at scan and sex. For network analysis, fMRI scans from the Conte Center sample and weighted signed adjacency matrices were derived. Betweenness centrality (BC) of each ROI in these networks was computed using the Brain Connectivity Toolbox. Linear regression analyses examined the relationship between BC and ELA (QUIC) measures, as well as the relationship to anhedonia, one important mental health outcome linked to ELA.

Results: Preliminary findings suggest that ELA is associated with reduced PVT-amygdala FC in both Conte Center ($T = -2.9$, $p = 0.004$) and ABCD ($T = -2.3$; $p = 0.02$) cohorts. PVT-hippocampus FC was negatively associated with ELA measures in females across both cohorts (Conte Center QUIC: $T = -2.37$, $p = 0.02$, Conte Center ACEs: $T = -1.91$; $p = 0.06$; ABCD ACEs: $T = -2.82$, $p = 0.005$). Linear regression analysis of the Conte Center cohort revealed that QUIC and significantly sex-assigned-at-birth interact, where males had a positive association with nucleus accumbens (NAc) BC and QUIC (QUIC*males-females, $\beta = 0.01$, p -value = 0.006). In a second model, QUIC positively predicted anhedonia in this cohort (QUIC, $\beta = 0.3$, p -value < 0.001), where

NAc BC and sex-assigned-at-birth did not significantly contribute to the model.

Conclusions: The "PVT network" and ELA have an important relationship in the neurobiological origins of mental health in adolescence and beyond. Two measures of ELA—unpredictability of early environment and adverse childhood experiences—across two cohorts, are associated with reduced FC of the PVT to key nodes in the medial temporal lobe emotional memory system. Early life adversity has been shown to induce synaptic pruning in the hippocampus and impact the structure and function of the PVT circuitry in animal models. The NAc betweenness centrality results are supported by preclinical literature, in that ELA has a sex-dependent effect on the PVT and subsequent development of anhedonia in preclinical rodent models. The NAc is thought to have a critical role in the relationship between ELA, PVT, and anhedonia. These data in humans are consistent with data from animal models and suggest that PVT and its connectivity with emotional memory circuitry may be a key hub of vulnerability in the adolescent brain. Future research will consider network analysis measures appropriate for assessing the PVT network, ELA, and mental health outcomes across cohorts and timepoints.

Keywords: paraventricular nucleus of the thalamus, early-life adversity, anhedonia, Adolescence, unpredictability

Disclosure: Nothing to disclose.

P26. Effects of Racial Discrimination on Cortical Oscillatory Dynamics Subservient Affective Processing During Adolescence

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Background: Experiencing racial discrimination (RD) potentially predicts adverse physical and mental health outcomes in Black-identifying adults, including obesity, cardiovascular disease risk, accelerated biological aging, oxidative stress, and a range of mental health disorders. Still, there is a dearth in knowledge about how RD affects Black youths' development, particularly their neural functioning. Thus, this preliminary study investigated how RD experiences affect neural oscillatory dynamics during an emotional face processing task, with the core hypothesis being that elevated RD exposure would alter stress-sensitive circuitry (e.g., fronto-limbic networks).

Methods: A sample of 37 Black-identifying youth (9–16 years old; 18 females) completed an emotional face processing task during an magnetoencephalography (MEG, 306 magnetic sensor TRIUX neo system) scan, and a T1-weighted MRI structural scan for co-registration. In the MEG task, participants viewed angry, happy, and neutral faces (1500 ms, 1600 (± 100) ms fixation; 240 trials) from the demographically-diverse RADIATE face database. Participants were asked to make a gender attribution for each face (i.e., button press for male, or female), making the emotional processing orthogonal to the task response. In addition, they completed questionnaires about their perceived experiences of racial discrimination (RD). Data underwent noise and motion correction, and ocular and cardiac artifacts were removed. Next, the data were transformed into the time-frequency domain and significant oscillatory windows were identified for source imaging. A beamformer (Dynamic Imaging of Coherent Sources) was used to source image oscillatory responses, and whole-brain linear mixed-effects models were used to analyze interactions between task activity and RD experiences (age and reaction time were entered as covariates of no interest).

Results: Sensor space analyses revealed significant oscillatory responses ($p < .001$, following cluster-based permutation testing) in the alpha (8 – 14 Hz, 300-750ms) and beta (18 – 24 Hz, 350-750ms) frequency bands. Linear mixed-effects models (all $p < .005$, $k = 10$) revealed main effects of emotion in both the alpha and beta bands in regions previously implicated in affective processing (i.e., alpha: left parahippocampal gyrus, beta: left ventromedial prefrontal cortex, left precuneus). Post-hoc comparisons between emotion largely showed blunted responses for neutral compared to happy or angry faces (all $p < .05$). Of most interest, there was an interaction between racial discrimination exposure and emotion in the beta band within the left orbitofrontal cortex. Post-hoc analyses revealed that for neutral faces only, youth with greater levels of RD exhibited diminished beta oscillatory responses compared to youth with lower levels of RD exposure ($p < .001$).

Conclusions: These preliminary results suggest that greater levels of racial discrimination (RD) exposure during development are linked to blunted beta band activity in response to ambiguous emotional stimuli (i.e., neutral faces). This result was specific to the left orbitofrontal cortex, which is known to be rich in glucocorticoid receptors across the lifespan and across species, suggesting that these results may be due to targeted stress effects. Moreover, the orbitofrontal cortex is among the last to mature structurally and functionally, with developmental fine-tuning occurring well into adolescence, making it especially sensitive to potential developmental insults. In addition, the specificity of these findings for neutral faces is in line with prior literature documenting stress- and psychiatric-related individual differences in processing ambiguous stimuli (e.g., negative attribution biases). Finally, increased beta band activity has previously been implicated in helping to orchestrate processing of affective images and dynamic emotional displays, suggesting that the blunted activity linked with RD exposure revealed in the current study could be indicative of stress-related alterations. Taken together, these findings highlight RD exposure as dimension of early life stress that is likely consequential for affective circuitry undergoing protracted development. Next steps in this line of work will be to examine how these aspects of affective processing intersect with RD experiences and risk for elevated psychopathology symptoms.

Keywords: stress in adolescence, racial discrimination, Early life stress (ELS), magnetoencephalography, Emotion Circuitry

Disclosure: Nothing to disclose.

P27. Juvenile Chronic Social Defeat Stress (jCSDS) Alters Fear Responding and Neural Circuit Activation in Adult Male Mice

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Background: Adolescent stress exposure is known to contribute to adult psychiatric symptomology. Although recent studies using early life trauma models have yielded crucial insights, the neural mechanisms mediating this relationship are largely unknown. In the present study, we investigate how adolescent stress alters neural activation upon exposure to fear-inducing stimuli in adulthood.

Methods: C57BL/6 male mice were exposed to juvenile chronic social defeat stress (jCSDS) from P29 to P38. jCSDS involves 5 minutes of direct exposure to a resident aggressive male CFW mouse daily, as well as housing adjacent to (though physically separated from) residents. Fear responses in adult jCSDS-exposed mice, as well as ongoing age matched naïve controls, were evaluated using several behavioral measures, such as social interaction and auditory fear generalization. Neural activation was probed broadly through whole-brain cFos staining, as well as in a region- and circuit-specific manner using virally delivered DREADD chemogenetic inhibition.

Results: Adult jCSDS-exposed mice showed significantly increased fear generalization to novel tones ($p = 0.022$, $n = 11$) and decreased social interaction when exposed to non-aggressive CFW males ($p < 0.0001$, $n = 95$ across multiple experiments). DREADD-mediated inhibition of the anterior insula resulted in increased social interaction to non-aggressive CFW males in adult jCSDS-exposed mice ($p = 0.034$, $n = 16$).

Conclusions: jCSDS results in robust increases in fear responding and generalization for social and non-social fear into adulthood. Adult jCSDS-exposed mice also show widespread changes in neuronal activation upon exposure to a fear-inducing social stimulus. The anterior insula is involved in mediating social fear responses in adult jCSDS-exposed mice, as demonstrated in whole brain correlational (c-fos) and causal (DREADD) analyses.

Keywords: Chronic social stress, Early life stress, Anterior insula, cFos, DREADD

Disclosure: Nothing to disclose.

P28. Inhibition of Post-Lanosterol Biosynthesis by Fentanyl: Implications for Fetal Fentanyl Syndrome (FFS)

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Background: A recent study discovered a novel, complex developmental disability syndrome, most likely caused by maternal fentanyl use disorder. This Fetal Fentanyl Syndrome (FFS) is biochemically characterized by elevated 7-dehydrocholesterol (7-DHC) levels in neonates, raising the question if fentanyl inhibition of the dehydrocholesterol reductase 7 (DHCR7) enzyme is causal from the emergence of the pathophysiology and phenotypic features of FFS.

Methods: To test this hypothesis, we undertook a series of experiments on Neuro2a cells, primary mouse neuronal and astrocytic cultures, and human dermal fibroblasts (HDFs) with DHCR7 +/+ and DHCR7 +/- genotype ($n = 5-6$ replicates per group). We used ANOVA to determine statistical significance.

Results: Our findings suggest that at baseline (vehicle-treated cells) developing neurons had the highest post-lanosterol biosynthesis. This was true for every analyte measured, with the exception of 7-DHD, which was found at higher levels in astrocytes. In addition, Neuro2a cells had generally the lowest levels of analytes of the three cell types we queried. In the light of these findings, perhaps it is not surprising that developing neurons showed the most robust response to fentanyl, followed by astrocytes and Neuro2a cells. These results revealed that fentanyl is an inhibitor of DHCR7 all three systems. However, while the increases of 7-DHC and 8-DHC and DES decrease could be attributed to DHCR7 inhibition, we also observed robust increases in Zyme, Zym, 14-dZym, and 14-dZyme, which are not features of the known DHCR7 inhibition pattern. This suggests that fentanyl has the potential to impede the developmental accumulation of cholesterol in the brain by targeting multiple post-lanosterol enzymes, potentially also inhibiting DHCR14 and/or EBP.

Sterol levels in untreated control and DHCR7 +/- human fibroblasts were different, with DHCR7 +/- HDFs having higher baseline 7-DHC and reduced DES and CHOL levels. In follow-up experiments we found that heterozygous DHCR7 +/- HDFs were significantly more susceptible to the sterol biosynthesis inhibitory effects of fentanyl than wild-type DHCR7 +/+ fibroblasts. Both control HDF lines and DHCR7 +/- HDF lines responded to fentanyl with the hallmark signature of DHCR7 inhibition in a dose-dependent fashion (increased 7-DHC). However, the magnitude of the change in 7-DHC levels in response to fentanyl exposure was

greater in the DHCR7 +/- HDF lines. These data suggest that DHCR7 +/- heterozygosity (present in approximately 3% of the human population) of the mother and/or developing child (and potentially other sterol biosynthesis genes), when combined with maternal fentanyl use disorder, might be significant contributory factors to the emergence of FFS in the exposed offspring.

All above-listed findings exceeded significance of $p < 0.01$.

Conclusions: The complex biochemical disruption we observed would likely alter many molecular processes through an imbalance of sterol homeostasis and altered generation of biologically active oxysterols. The overall findings suggest that maternal fentanyl use in the context of an opioid use disorder leads to FFS in the developing fetus through a strong disruption of the whole post-lanosterol pathway that is more complex than a simple DHCR7 inhibition. In a broader context, we believe that evaluation of new and existing medications for their effects on sterol biosynthesis should be an essential consideration during drug safety determinations, especially in pregnancy.

Keywords: Fentanyl, Fetal Fentanyl Syndrome, Sterol Biosynthesis, Opioid abuse

Disclosure: Nothing to disclose.

P29. Anti-Aggressive and Pro-Social Effects of PGI-7043, a 5-HT1B/1D Receptor Agonist, in Rodents

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Background: The neurobiology underlying agitation in psychiatric, neurologic and neurodevelopmental disorders is currently unknown. However, there is substantial non-clinical evidence supporting the hypothesis that stimulating central serotonin (5-HT) 1B/1D receptors can suppress aggressive behavior without inducing sedation: (i) 5-HT1B receptor knock-out mice are more aggressive than wild-type controls, and (ii) selective 5-HT1B receptor agonists such as CP94253 suppress aggressive behavior in a wide range of rodent models without sedative effects. To date, there are no approved brain-penetrant, selective 5-HT1B receptor agonists. The triptans, a class of drugs used to treat migraine, activate 5-HT1B/1D receptors, but they poorly penetrate the blood-brain barrier, and their mechanism of action is likely mediated at the level of the meningeal vasculature. Here we describe the characterization of PGI-7043, a potent, brain-penetrative 5-HT1B/1D receptor agonist in rodent models along with its target engagement and a biomarker to support clinical studies.

Methods: Iterative medicinal chemistry design and synthesis of analogs of a hit compound was conducted, and compounds tested for in vivo activity in PsychoGenics' mouse SmartCube® platform. Briefly, mice (8-10 male/dose) were treated with test or reference compounds, administered i.p. 15 min prior to the study, and then placed in the SmartCube® system, which runs through an automated sequence of stimulatory challenges for 45 min. Behavioral features were captured with optical and mechanical sensors using various machine-learning techniques, and the resulting behavioral profiles were compared at the dose level using machine-learning derived similarity scores. Compounds' profiles were assigned to a putative therapeutic class using a deep-learning model trained and tested using more than 100 reference drugs. Compounds of further interest were evaluated for in vitro functional activity at serotonin receptors (EuroScreen), and at anti-targets (Eurofins' Safety47). Metabolic stability was evaluated using hepatocytes from human, rat, mouse and dog in vitro, and in vivo pharmacokinetics was determined in rodents

and in dog. Approx. 300 analogs were synthesized in the program, resulting in the identification of the development candidate, PGI-7043 as well as several backup molecules. Activity in rat and mouse aggression models was determined in the resident intruder offensive model, in which a 5-min interaction between the resident male, previously housed with a female, and a male intruder are recorded and scored blinded. This study was powered with $n = 24$ (rat) and 18 (mouse) per dose group. EEG studies were conducted at PsychoGenics in rats implanted with DSI-SO2 telemetry transmitter system with $n = 12$ per group. Ex vivo receptor occupancy studies were conducted in mouse and rat by quantifying radioactivity in striatal slices following incubation with 0.6 nM 3H-GR125743 using phosphoimaging. All studies involving animals were conducted under approved IACUC protocols.

Results: PGI-7043 produced an antidepressant phenotype in SmartCube®, with a minimal effective dose (MED) of 0.3 mg/kg (i.p.). In contrast, zolmitriptan was weakly effective in SmartCube®, with an MED of 30 mg/kg (i.p.). PGI-7043 was a full agonist at human 5-HT1B and 5-HT1D receptors in a functional GTPγS assay with EC50 values of 1.3 and 0.5 nM, respectively. In the Groningen rat resident intruder model, PGI-7043 at 0.3 and 1 mg/kg (i.p.) extended the latency to the first attack of the resident and reduced offensive behaviors without causing sedation (one-way ANOVA using Dunnett's multiple comparisons test. $P < 0.01$). Strikingly, PGI-7043 also increased social exploration at 0.3 and 1 mg/kg (i.p.). This contrasted with the profile of the atypical antipsychotic, olanzapine (2.5, 10 mg/kg, i.p.), which attenuated offensive behavior, but at doses that were sedative, reducing social interaction and increased inactivity time. PGI-7043 was similarly active in the mouse resident intruder model: latency to first attack was increased, number of attacks was reduced, and social interactions were increased at 1 and 3 mg/kg (p.o.).

In rat EEG studies, PGI-7043 suppressed REM sleep at 0.3, 1 and 3 mg/kg (p.o.), suggesting its utility as a non-invasive translational biomarker for clinical studies. Ex vivo receptor occupancy studies confirmed occupancy of central 5-HT1B/1D receptors with an ED50 of 0.3-1 mg/kg p.o., consistent with behavioral and EEG studies.

Conclusions: PGI-7043, discovered using SmartCube®, is a highly brain-penetrant 5-HT1B/1D agonist with excellent drug-like properties. PGI-7043 exhibited a novel profile in resident intruder models of aggression, reducing aggressive behaviors while enhancing social interactions. This profile suggests a potential therapeutic in clinical populations of agitated patients such as those with Alzheimer's disease, bipolar disorder and schizophrenia, and in patients with reduced social behaviors such as those diagnosed with autism spectrum disorders.

Keywords: Irritability/Aggression, Sociability, Alzheimer's disease, Schizophrenia, Autism Spectrum Disorder

Disclosure: PsychoGenics: Employee (Self). Acetel Inc., Blue Oak Pharmaceuticals: Advisory Board (Self). Luminous Mind Inc.: Consultant (Self). Eli Lilly and Company: Stock / Equity - Publicly Traded Company (Self). Eli Lilly and Company: Employee, Stock / Equity - Publicly Traded Company (Spouse/Partner)

P30. Targeting Opioid Neurotransmission in Borderline Personality Disorder With Self-Harming Behavior: Preliminary Findings of a [11C]NOP-1A Positron Emission Tomography Study

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Background: Non-suicidal self-injury (NSSI) is a core symptom of borderline personality disorder (BPD). Reduced pain sensitivity

and increased pain threshold were reported in BPD pointing to an altered pain perception. Though, research investigating biomarkers contributing to attenuated pain sensation in mental disorders is limited. The nociceptin/orphanin FQ (N/OFQ) peptide (NOP) receptor is involved in pain transmission. Supraspinal administration of N/OFQ in animal studies demonstrated an elevated pain sensation. Our study focuses on measuring NOP receptor in BPD with NSSI using positron emission tomography (PET) imaging and the novel radiotracer [^{11}C]NOP-1A. We hypothesize BPD + NSSI would have lower levels of NOP compared to healthy controls (HC) in brain regions altered in BPD and emotional pain processing including anterior cingulate cortex, amygdala, and thalamus.

Methods: 13 BPD + NSSI participants (all females) were compared with 16 age and sex-matched HCs (all female). Participants were between 18 to 50 years of age. BPD + NSSI participants were not actively taking psychotropic medication or recreational substances. BPD + NSSI participants met the diagnostic criteria for BPD and at least 7 NSSI episodes in the past 4 months before their enrollment in the study. All participants completed a structural magnetic resonance imaging scan. Using the high resolution research tomograph (HRRT) scanner and arterial blood sampling, NOP binding was recorded following an intravenous injection of the radiotracer [^{11}C]NOP-1A. PMOD (v4.203) and PELI (v2019) were used to calculate the total tissue volume of distribution of NOP (NOP VT) reflected via the Logan graphical analysis method. A linear mixed effects model was used for statistical analysis.

Results: NOP VT levels were 4.9% lower in amygdala (p -value = 0.048) and Brodmann Area 25 (p -value = 0.046) in BPD + NSSI participants compared to HCs. Moreover, there was a 4.8% and 4.4% mean difference in thalamus (p -value = 0.054) and ventral striatum (p -value = 0.073), respectively.

Conclusions: Although these results are preliminary, attenuated NOP signaling in limbic areas indicates that an endogenous imbalance may be present, which could be a contributing factor to dampened pain sensation in BPD + NSSI. The limbic regions investigated in our study are known to be involved in processing affective aspects of pain, while emotional instability is a cardinal feature of BPD. We aim to enroll more participants in the study to strengthen our findings.

Keywords: Borderline Personality Disorder, Positron Emission Tomography (PET) Imaging, Opioid system

Disclosure: Nothing to disclose.

P31. Resource Scarcity Affects Postpartum Defensive Behaviors and Related Circuits

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Background: Postpartum affective disorders (PAD) are characterized by a suite of mood changes including altered threat processing. Low socioeconomic status (SES) increases the risk for PAD. Despite the impact of PAD and a dearth of treatments, few preclinical studies focus on postpartum behavior and brain changes using translational animal models. Here we used a resource scarcity manipulation, the limited bedding and nesting (LBN) procedure, that mimics aspects of a low SES environment during the postpartum period in rats. We assessed how LBN altered defensive behaviors to a threatening stimulus and associated circuits.

Methods: In LBN, dams and pups (postnatal day, PND2) were housed with no enrichment and limited nesting materials. Control dams had ample nesting material and enrichment. Prior work from our lab and others found that LBN alters maternal behavior

causing hypervigilant parenting and fragmented care. Yet whether LBN affected threat responses was unknown. Here we addressed this gap by introducing a late adolescent male, considered a mild threat, into the home cage of LBN or control dam on pups' PND 10. We scored defensive behaviors including offensive (pin, wrestle, shove) and defensive (kick, box, bite) aggression. We next processed brains for whole brain cFOS analysis to determine circuits differently activated in response to threat in LBN v. control dams. Females are a focus of the work as we are interested in postpartum defensive behaviors and analyses were conducted with independent samples t-tests.

Results: LBN dams ($n=11$) spent less time attacking the intruder than control dams ($n=15$) [$t(24)=2.43$, $p<.001$, $\eta^2=.20$], an effect due to reduced offensive [$t(19.05)=2.73$, $p=.004$, $\eta^2=.28$] but not defensive aggression [$t(22.07)=0.93$, $p=.40$, $\eta^2=.04$]. When individual offensive behaviors were assessed, the duration of pinning [$t(14.93)=3.01$, $p=.009$, $\eta^2=.38$] and wrestling [$t(16.03)=2.41$, $p=.03$, $\eta^2=.27$] but not shoving [$t(21.01)=0.71$, $p=.49$, $\eta^2=.03$] were reduced in LBN v. control dams. This reduced pinning in LBN dams suggested diminished dominance, even though the intruder should be perceived as a minor threat. Preliminary cFOS data reveal that this encounter reduces cFOS in the frontal cortex [$t(8)=4.778$, $p=.0014$, $\eta^2=.74$] and the lateral habenula [$t(8)=2.18$, $p=.061$, $\eta^2=.37$], which mediates male aggression, in LBN v. control dams.

Conclusions: These findings suggest resource scarcity alters threat perception and defensive behavior in rats. We are the first, to our knowledge, to link different patterns of maternal defensive behavior to brain circuits. These studies may reveal novel mechanisms involved in altered threat perception in PAD, particularly in low SES moms. Ongoing studies are integrating human neuroimaging data from low SES moms during a threat perception task with current findings to choose regions for follow-up molecular analysis with high translational potential

Keywords: Social Threat, maternal behavior, aggression, maternal stress

Disclosure: Nothing to disclose.

P32. The Aggressive Amygdala: A Crh+ Cell Activity Signature for Attack Initiation

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Background: Physical aggression between members of the same species represents the last resort for settling a dispute; while specific brain areas and pathways are known to evoke and sustain attacks, less is known about the neural signaling substrates that mediate aggressive escalation. Amygdala corticotropin releasing hormone cells (Amy-Crh) are known to facilitate active reactions to threatening stimuli and may be more broadly implicated in fight-or-flight responding.

Methods: We examined the role of Crh signaling during agonistic confrontations in male or female mice. To examine the role of Amy-Crh cells in the escalation of territorial inter-male aggression, we combined naturalistic behavioral observations with in vivo single-cell calcium imaging, chemogenetics, in vivo pharmacology, localized knockout, and cutting-edge state-dependent optogenetics ($n=5-11$ mice/condition). Novel brain regions involved in inter-female aggression were also uncovered using whole brain activity mapping and in vivo pharmacology.

Results: Using in vivo single-cell calcium imaging, we identified an Amy-Crh cell activity signature that intensifies with increasing

attack probability during inter-male territorial aggression. Chemo-genetic Amy-Crh activation produced aberrant attacks toward non-threatening social stimuli whereas Amy-Crh cell inhibition prevented attacks. Using closed-loop optogenetics triggered by attack-predictive neural activity, we found that clipping attack-predictive cell activity could block attacks. Through localized genetic knockout strategies, we also observed that depleting amygdala CRH persistently blunted the acquisition of experience-escalated aggression. In female mice, we identified a sex-specific pattern of behavior and found a downregulation of inter-regional connectivity in aggressive vs. non-aggressive mice. Importantly, blocking CRH signaling through CRH-R1 antagonism blunted inter-male aggression without affecting inter-female fighting.

Conclusions: Our findings converge on Amy-Crh cell activity as a sex-specific neurobiological readout of aggressive internal state that predicts attack onset in male mice. This cell population may serve as a unique and potent site for the selective treatment of pathological aggression in specific patient populations.

Keywords: Aggression, CRH+ neurons, Amygdala, Active Avoidance, In vivo calcium imaging

Disclosure: Nothing to disclose.

P33. A Neural Signature of Sleep Deprivation in Human Brain

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Background: Sleep disturbances and sleep deprivation (SD) are common complaints in general population and interfere with normal cognitive and emotional functioning. However, the neural mechanisms underlying the functioning deficits are unclear and reliable neural signatures of SD are lacking. Here, we combined functional magnetic resonance imaging (fMRI) and predictive machine learning modeling to establish a neural signature of SD.

Methods: We analyzed human fMRI data from healthy adults (dataset 1, $n = 152$) that were randomized into 3 groups: normal sleep (NS, $n = 48$), sleep restriction (SR, $n = 52$), or SD ($n = 52$). To construct and validate a neural signature of SD, we combined intrinsic whole-brain functional connectivity (FC) with a machine learning algorithm. The neural signature of SD was cross validated by decoding SD from NS, and the significance of its performance was assessed using a permutation test. To test the generalizability of the constructed decoder, we applied it to two external validation datasets in which participants underwent both pre- and post-SD resting-state scanning sessions (dataset 2: $n = 68$; dataset 3: $n = 54$). We further examined the neural signature after recovery sleep (SD group from dataset 1) and after SR (SR group from dataset 1, and dataset 4: $n = 76$), and its association with individual's total sleep time as measured by actigraphy (without sleep manipulation; dataset 5: $n = 109$; dataset 6: UK Biobank, $n = 39,799$). The connections that contributed the most to the signature of SD were examined.

Results: The constructed classifier significantly discriminated between SD and NS (area under the receiver operating characteristic curve [AUC] = 0.91, $p < 0.001$) and successfully generalized to external datasets (dataset 2: force-choice accuracy = 94.1%, $p < 0.001$; dataset 3: 83.3%, $p < 0.001$). The neural signature of SD remained significant after a night of recovery sleep (SD vs. NS, AUC = 0.73, $p < 0.001$), and in individuals that underwent SR (dataset 4: SR vs. NS, $p < 0.001$). The neural signature of SD was negatively correlated with total sleep time (dataset 5: $r = -0.39$, $p < 0.001$; dataset 6: $r = -0.068$, $p < 0.001$). Distributed neural systems, especially the default mode, dorsal attention, subcortical, and cerebellum networks, contributed to

the neural signature of sleep deprivation. These connections were partially restored after a night of recovery sleep and are responsive to sleep manipulation (restriction vs. deprivation).

Conclusions: We show that sleep disturbances and deprivation interfere with normal brains connectivity patterns and that the sleep related changes to functional connectivity can be used to identify sleep-deprived individuals. These findings highlight a distributed neural basis for sleep deprivation and provide a potential neural signature for identifying sleep-deprived brains, offering new insights into the neural impacts of sleep loss.

Keywords: Sleep disturbances, machine learning, neural signature

Disclosure: Nothing to disclose.

P34. Towards Multi-Arm Prediction of Remission to Antidepressant Therapy Using Multi-Modal Integration: A Case Study Using EMBARC Study

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Background: Remission rates of first-line antidepressant treatments for Major Depressive Disorder (MDD) are low and clinical and sociodemographic measures along challenge the predictability of treatment outcomes for individualizing treatment. This work sought to integrate baseline clinical and demographic measures with week-1 imaging, electroencephalogram, and immune biomarkers to assess the ability to simultaneously predict 16-week remission to sertraline, or bupropion (after failure to respond to sertraline), or placebo treatments.

Methods: All patients with MDD, clinical remission status at 16 weeks, and complete biological datasets in the 'Establishing Moderators and Biosignatures of Antidepressant Response for Clinical Care for Depression' (EMBARC) study were included ($N = 88$). Patients were initially treated with sertraline or placebo and depending on response status at 8 weeks of therapy, their therapy was maintained or switched (to Sertraline, if a non-responder to placebo, or to Bupropion, if a non-responder to Sertraline). Resting state fMRI, EEG, plasma protein, and clinical items (8,949) collected at baseline were used as predictors. EEG features collected at 1-week of therapy and changes from baseline to 1 week were also included as predictors. Feature selection was performed utilizing a wrapper-based algorithm. Selected features were incorporated into several supervised ML approaches (random forest, penalized regression, naïve bayes, K-nearest neighbors) and compared on predictability of remission after 16 weeks. Initial models were built using data from patients treated with sertraline during both stages of the study ($N = 54$) (sertraline responders and placebo non-responders switched to sertraline). Five-fold nested cross validation was used to train and test models, with 75% of the sample allocated for training. The training and testing split was repeated 10 times, and results were averaged over splits. External validation of models was done using the subset of patients who were placebo responders ($N = 19$) and sertraline non-responders ($N = 15$).

Results: A wrapper-based feature selection algorithm yielded 16 features (of the 8,949) for predicting remission of MDD at 16 weeks. Random-forest based integration of blood, EEG, fMRI brain connectivity, and clinical data resulted in the highest area under the receiver operating characteristic curve (AUC) when trained and tested on patients taking sertraline during stage 2 (mean AUC [95% CI] = 0.73 [0.63-0.83]). Sensitivity was 0.92 and specificity was 0.30. When externally validated on placebo-

responders the model achieved a mean AUC of 0.71 [0.64-0.77], with a sensitivity of 0.964 and specificity of 0.05. When validated on sertraline non-responders the mean AUC was 0.77 [0.71-0.84] with a sensitivity of 0.72 and specificity of 0.34.

Conclusions: Integration of baseline and week-1 blood, and brain features improve prediction of MDD at 16 weeks. Models were externally validated, with minimal reduction in AUC and accuracy. Specificity and sensitivity values suggest the utility of these features as treatment-nonspecific biomarkers for predicting remitters, yet further emphasize the need for biomarkers to predict non-remitters. Future work will aim to understand the interactions between these brain and blood predictors and further validate findings.

Keywords: Antidepressant, machine learning, Treatment Prediction

Disclosure: Nothing to disclose.

P35. Q-MRS: Quantitative Magnetic Resonance Spectra Processing and Analysis Deep-Learning Framework

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Background: Magnetic Resonance Spectroscopy (MRS) is a non-invasive technique for measuring brain metabolite concentrations, offering insights into neurometabolic processes. Traditional MRS methods face challenges like low signal-to-noise ratios and complex modeling, affecting accuracy and reliability. Accurate metabolite quantification is crucial for both research and clinical applications, particularly in neurological disorders.

Deep learning technologies, especially Convolutional Neural Networks (CNNs) and Long Short-Term Memory (LSTM) networks, have shown promise in enhancing spectral analysis. These models can capture both local and global data patterns, potentially overcoming traditional MRS limitations.

This study introduces and validates a CNN-LSTM-based MR spectra fitting framework, Q-MRS, for improved MR spectral analysis. We tested its effectiveness using simulated and in vivo datasets and applied it to a clinical cohort at high risk for psychosis, where previous research identified abnormal Glutamate/Glutamine (GLX) levels. Our goal is to establish Q-MRS as a robust tool for better diagnostic and monitoring capabilities in neurological conditions.

Methods: A basis set containing fourteen metabolites was simulated using Osprey with the same scanning parameters as the in vivo data.

For the in vivo dataset, GABA-edited MEGA-PRESS datasets from the Big GABA repository, acquired on a 3T Philips scanner, were collected from 101 subjects (age range: 18-34 years). Raw data was pre-processed using Osprey's Proc module, including spectral registration, eddy-current correction, Fourier transform, and linear baseline correction.

The simulated dataset contained 100,000 pairs of edit-OFF and DIFF spectra, with amplitude ranges derived from a meta-analysis on healthy young adults. Parameters were derived from initial fitting of the in vivo data using Q-MRS-net. Spectra were simulated from a uniform distribution with added randomness using Sobol sequences, with Gaussian noise added before normalization.

The Q-MRS framework involved the initial fitting of the in vivo data to obtain parameter ranges, training on simulated data, and transfer learning where pre-trained model weights were used, freezing all but the final output layers in each head of the Multilayer Perceptron (MLP).

The network architecture included a convolution and max-pooling layer, three inception modules, a bidirectional LSTM, and multi-headed MLP layers. The loss function for in vivo data was the

mean squared error (MSE) between the predicted and target spectra.

The clinical utility of Q-MRS was evaluated with a cohort of 'clinical high risk' (CHR) patients. In vivo brain spectra were acquired using 1H-MRS at 3 Tesla, allowing reliable measurement of glutamate peaks. The dataset included 23 CHR patients and 11 healthy controls.

For statistical analysis, demographic differences were assessed using t-tests. ANOVA compared GLX, GABA, and GLX + GABA levels between CHR patients and controls. Logistic regression evaluated the predictive power of these metabolites.

Results: For the performance evaluation of our proposed Q-MRS framework, we compared the Mean Absolute Percentage Error (MAPE) of metabolite amplitudes predicted by four methods against ground truth values. The Q-MRS framework, utilizing a CNN-LSTM architecture, demonstrated the lowest MAPE across all metabolites. Q-MRS also produced the lowest coefficient of variation (CV) for most metabolites, indicating stable and consistent estimations across the healthy population. Our Q-MRS framework, which integrates local and global spectral features, showed enhanced accuracy and reproducibility in metabolite quantification.

For the CHR data analysis, we were able to replicate our previously published finding on the hippocampal neurometabolic pathophysiology of CHR patients. Compared with control subjects, patients were found to have an elevation of GLX ($p = 0.049$), and the elevation is dominated almost exclusively by Glutamate ($p = 0.030$). We also observed a co-elevation in both Glutamate and GABA, and the elevation in Glutamate+GABA most reliably distinguishes CHR patients from controls ($p = 0.016$).

Conclusions: This study demonstrates a new deep learning-based LCM fitting framework using a CNN-LSTM model to enhance MRS spectral analysis accuracy and reliability. Using a robust architecture and simulated and in vivo datasets, we addressed key challenges in traditional MRS methods, such as low signal-to-noise ratios and complex modeling.

The Q-MRS framework excelled in metabolite quantification, showing the lowest MAPE and CV across almost all tested metabolites. This indicates improved measurement precision and consistency within the healthy population. Our framework's integration of local and global spectral features ensures accurate and reproducible results, meeting critical needs in MRS research and clinical applications.

Applying Q-MRS to a high-risk psychosis cohort replicated neurometabolic findings, particularly GLX elevations. Q-MRS distinguished Glutamate as the main driver of GLX and identified Glutamate and GABA co-elevation as key markers.

In summary, the CNN-LSTM-based Q-MRS framework advances MR spectral analysis, enhancing diagnostic and monitoring capabilities in neurological conditions. Future work will validate its use across diverse populations and clinical settings.

Keywords: Magnetic Resonance Spectroscopy (MRS), AI, Metabolite Quantification, Clinical high-risk for psychosis, glutamate and gaba

Disclosure: Nothing to disclose.

P36. Detecting Infant Cry From Naturalistic Audio Recordings: Validity and Extension of a Binary Infant Cry Classifier in a Sample of 12-Month-Old Infants Enriched for Irritability and Language Delay

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Background: Features of vocalizations early in life are promising markers of later psychopathology. Wearable devices can be used to passively collect naturalistic audio recordings that capture vocal sounds from at-risk infants, such as cry, laughter, and early language. However, it is challenging and tedious to annotate the large volumes of resulting audio records manually, and machine learning algorithms may be able to automate this process. With datasets annotated automatically via machine learning algorithms, researchers will be able to leverage highly ecologically valid datasets for the evaluation of specific features of infant vocalizations with potential predictive utility. Here, we developed a novel, open source implementation of an existing algorithm (Micheletti et al., 2023; Yao et al., 2022) that can be used for infant cry detection and other audio detection applications. We examined the validity of this novel, open source implementation for cry detection in a new sample of 12-month-old infants enriched for irritability and language delay.

Methods: We recruited 356 12-month-olds (54.21% male, 49.44% White) enriched for irritability and language delay and their parents from the community as part of the When2Worry study at Northwestern University (PIs: Lauren Wakschlag, PhD, Elizabeth Norton, PhD). Using infant-worn devices that are part of the Language ENvironment Analysis system (Gilkerson et al., 2017), 295 families completed daylong, naturalistic audio recordings. In addition, infants completed the Disruptive Behavior Diagnostic Observation Schedule (DB-DOS), which is a lab-based task designed to elicit disruptive behaviors (Wiggins et al., 2018).

We divided infants into quartiles of low ($n = 92$), moderate ($n = 89$), high ($n = 77$), or very high ($n = 84$) irritability based on their DB-DOS scores and randomly selected 25 infants from each quartile. When examining the files of the selected subjects, if any files were found to lack information or were deemed unusable (e.g., perfectly silent, very noisy, or containing only a small portion of useful data), we replaced them with new files from the same category. We randomly extracted a 10-min audio recording from each infant's daylong audio recording. Next, we conducted automated quality testing using traditional detection and signal processing algorithms. After applying a low (250 Hz) and high (3000 Hz) frequency bandpass filter to extract frequency components in the range of infant cry, we computed the energy of the filtered signal using a sliding window. Then, we calculated the energy ratio between the short-term and the long-term average energy. If > 1.5 , it was considered that the window may contain a cry event. If the 10-min dataset failed to meet this sound quality threshold, we extracted another 10-min segment from the same recording. We repeated this step up to 10 times before selecting a new audio recording.

Trained research assistants annotated 2,429 secs of "cry" vocalizations (including cries, whines, and fusses, consistent with coding methods reported in Micheletti et al., 2023) from 60,000 secs of audio data using ELAN open-source software. A subset ($n = 20$, 20%) of 10-min audio recordings were double coded; Holley and Guilford's G (Cohen's Kappa alternative for skewed datasets) = .96, representing a strong level of agreement among coders.

We developed a novel implementation of an existing cry detection algorithm (Micheletti et al., 2023; Yao et al., 2022). The implementation is based on scikit-learn and Pytorch, and the code is publicly available. We adapted the existing cry detection algorithm from TensorFlow to PyTorch with parameter optimizations. We used a publicly available dataset (Micheletti et al., 2023; Yao et al., 2022) to develop the algorithm. We split the 100 10-min annotated audio recordings into training ($n = 70$), validation ($n = 10$), and test ($n = 20$) sets that were balanced in the number

of cry and no-cry events. The algorithm is a support-vector machine classifier that operates on a combination of acoustic features and features derived from mel spectrograms with a neural network. We evaluated the performance of the algorithm by calculating precision, recall/sensitivity, specificity, and F1 scores. Analyses were conducted in the original dataset in which it was developed and the current validation dataset.

Results: In the original dataset in which the algorithm was developed, accuracy = .789, precision = .739, recall/sensitivity = .895, specificity = .684, and F1 = .810. In the current dataset, relative to hand-annotated ground truth, accuracy = .871, precision = .211, recall/sensitivity = .631, specificity = .888, and F1 = .316.

Conclusions: Leveraging PyTorch's dynamic computation graph and efficient memory management, we achieved increased training speeds and improved scalability. The binary cry classifier performed very well in identifying no-cry (specificity) and performed moderately well in identifying cry (recall/sensitivity) in the current dataset. The classifier was severely limited in precision; that is, the model produced many false positives. Poor precision was, in part, due to limitations surrounding our highly unbalanced validation dataset; there were relatively few cases of cry compared to many cases of non-cry, which we would expect naturalistically. Future work will focus on optimizing the current model, developing a new model to enhance precision, and examining individual differences in model performance (e.g., sex).

Keywords: machine learning, Infancy, cry

Disclosure: Nothing to disclose.

P37. External Validation of a Latent Diffusion Model for Brain Imaging Generation

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Background: Acquiring MRI data is resource intensive; this limits large-scale data collection. Generative models, particularly latent diffusion models (LDMs), have shown promise in producing realistic images and can be conditioned on text or labels, offering a way to expand and complement real datasets. This study aims to externally validate an LDM trained to generate synthetic brain MRI volumes, using an independent dataset.

Methods: Latent Diffusion Model

Pinaya et al. trained a LDM using data from the UK BioBank (UKB), including T1-weighted images from 31,740 participants. The model was conditioned on the following labels: age, gender, ventricular volume, and brain volume (normalized by head size) (https://github.com/Warvito/generative_brain).

Validation sample: We included T1w images acquired from 246 healthy individuals from three sites. The above-mentioned labels were used to condition the model and generate pairs of real – synthetic images.

Validation strategies: The following strategies were employed to evaluate the quality of the synthetic images:

1. Degree of realism:

- a. Fréchet Inception Distance (FID) and t-Distributed Stochastic Neighbor Embedding (t-SNE) were computed using image features extracted using a Convolutional Neural Network (CNN) trained on medical images (<https://github.com/Tencent/MedicalNet>). To account for potential differences in scan acquisition, both the original and synthetic images were preprocessed (intensity correction and normalization, skull stripping, rigid transformation from synthetic to original).

- b. Neuroanatomical features - including cortical and subcortical volumes, and cortical thickness - were extracted using SynthSeg (<https://github.com/BBillot/SynthSeg>). These features were then mapped using t-SNE.
2. Efficacy of conditioning:
 - a. Age: Brain age was estimated using a pretrained CNN model (https://github.com/irimia-laboratory/USC_BA_estimator), and compared across the pairs of real – synthetic images.
 - b. Gender: We trained a Support Vector Machine (SVM) classifier based on the SynthSeg output from the original images; the best model was then validated on synthetic images, and vice versa.
 - c. Brain Volume, Ventricular Volume: Metrics were extracted and compared across the pairs of real – synthetic images.

Results: Degree of realism: FID was computed using the same image features used in the original paper. However, between our two samples (real vs synthetic), the FID metric was very high (FID = 81971.28) compared to what was reported in the original paper (FID = 0.0076). This was detected despite using the features from the preprocessed images, something we thought could have minimized differences in scan acquisitions. On the other hand, t-SNE mapping of the same features did not seem to separate the two groups.

The t-SNE maps based on neuroanatomical features showed a clear separation between real and synthetic images on all levels of perplexity. While the LDM was not conditioned on these features, this seems to suggest that the distribution and/or characteristics of brain tissues were different across the pair (real – synthetic).

Efficacy of conditioning: Brain age estimates showed only a modest correlation within pairs ($r = 0.34$). However, brain age was a poor predictor of chronological age in both the real and synthetic datasets ($r = 0.33$ and $r = 0.36$, respectively), raising doubts about the validity of this metric for evaluating the accuracy of age conditioning. The SVM model for gender classification achieved similar high accuracy for real (75.25%) and synthetic (78.44%) brain scans in the discovery sample. In the validation sample, accuracy drops for both, with synthetic scans (50.81%) performing only slightly worse than real scans (56.50%). This suggests that the SVM generalizes similarly for gender prediction across both real and synthetic images, albeit with reduced overall performance.

In agreement with the original paper, brain and ventricular volumes showed high correlations across pairs of real and synthetic images ($r = 0.75$ and $r = 0.92$, respectively), indicating good accuracy for both types of conditioning.

Conclusions: This study validated an LDM for generating synthetic brain MRI volumes with key findings in realism and conditioning efficacy. Firstly, the synthetic images were visually very realistic based on internal ratings, though realism metrics yielded mixed results, particularly in neuroanatomical feature distributions that the model was not conditioned upon. Secondly, the conditioning efficacy was unclear for age and gender but showed good results for key neuroanatomical features like ventricular and brain volumes. These findings suggest that future work should focus on more granular neuroanatomical conditioning, incorporating global and regional features such as thickness, area, volume, and curvature. Conditioning on topology using control nets also appears promising. Such refinements could enhance anatomical accuracy and overall performance, broadening the application of LDMs in neuroimaging research.

Keywords: Generative Artificial Intelligence, Human Neuroimaging, Magnetic Resonance Imaging

Disclosure: Nothing to disclose.

P38. Digital Phenotyping to Cluster and Predict Clinical High Risk Patients in the Accelerating Medicines Partnership® Schizophrenia (AMP® SCZ) Observational Study

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Background: Digital phenotyping enables data capture with higher temporal precision than traditional and sporadic interviews. The Accelerating Medicines Partnership® Schizophrenia (AMP® SCZ) Observational Study uses the mindLAMP smartphone application to collect GPS, accelerometer, and screen state (off/on) measures that can inform behavioral features like sleep, mobility, sociability, etc. While data collection is still ongoing, exploring the potential of clustering approaches can help propose novel means to distinguish clinical high risk for psychosis (CHR) syndrome patients from community controls (CON) that can be utilized later with the full dataset to help predict conversion and to inform power analyses for digital outcomes in clinical trials.

Methods: Participants in our sample are from the ongoing international AMP SCZ Observational study ($n = 102$ with full sensor, survey, and clinical data at baseline, 90 CHR and 12 CON), which uses mindLAMP to collect smartphone data in both passive (e.g. GPS, home time, entropy, device usage) and active (e.g. daily EMA surveys and journaling) streams. 42 traditional clinical surveys were also administered as part of the study baseline measures.

Results: Within participant analysis revealed weaker correlations between EMA surveys and sleep duration, entropy and home time in CHR individuals compared to community controls (CON). Performing logistic regression to predict CON or CHR as the outcome using only variables from clinical measures revealed high accuracy (Pseudo-R-squared = 0.894) and PSYCHS Positive, SOFAS, Schizotypal and Calgary Depression scales as significant predictors. Random forest predictions and principal component analysis (PCA) also revealed similar sets of feature importances. When combining the top clinical predictors alongside passive digital measures like home time, screen time and sleep duration, partial correlations for CHR individuals show a negative relationship between screen time and sleep duration ($r = -0.45$), a positive relationship between home time and sleep duration ($r = 0.32$), a positive relationship between SOFAS score and screentime ($r = 0.24$), and a negative relationship between SOFAS scores and hometime ($r = -0.25$). In CON individuals, we saw positive correlations between OASIS scores and screen time ($r = 0.89$), positive correlations between depression and sleep duration ($r = 0.86$) and negative correlations between depression and screen time ($r = -0.85$).

Conclusions: The differences in relationships between digital and clinical variables across CHR and CON groups highlight the significant associations that emerge when incorporating active and passive digital measures, hence providing a unique predictor of CHR. Even at baseline, this integration offers a more comprehensive phenotype of CHR patients, revealing distinct patterns both at the level of individual variables and across all correlations within participants. Ongoing data collection and availability will enhance the power of these analyses and help identify clearer trends for clustering individuals over time. Future work will continue to integrate digital and clinical measures to further elucidate the longitudinal trajectories of CHR patients,

helping identify predictors of psychosis conversion and develop more holistic, personalized interventions.

Keywords: smartphone, Digital phenotyping, Clinical trial, Data quality, Schizophrenia, Within Person Variance, Clinical high-risk for psychosis

Disclosure: Otsuka: Grant (Self)

P39. Artificial Intelligence Assisting Psychiatric Diagnosis: A Simulated Patient Study

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Background: Despite speculation about the potential benefits of AI in mental health, empirical research on its application remains limited. This study tested the ability of a AI based application to perform a psychiatric examination generating a psychiatric diagnosis including level of severity, and recommendations for medication and psychotherapy.

Methods: Four standardized/simulated patients (SPs - role playing actors) were trained, each simulating a patient: in a major depressive episode, with an anxiety disorder, with PTSD with cannabis abuse, or in a manic episode; each with either mild-moderate or severe symptoms. A fifth SP was trained as a control, with complaints not severe enough to warrant a psychiatric diagnosis. Each actor was examined once by 10 board-certified psychiatrists and 10 times by the application.

Results: The "gold standard" were the diagnoses, severity and recommendations for treatment that the simulated patients were trained for. The percent agreements of the application with the gold standard was 94% (95% CI: 83.5%-98.7%), between the application and the psychiatrists was 93% (95% CI: 90.0%-95.9%), and between the psychiatrists and the gold standard was 100%. Regarding severity, the percent agreement between the application and the gold standard was 82.5% (95% CI: 67.2%-92.7%), between the application and the psychiatrists was 66.5% (95% CI: 61.5%-71.5%) and between the psychiatrists and the gold standard was 75% (95% CI: 58.8%-87.3%). The percent agreement with the gold standard on recommendations for pharmacotherapy and psychotherapy ranged between 78%-84% for the application and the psychiatrists.

Conclusions: There was a high degree of agreement between the application and psychiatrists in diagnosing psychiatric disorders; both the application and the psychiatrists were less successful, and less likely to agree with each other in identifying the degree of severity and recommendations for treatment. The next step of the project is to assess real patients.

Keywords: artificial intelligence, clinical assessment, digital psychiatry, remote assessment solution, Diagnosis

Disclosure: Teva, Minerva, Dexcel, Lundbeck, Clearmind, Medipharma: Consultant (Self). Janssen: Speakers Bureau (Self).MSD: Grant (Self),

P40. Synthesizing PET Images From fMRI Data Using AI

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Background: Positron Emission Tomography (PET) imaging is essential for evaluating neurotransmitter systems in the brain, but

the high cost, limited availability of PET radiotracers, and potential radiation exposure presents considerable challenges. Recent studies have shown that fractional amplitude of low-frequency fluctuations (fALFF), derived from a more accessible functional Magnetic Resonance Imaging (fMRI), exhibits a high correlation with 18F-FDG PET, suggesting the potential for fMRI to synthesize 18F-FDG PET.

Methods: We propose an advanced deep learning technique using a Unet-based generative adversarial network (U-GAN) model to predict 18F-FDG PET images from resting-state fMRI data. Our study included 94 healthy participants (39 female) who underwent both dynamic 18F-FDG PET and fMRI scans. We employed a 5-fold cross-validation to assess the model's performance. Evaluation metrics included normalized root mean square error (NRMSE), structural similarity index (SSIM), and Pearson's correlation coefficient to assess overall image prediction accuracy. Additionally, we computed mean bias and correlation plots for various brain regions of interest (ROIs) to evaluate ROI-based prediction accuracy.

Results: The deep learning model demonstrated satisfactory performance in synthesizing 18F-FDG PET images, achieving an NRMSE of $6.3\% \pm 1.0\%$, an SSIM of $76.6\% \pm 14.2\%$, a Pearson correlation coefficient of $97.1\% \pm 0.3\%$, and a mean ROI bias of $1.3\% \pm 10.6\%$.

Conclusions: To the best of our knowledge, this study is the first attempt to utilize fMRI for synthesizing PET images, demonstrating the feasibility of this approach with reasonable prediction accuracy. Future research could enhance the quality of synthesized PET images by integrating T1-weighted MRI, extend the deep learning model to other radiotracers, and evaluate its potential applicability in clinical practice.

Keywords: Deep learning, PET, Resting-state fMRI, Synthesis

Disclosure: Nothing to disclose.

P41. Machine Learning Classification Across Multiple Psychiatric Disorders Using Objective Speech and Language Features

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Background: Psychiatric disorders account for 8-12% of primary diagnoses in emergency room visits across the U.S., and over 15% of primary care visits. Individuals with psychiatric disorders are actually more likely to present to primary care physicians (58%) than they are to psychiatrists (42%). In these settings, an automated tool providing diagnostic guidance may assist emergency and primary care physicians in making mental health diagnoses, triage, and treatment decisions. Our objective was to evaluate the use of automated speech and language analysis in making differential diagnoses across multiple psychiatric presentations.

Methods: We evaluated 235 participants in total, including 202 with one or more psychiatric diagnoses participating in treatment with inpatient and outpatient services at Zucker Hillside Hospital, and 54 healthy volunteers (HV). Participants were 14 to 50 years old (mean 26 years). Primary diagnoses included 85 individuals with unipolar depressive disorders (Dep), 52 with bipolar disorders (BPD), and 44 with schizophrenia spectrum disorders (SSD). However, as expected, there was a high degree of comorbidity and many participants had more than one diagnosis.

Speech was assessed for each participant using paragraph reading, verbal fluency, picture descriptions, and open-ended

journaling tasks. Features were computed with a range of acoustic analysis and Natural Language Processing (NLP) methodology, and included pitch and voice quality, tempo and pausing, parts-of-speech, sentiment, task-specific features (e.g., fluency totals, identification of picture elements on picture descriptions), and quantification of speech organization through universal dependencies, speech graph metrics, and semantic coherence using word and sentence embeddings. These features were used with and without demographic information to classify a series of diagnostic distinctions. The data underwent 80/20 stratified splits for training and test sets. Machine learning was conducted in Python and an LGBMClassifier was chosen. SHAP values (SHapley Additive exPlanations) were calculated to evaluate relative feature importance and the nature of their relationship to the classification problem at hand.

Results: We first classified individuals who had any Axis I disorder vs. HV with the speech features alone and obtained 86% accuracy in the test set, with 95% sensitivity and 56% specificity. Adding demographic information led to a negligible decline in model performance. Informative features included the number of interjections produced during fluency tasks (producing very few interjections was associated with having Axis I pathology), education level (having very high education level was associated with HV status), and local semantic coherence (having very low semantic coherence during picture description was associated with having Axis I pathology).

Using the speech features alone, individuals with unipolar depression could be distinguished from those with serious mental illness (SMI; SSD or BPD) with an accuracy of 72%, sensitivity of 74% and specificity of 70%. Adding demographic information did not substantially change the results. Informative features included articulation rate (very low articulation rate was associated with Dep), using of interjections in the journaling task (high interjections associated with Dep, low interjections associated with SMI), and correctly described picture elements and accurate semantic fluency responses (low performance associated with SMI).

Conclusions: Individuals with Axis I psychiatric disorders can be distinguished from health volunteers with relatively high sensitivity and performance approaches clinical viability. While classification of individuals with unipolar depression vs. serious mental illness was also successful from a technical perspective, further development is needed to achieve clinically useful performance.

Keywords: speech, natural language processing (NLP), Diagnosis, machine learning

Disclosure: Winterlight Labs: Consultant, Contracted Research (Self). North Shore Therapeutics: Founder, Stock / Equity - Privately Held Company, Board Member (Self). LB Pharmaceuticals, Catholic Charities Neighborhood Services: Consultant (Self). Psyrin, Advisory Board, Consultant (Self)

P42. An EEG-Based, Artificial Intelligence/Machine Learning (AI/ML) Biomarker for Predicting Treatment Response in Depressed Patients Receiving an SSRI Antidepressant or TMS Therapy

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Background: Currently, selection of treatments for major depressive disorder (MDD) is hampered by the lack of biomarkers with adequate sensitivity and specificity to predict which patients will derive favorable responses. Delays to identifying effective therapies contribute further to disease burden. A crucial question is

whether pre-treatment biomarkers based on electroencephalogram (EEG) and machine learning (ML) have potential to improve treatment selection and optimize outcomes for individual patients. EEG offers a practical and potentially promising solution by measuring brain electrical activity and presumed underlying circuitry in the clinic setting. However, the massive datasets generated by EEG have yet to be effectively utilized to reliably predict individual patient outcomes and guide clinical decision-making. Leveraging artificial intelligence/machine learning (AI/ML) methods, we aimed to classify patient clusters based on clinical responses to an antidepressant medication and to a course of transcranial magnetic stimulation (TMS) therapy. By interrogating EEG datasets generated by two separate patient samples and two different treatment modalities, we aimed to test the versatility of the AI/ML approach for identifying treatment response biomarkers.

Methods: We conducted a secondary analysis in patients with MDD using EEG data from (1) the EMBARC (Establishing Moderators and Biosignatures of Antidepressant Response in Clinical Care) randomized placebo-controlled trial of sertraline (N = 215), and (2) a clinical sample receiving naturalistic (i.e., open-label, uncontrolled) TMS treatment (N = 127). In both cases, the EEG recordings at the baseline (prior to treatment) and with resting-state (patients sitting stably and comfortably) were utilized. Brain functional connectivity measures, including coherence, power envelope correlation, and covariance, were used as feature sets, and a ML data analytic platform with combined unsupervised (Kmeans) and supervised learning (XGBoost) was employed to discover response biomarkers for the SSRI antidepressant and for the TMS therapy. Change in HAM-D-17 and IDS-SR scores from baseline to treatment endpoint were used as the outcome variables for statistical analyses. Data from EMBARC patients in both the active treatment and placebo arms were used for AI/ML steps, with only the active treatment arm data used for response calculation. Cross-validations performed on both datasets assessed the robustness of findings.

Results: For patients who received sertraline in the EMBARC study, an AI/ML-empowered EEG biomarker was identified for prediction of treatment outcome with a balanced accuracy of 81%, with 83% sensitivity and 80% specificity, and an AUC of 0.87. For outpatients who received naturalistic TMS treatment in a clinic setting, a distinct biomarker with 79% balanced accuracy was identified for predicting TMS treatment outcome, with 88% sensitivity and 70% specificity, and an AUC of 0.89. For both datasets, the 10-fold cross-validations showed > 90% consistency.

Conclusions: Utilizing brain functional connectivity features extracted from baseline resting state EEG data and leveraging AI/ML technology presents an innovative opportunity for predicting antidepressant pharmacotherapy and TMS treatment outcomes in patients with MDD. The versatility of this AI/ML-powered EEG biomarker approach holds potential for identifying the optimal treatment type for individual patients presenting with depression, potentially shortening suffering, reducing the risk of disability and suicide, and lowering healthcare and societal costs. Ongoing efforts aim to further test these findings across different treatment modalities for MDD patients.

Keywords: artificial intelligence, Machine learning clustering, Major Depressive Disorder (MDD), EEG biomarkers

Disclosure: Neumarker: Consultant (Self).

P43. Higher ADRD Phenotyping Scores are Associated With Faster Conversion to ADRD in Veterans Without ADRD Diagnoses

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Background: Alzheimer's disease and related dementias (ADRD) are underdiagnosed, and that underdiagnosis is detrimental to patients, their families, and the VA health care system. This crisis of underdiagnosis exacerbates existing disparities in health care, as dementia underdiagnosis may disproportionately affect Black Americans (BAs) compared to White Americans (WAs). Using a support vector machine (SVM) machine-learning model to assign ADRD-related scores may help identify Veterans with undiagnosed ADRD and their risk of developing dementia in the near future.

Methods: Shao et al. (2023) developed an SVM model based on over 850 variables (e.g. from chart notes and features) collected from the Veterans Health Administration's electronic medical record (EMR) system using a training sample of 20,000 BA and WA Veterans.

ADRD risk scores were calculated for WA Veterans and BA Veterans age 65+, lacked ADRD ICD codes or prescribed ADRD-related medication prescriptions, and were not in the training sample ($n = 4,038$) and followed until ADRD ICD diagnosis, death, or for up to six years (i.e., until 9/12/2023).

Kaplan-Meier curves were calculated separately by race and SVM score quintile group and a Cox proportional hazards model was used to obtain an estimate of the hazard ratio (HR) for the risk of developing ADRD after the index date for individuals with different scores, adjusted for race. Individuals who died before the end of follow up were considered censored at the date of death.

Results: The HR comparing the 75th to and 25th percentile of scores is estimated to be 2.20 (95% CI: 1.93–2.51; p -value < 0.01).

The HR comparing BAs to WAs is estimated to be 1.74 (95% CI: 1.25–2.45; p -value < 0.01).

The Kaplan-Meier plot shows the probability of remaining dementia-free in the years after the index date.

- o For BAs, the curves of the highest two risk quintiles have visibly steeper slopes than those of the other quintiles.

- o For WAs, the curve of only the highest risk quintile is visibly steeper than those of the other quintiles

Conclusions: Higher SVM scores were associated with higher risk of developing ADRD in the years following the index date.

BAs in our sample were at a higher risk of developing ADRD in the years following the index data compared to WAs with the same score.

SVM risk scores may perform differently in BAs and WAs

- o This is further evidence that more BAs with undiagnosed ADRD are at risk of developing dementia compared to WAs.

This work suggests our SVM model may be a useful tool for identifying patients at a high risk of future conversion to ADRD.

Keywords: Alzheimer disease, machine learning, veterans, Dementia, Health Disparities

Disclosure: Nothing to disclose.

P44. Can Large Language Model-Based AI Reason About Behavioral Health? Preliminary Evaluation of a Decision Tree-Based LLM Algorithm for Psychiatric Case Diagnosis

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Background: Large Language Models (LLMs) have recently garnered enthusiasm about their potential use in mental health care. However, the promise of LLMs is predicated on the quality of the knowledge and reasoning capability encoded within the models and the specific prompting approach used for any inquiry. Previously early work has suggested that combining LLMs with expert knowledge may enable effective automated reasoning. Here, we perform a preliminary evaluation to determine the promise of a decision tree-based approach to prompting LLMs,

drawn from structured diagnostic pathways, to provide DSM diagnoses for standardized psychiatric scenarios and for real-world psychiatric intake assessment notes.

Methods: Data: 28 full-text case diagnosis vignettes and associated author-designated diagnoses were retrieved from the DSM-5-TR Clinical Cases book using stratified random sampling without replacement. Stratification was performed by the diagnostic group chapter, excluding chapters 11, 14, 18, and 19 as these DSM categories were not represented in the structured diagnostic pathways. 21 randomly selected de-identified outpatient psychiatric intake assessment notes were retrieved from the UCSF Information Commons electronic medical record database. The HPI and diagnoses were extracted from each note, with manual censoring of all DSM diagnoses within the HPI.

LLM Prompting: 28 decision trees were extracted from the DSM-5-TR Handbook of Differential Diagnoses and implemented as gpt-4-turbo LLM prompts as in the following example:

- Are there at least 2 weeks of depressed mood or diminished interest plus associated characteristic symptoms (e.g., changes in weight and appetite, fatigue, feelings of worthlessness or guilt, changes in sleep, suicidal thoughts)?

Each tree was implemented as an iterated series of yes/no prompts, leading to a diagnosis node that contained zero, one, or two diagnoses.

Experiments: The decision tree models processed each vignette and HPI to generate candidate diagnoses. Every case was processed through the 28 trees, creating a list of candidate diagnoses. The LLM was then used to refine these candidates pairwise by presenting pairs of diagnoses to the LLM and prompting it to determine if one diagnosis was more appropriate than the other or if both were equally appropriate. Once a final diagnosis list was obtained, the positive predictive value (PPV) and true positive rate (TPR) were calculated on a per-case basis and averaged across all vignettes and HPIs.

Results: All inputs were successfully processed by the LLM-based decision tree model. The case vignettes had a mean of 1.5 author-designated diagnoses per vignette, and the model predicted a mean of 1.7 diagnoses. The real-world intakes had a mean of 2.1 author-designated diagnoses per note, and the model predicted a mean of 2.4 diagnoses. For the diagnosis of case vignettes, the model had a mean positive predictive value [PPV, true positives / (true positives + false positives)] of 78.6% (SD 37.1%) and a mean true positive rate [TPR, true positives / (true positives + false negatives)] of 76.2% (SD 36.7%). For the diagnosis of real-world intake HPIs, the mean PPV was 61.5% (SD 41.0%), and the mean TPR was 57.0% (SD 39.2%).

Conclusions: The LLM-based decision tree model exhibited high performance in the diagnosis of case vignettes and moderate performance in the diagnosis of real-world intake HPIs. There was significant inter-case performance variability. We attribute the performance limitations to the diagnostic uncertainty inherent within the field of psychiatry (as demonstrated by the DSM field trials). Real-world intake assessment is further limited by the reality that insufficient information often exists at a first visit to confirm a diagnosis. There may also be psychiatric domain-specific limitations of the model's performance that could be detected by a higher-powered study.

Given these challenges, we see the positive LLM performance on this task as a signal that the combination of structured clinical knowledge with LLM technology could enable high-quality automated psychiatric reasoning capabilities, and further study is indicated. Such future efforts might better focus on the ability of LLMs to evaluate symptoms and behaviors, rather than diagnoses, and to make appropriate psychotherapeutic recommendations based on these findings. Such a model could be deployed as a clinical decision support system to support psychotropic prescribing by primary care providers and allied health professionals, enhancing access to appropriate mental health interventions.

KVS and KEH acknowledge support from NIMH R25 MH060482. We thank the UCSF AI Tiger Team, Academic Research Services, and the Chancellor's Task Force on Generative AI. The authors appreciated the opportunity to give unpublished presentations of earlier components of that work at AMIA, NCPS, and TIPS.

Keywords: Generative Artificial Intelligence, Large Language Models, Diagnosis, DSM, informatics

Disclosure: Nothing to disclose.

P45. Personalized Quality of Life Assessment in Neuropsychiatry

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Background: There is a current lack of quality of life (QOL) measures that are specific to mental health (MH). This gap impedes progress in clinical neuropsychiatry in several ways. First, clinicians and researchers cannot evaluate and recommend treatments based on overall impact, but rather they are restricted to narrowly focused symptom and outcome measurements. Current QOL measures that could more broadly inform these recommendations contain minimal MH content. As a result, they are relatively insensitive to MH-specific change. Societal-level approaches are also impacted, because regulatory bodies and third-party payers do not have a “common denominator” that they can use to compare the impact of MH symptoms and treatment options across all MH and physical health (PH) conditions. For example, we are limited in how we can compare the overall impact of treatment for depression to treatment for other MH conditions such as schizophrenia, or to PH conditions such as diabetes. QOL measurement also underpins cost-effectiveness research, and without valid QOL measurement we cannot accurately allocate resources needed for a nationwide MH strategy. Without addressing problems in outcome-focused quality measures, patients will continue to face a disjointed MH care system where sufficient resources are not apportioned to their needs, their clinicians cannot select treatments in a way that maximizes overall functioning, and research to improve their care cannot consistently demonstrate comparative effectiveness.

Modified QOL measures provide a promising approach to serve as a common denominator across conditions. However, current nomothetic approaches are not specific to MH symptoms, which creates measurement insensitivity and substantially reduces measurement accuracy. There are also many idiographic QOL measures that are tailored to specific disorders, but they are not nomothetically comparable across MH or PH conditions. Multiple approaches have been proposed to convert scores from these specific symptom measures into more general QOL domains, but they involve multiple procedures that introduce measurement bias. New QOL measurement approaches are needed that are both nomothetically comparable across disease conditions and ideographically tailored to MH phenomenology.

Methods: We used unsupervised machine learning to address the aforementioned gaps created by inadequate QOL measurement. Specifically, we used an approach based on partial least squares estimation of a formatively-modeled latent QOL factor combined with finite mixture modeling (PLS-FIMIX). This approach is used to create two types of QOL scores.

First, we create a group-level score (GLS) that assigns an individual to a subgroup of people with similar characteristics, allowing for a nomothetic comparison. Second, we create an individual-level total score (ILS), which ideographically reflects differential QOL item weights based on an individual's GLS group assignment. For example, some patients may be assigned to a GLS

subgroup that shows a predominant focus on MH symptoms. This GLS group assignment indicates that their ILS should weight MH content more than PH content. Together, the GLS and ILS provide a personalized QOL (PQOL) assessment that simultaneously offer nomothetic and idiographic information for an individual patient.

These goals are achieved by relying on QOL measures already implemented in everyday clinical practice, which minimizes implementation burden. Preliminary results were used in NIH-funded grant R01MH137075 using data from the National Epidemiologic Survey on Alcohol and Related Conditions-III (NESARC-III; N = 36,309). The NESARC-III provides a diverse sample that uses complex survey weighting to achieve representativeness of the American population. We sought to provide proof of concept that GLSs and ILSs could be generated to form a comprehensive PQOL assessment approach. To create PQOL scores, we applied our unsupervised ML approach to 12-Item Short Form Survey (SF-12) QOL scores. To show real-world relevance, we correlated ILS scores with emergency room visits.

Results: Two GLS groups were identified, one focusing on MH and one focusing on PH. ILS scores allowed for effect sizes that were almost tripled compared to conventional QOL assessment measurement ($r = 0.46$ vs. $r = 0.16$). At this level of effect size difference, we found that required sample sizes were reduced in some cases by 89%.

Conclusions: We provide proof of concept that unsupervised ML can be used to create PQOL assessments that are both tailored to an individual patient's perspective of QOL (ILSs) and allow for normative comparisons (GLSs). This approach substantially improved effect sizes and reduced the sample sizes needed to recruit. Our new PQOL assessment approach also allows for better characterization of an individual patient's overall QOL, creates a more precise target for treatment outcomes, and enables direct comparison between various MH conditions and between MH and PH conditions.

Keywords: Quality of Life (QoL), Machine Learning, Epidemiology

Disclosure: Nothing to disclose.

P46. Towards an Objective Assessment of Spoken Language Changes in Mania

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Background: Spoken language is able to convey a wealth of information regarding one's internal mental state to listeners. Attempts at harnessing this information to help guide psychiatric diagnosis and treatment has been a goal for the past century. Recent advancements in the fields of computational linguistics and artificial intelligence allow us to come closer to the goal of quantifiable and diagnostically valid markers for mental illness. We present the data collection, raw audio processing and analysis pipeline, and the unpublished results of a recent multinational collaborative study tracking linguistic changes over the course of mania in multiple languages.

Methods: This pilot study of speech changes was conducted with ethics board approval and oversight at the Mayo Clinic St. Marys hospital system and at the Clinical Institute of Neurosciences of the Hospital Clínic de Barcelona. Patients with a diagnosis of Bipolar 1 disorder, current episode manic were recorded using high fidelity audio equipment while completing several standard linguistic tasks in their native language. Interviews were continued over the course of hospitalization and following discharge as the symptoms of mania resolved.

Results: Acoustics measurements of speech including rates of speech and pausing were reliably calculated with precision measured in milliseconds and generally aligned with subjective, clinician assessment. Artificial intelligence guided voice to text transcription enabled analysis of semantic and syntax linguistic domains and comparison with clinician rated assessments of cognitive processing.

Conclusions: Accurately and reliably quantifying speech disturbances in mania is now possible given recent technological advancements. This enables augmentation of clinician expertise and extension of psychiatric knowledge into novel settings for improved management of Bipolar 1 Disorder and other mental illnesses.

Keywords: Bipolar I disorder, artificial intelligence, Automated natural speech analysis

Disclosure: Nothing to disclose.

P47. Dampening Activity of Interpeduncular Nucleus Somatostatin Neurons via GABAA Receptor Signaling Reduces Stress-Induced Anxiety-Like Behavior in Mice

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Background: Stress exposure engages neural circuits that detect homeostatic challenge and imminent threat, resulting in behavioral outputs that restore homeostasis and increase survival. Responses inconsistent with stress levels often result from maladaptive changes in coping behavior, leading to an inability to manage stress, like what is observed in anxiety-related illness and depression. We recently identified the interpeduncular nucleus (IPN), as an inhibitory midbrain area that is activated by acute stress, particularly in somatostatin (SST)-expressing GABAergic neurons, triggering anxiety-like behavior. Optogenetically inhibiting stress-induced IPN GABAergic neuron activity was anxiolytic, suggesting that pharmacological interventions that reduce stress-induced activation of the IPN may alleviate anxiety-like behavior. In addition, analysis of single-cell sequencing IPN data revealed robust expression of GABAA $\alpha 1$ and $\alpha 4$ receptor subunits in SST GABAergic neurons, revealing potential molecular targets to dampen IPN activity induced by stress.

Methods: To test if a GABAergic positive allosteric modulator reduced stress-induced anxiety-like behavior in C57Bl/6J mice, we intraperitoneally injected (i.p.) male and female animals with SGE-516 (1, 2, or 3 mg/kg, $n=8-33$), a synthetic analogue of allopregnanolone developed by Sage pharmaceuticals and a positive allosteric modulator of GABAA receptors that has been shown to augment $\alpha 1$ and $\alpha 4$ subunit-containing GABAA receptors. Mice were challenged with drug or vehicle and 25 min post-injection, mice were subjected to 5 min of restraint stress followed by recording of anxiety-like behavior in the elevated plus maze (EPM). To test if SGE-516 reduced stress-induced activity of IPN SST GABAergic neurons, we expressed genetically encoded calcium indicators (GCaMPs) in the IPN of SST-Cre mice using AAV-mediated gene delivery and used in vivo fiber photometry to measure calcium signal in these neurons as a proxy of neuronal activity. IPN SST GABAergic neuron activity was monitored during and post-restraint in animals that were administered an i.p. injection of vehicle or SGE-516 (1 and 3 mg/kg i.p., $n=4$ males and $n=4$ females). In addition, stress induced grooming was also quantified.

Results: Acute systemic administration of SGE-516 significantly increased time spent in the open arms of the EPM at all doses tested in male and female mice (One Way ANOVA $F(3,$

$63) = 15.01$, $p < 0.0001$). Total arm entries in female mice were not significantly different between vehicle and SGE-516 groups at the 1 and 2 mg/kg dose. In males, the 1 mg/kg dose did not significantly increase total arm entries compared to vehicle. In fiber photometry experiments, acute restraint stress significantly increased activity of SST-Cre male and female mice during and post restraint compared to baseline in mice receiving vehicle injection (Repeated Measure One Way ANOVA $F(1.79, 12.53) = 16.05$, $p = 0.0004$). In contrast, acute restraint stress did not significantly increase activity of IPN SST GABAergic neurons in mice receiving a pre-injection of SGE-516. In addition, SGE-516 also significantly reduced time spent grooming post-restraint compared to vehicle injected control mice ($t(14) = 2.70$, $p = 0.017$).

Conclusions: The GABAA receptor positive allosteric modulator, SGE-516 acutely reduced stress-induced anxiety like behavior in the EPM and also blocked stress induced activity of IPN SST GABAergic neurons in mice. These data suggest that pharmacologically inhibiting activity of IPN SST GABAergic neurons via modulation of GABAA receptors may provide a novel therapeutic strategy to alleviate stress-induced anxiety.

Keywords: Anxiety, Somatostatin, interpeduncular nucleus, GABA

Disclosure: Nothing to disclose.

P48. Individual-Level Associations Between White Matter Microarchitecture and Anxious Temperament in Infant Rhesus Macaques

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Background: Understanding the neurodevelopmental correlates of early-life pathological anxiety may inform novel, targeted interventions for the prevention and treatment of childhood anxiety disorders (ADs). In this regard, white matter (WM) microarchitecture is particularly promising, as alterations in WM have been linked to psychiatric illnesses, including ADs, and, critically, WM is sensitive to behavioral, environmental, and pharmacological factors. To explore mechanisms underlying the development of ADs, we developed a highly validated young rhesus monkey model of anxious temperament (AT), incorporating behavioral and physiological measures induced by potential threat: increased freezing, decreased vocalizations, and increased cortisol. This translational nonhuman primate (NHP) model enables the characterization of the neurobiological substrates of pathological anxiety, including alterations in WM microstructure. Our previous work in NHPs and in preadolescent children with pathological anxiety demonstrated region-specific and distributed anxiety-related WM microstructural reductions. From these data, we posited that these WM alterations may function to modulate threat-related neuronal signaling. The current study, performed in the first year of primate life, uses DTI and quantitative relaxometry (QR), an imaging method that may be more sensitive to myelination, to comprehensively and longitudinally characterize the relations between WM microstructural parameters and individual differences in early-life anxiety.

Methods: 34 rhesus monkeys (23 F, 11 M) underwent 30 minutes of the No-Eye-Contact (NEC) session of the Human Intruder Paradigm 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. AT-related behaviors, namely freezing duration and cooing frequency, were scored during each 30m NEC session. After each NEC session, plasma

cortisol was measured. Freezing durations and cooing frequencies were log- and square-root-transformed, respectively, and then converted to z-scores. Plasma cortisol levels were corrected for time of day at collection and then also converted to z-scores. Finally, the 3 resultant z-scores were averaged (incorporating the inverse of cooing) to generate composite AT scores for each animal at each timepoint, described by the following formula: $AT = (\text{Freezing} + \text{Cortisol} - \text{Cooing}) / 3$. 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. Three sets of ROI-specific linear mixed-effects (LME) models were built in R to assess within-subject associations among: 1) FA and AT; 2) qR_1 and AT; and 3) both FA and qR_1 in relation to AT to determine the unique contribution of each metric to early-life AT. All analyses controlled for gestational age at scan.

Results: On a within-subject level, FA in 2 out of 5 ROIs – the uncinate fasciculus and cingulum – negatively predicted AT across the first year of life (mean $R^2 = 0.02$, $p = 0.05$). QR analyses revealed that qR_1 measures in all 5 ROIs were negatively associated with AT on a within-subject level during the first year of life (mean $R^2 = 0.04$, $p < 0.05$). Notably, the relationship between qR_1 and AT was generally stronger, reflected by slightly larger effect sizes (compared to analyses with FA). Accordingly, LME models that simultaneously estimated the effects of FA and qR_1 on AT showed that, in 3 out of 5 ROIs (superior longitudinal fasciculus, internal capsule, corpus callosum), the qR_1 metric was a more robust predictor of AT relative to FA. In other words, when assessed alongside qR_1 , FA was a nonsignificant predictor of AT, while qR_1 remained highly significant and negatively predicted AT in these ROIs (qR_1 : mean partial- $R^2 = 0.03$, $p < 0.05$).

Conclusions: These results suggest a dynamic, within-subject relation between WM microarchitectural integrity and anxious temperament during the first year of primate life. They serve as a cross-species replication of our longitudinal work in preadolescent children with pathological anxiety and indicate an evolutionary conserved relationship between WM microstructure and maladaptive anxiety at the individual level. Importantly, the current work extends this association to the earliest postnatal developmental period, suggesting the importance of WM in the neurodevelopmental underpinnings of pathological anxiety and anxiety disorders. Lastly, combined analyses of multimodal WM metrics (FA vs. qR_1) reveal that qR_1 is generally a more robust predictor of AT in the first year of life. Taken together with data from other labs suggesting that QR metrics may be a more sensitive marker of myelination relative to those derived from DTI, this result suggests the possibility that myelination-related processes are particularly relevant to the emergence and course of anxious temperament in very early life.

Keywords: Anxiety, Nonhuman Primate Models, longitudinal multimodal imaging, Translational research, early brain development

Disclosure: Nothing to disclose.

P49. The Effects of Herbicide Consumption on Avoidance Behaviors in Female Rats

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Background: The use of glyphosate-based herbicides (Glyph) increases each year in the United States. Interestingly, there is a correlation between Glyph exposure and anxiety disorders. Unfortunately, increases in anxiety may exacerbate behaviors such as avoidance. Avoidance is a defensive response where an individual takes actions to avert potential harm.

Methods: Notably, avoidance can be modeled in rodents using platform-mediated avoidance. Here, rodents learn to avoid a foot shock by stepping onto a safe platform during presentation of a conditioned auditory stimulus (e.g. tone). Stepping on the platform protects the rodent from the shock, but does not eliminate the auditory stimulus. Of note, when the animal steps onto the avoidance platform, it cannot access a sugar-pellet reward. Thus, platform-mediated avoidance creates a conflict that requires rodents to make a choice between avoidance (no shock) and reward (receiving sugar pellets).

Results: In the current study, we hypothesized that glyphosate exposure would result in excess avoidance as observed by more time on the platform. To test this idea, female rats were trained on platform-mediated avoidance. Next, rats were exposed to Glyph (2.0 mg/kg/day) through drinking water for 12 weeks ($n = 8$). Control rats received filtered water during this period ($n = 8$). At the end of exposure period, rats were tested for avoidance behaviors over four days. Anxiety levels were also measured through the open field and elevated plus maze. Contrary to our hypothesis, we observed that Glyph reduced the time spent on the platform during the avoidance test compared to controls ($p = 0.0294$, T-Test), but not across subsequent days. Furthermore, our results demonstrate that glyphosate increased anxiety-like behaviors in the elevated plus maze ($p = 0.0264$, T-Test), but not in the open field tests.

Conclusions: Together, considering our preliminary observations in male rats, our results suggest that exposure of glyphosate at levels thought safe by regulatory agencies increases anxiety-like behaviors in male rats, but not female rats. Thus, we are currently performing additional experiments and analysis to clarify the interpretation of our results. Moreover, we are using immunohistochemical techniques to assess for changes in brain regions implicated in avoidance.

Keywords: glyphosate, environmental contaminants, anxiety-like behaviors, rat

Disclosure: Nothing to disclose.

P50. Isolating Perceptual and Motor Contributions to Social Anxiety Disorder Using a Novel Social-Judgement Approach Avoidance Task

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Background: Social anxiety disorder (SAD) is characterized by debilitating fear and avoidance of social interactions. Despite rising rates of SAD, our understanding of the cognitive changes underlie SAD remains limited. A symptom-focused approach, in line with the Research Domain Criteria, will enable the development of treatment targets that can be targeted with non-invasive brain stimulation. A first step towards these novel interventions is to robustly drive cognitive processes in a manner that correlates with individual differences in symptom severity. Previous work demonstrated that people with SAD show a reduced ability to distinguish between levels of anger in morphed emotional faces. Others found altered approach-avoidance behavior. To investigate the differential contributions of altered perception and motor control to SAD symptoms, we developed a novel social judgement approach-avoidance task (SJ-AAT) and administered this task in

participants with SAD and in healthy control participants for comparison.

Methods: Participants (N = 28) were recruited to participate in a single session study comprising a clinical assessment phase followed by performance of the SJ-AAT while high-density 96-channel EEG was recorded. The Mini International Neuropsychiatric Interview for the DSM-V was used to diagnose SAD and screen healthy controls. The Liebowitz Social Anxiety Scale was used to quantify symptom severity of social fear and avoidance, which was used for individual differences analysis. In the SJ-AAT, participants were presented with two emotional faces simultaneously in the left and right visual hemifield. Both faces were either morphed angry-neutral or morphed happy-neutral. Participants had a joystick in each hand and responded with a joystick movement away from the angrier face or towards the happier face. Difficulty of the social judgement was manipulated by the degree of morph. When the fixation cross turned red, participants were required to respond with the incongruent motor response, i.e., behavioral approach to angry. First, we ran an omnibus ANOVA across all participants to assess that behavior was modulated as a function of task demands. Next, we investigated how individual differences in symptom severity of SAD related to perception (ANOVA for perceptual difficulty and valence) and motor control (ANOVA for approach-avoid and congruency).

Results: Three-way repeated measure ANOVA for accuracy across all participants revealed a main effect of perceptual difficulty ($F = 124.7$, $p < 0.001$, $\eta^2 = .85$), a main effect of approach-avoid ($F = 11.13$, $p = 0.003$, $\eta^2 = .34$), and an interaction between approach-avoid and valence ($F = 7.40$, $p = 0.013$, $\eta^2 = .25$). These results provide confirmatory evidence that participants were sensitive to the perceptual demands and the approach-avoid manipulation. For the SAD participants, the perception ANOVA for accuracy revealed an interaction between symptoms of fear of social interaction and emotional valence ($F = 10.5$, $p = 0.01$, $\eta^2 = .54$) such that greater symptoms were positively related to better accuracy for angry versus happy faces ($r = .73$, $p = 0.01$). The motor control ANOVA for response time revealed an interaction between symptoms of social fear and approach-avoid behavior ($F = 7.34$, $p = 0.02$, $\eta^2 = .45$), such that greater symptoms were related to reduced slowing during avoidance behavior ($r = -.67$, $p = 0.02$). These effects were specific to those with SAD and were not found with sub-clinical social fear symptoms in healthy controls.

Conclusions: In this study, we validated a novel cognitive task that integrates two existing constructs of altered perception of emotions and approach-avoid behavior. We provided evidence that the SJ-AAT quantifies individual differences in emotion perception and behavioral avoidance that are meaningfully related to symptom severity within SAD. The SJ-AAT provides a critical tool for investigating the neural basis of SAD. Consistent with the efforts of precision psychiatry, future studies can administer non-invasive brain stimulation targeted to the neural correlates of SAD symptom severity.

Keywords: Anxiety, Social Processing, Cognitive Neuroscience, Research domain criteria (RDoC)

Disclosure: Nothing to disclose.

P51. Anxious Arousal Symptoms are Uniquely Associated With Fusiform Gyrus Bold Signal During Fear Face Perception

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Background: The fusiform gyrus (FG) is a higher order sensory cortex associated with visual expertise, including face perception.

Previous studies have shown individuals with anxiety disorders demonstrate altered FG function during emotional face perception, particularly during fearful face perception. This is likely due to enhanced salience of threat detection in anxiety disorders. However, it is unclear what specific components of anxiety symptoms are associated with this phenomenon, and to what extent it is preattentive.

Methods: 214 adults ages 18-25 were recruited from the community for this fMRI study. Participants were either healthy or seeking treatment for psychological distress and ranged continuously in degree of trait-based risk factors for mood disorders. Participants completed an emotional face morph fMRI task designed to assess implicit emotional processing, as well as the Mood and Anxiety Symptoms Questionnaire (MASQ). The MASQ is based on the tripartite model of depression and includes subscales for anxious symptoms, depression symptoms, general mixed distress symptoms, anxious arousal, and anhedonia. FG BOLD signal was extracted during fearful face perception. Linear regressions were performed with extracted FG BOLD as the dependent variable, MASQ subscales as the independent variables, and age and gender demographic covariates.

Results: FG BOLD was positively associated with only the Anxious Arousal subscale of the MASQ ($F = 2.69$, $p = 0.007$). There were no significant associations between FG BOLD and any other subscale (all $ps > 0.2$) or demographic covariates (all $ps > 0.9$).

Conclusions: These findings suggest that sensory cortical regions are implicated in the physiological arousal component of anxiety, and that this may be a preattentive phenomenon. Future studies should assess coupling of the FG with limbic or ventral visual stream regions to ascertain the relationship between anxious arousal symptoms and network function.

Keywords: BOLD fMRI signal, Visual Processing, Anxiety, emotional arousal

Disclosure: Nothing to disclose.

P52. Evaluating Transcranial Focused Ultrasound Stimulation (tFUS) for Targeted Neuromodulation in Generalized Anxiety Disorder: A Double-Blind Feasibility Study

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Background: Generalized Anxiety Disorder (GAD) is a prevalent and debilitating mental health condition characterized by excessive and uncontrollable worry. Current pharmacological and cognitive-behavioral therapies often fail to provide satisfactory relief for many patients, necessitating the exploration of new treatment modalities. Transcranial Focused Ultrasound Stimulation (tFUS) offers high spatial precision and non-invasive modulation of deep brain structures such as the amygdala, crucial for emotion regulation and anxiety.

Methods: In this ongoing double-blind, randomized, sham-controlled study, we are evaluating the effects of tFUS on anxiety symptoms in patients with treatment-resistant Generalized Anxiety Disorder (trGAD). Forty-eight participants, aged 18-65, with moderate to severe trGAD, are randomly assigned to receive either active tFUS targeting the amygdala or sham stimulation once per week over four weeks. Anxiety levels are assessed using the Hamilton Anxiety Rating Scale (HAM-A) at each visit and during follow-ups. fMRI scans are conducted at the first and fourth visits to measure changes in brain activity and connectivity. Safety and side effects are monitored throughout. The primary endpoint

is the change in HAM-A scores from baseline to the end of the treatment phase, with secondary endpoints including changes in Clinical Global Impressions (CGI) and adverse event frequency.

This interim analysis presents data from the initial subset of the target 48 participants ($n=24$) as the study is still ongoing. Repeated measures ANOVA was used to evaluate the main effects of time and the interaction between time and condition (active tFUS vs. sham).

Results: Our interim analysis of 24 participants using repeated measures ANOVA reveals a significant main effect of time on anxiety symptoms ($p=0.0006$, $\eta^2=0.240$), indicating overall improvement in anxiety levels across both groups. However, the interaction between time and condition (active tFUS vs. sham) was not significant ($p=0.421$). This interim analysis was underpowered to detect a difference in the time by condition interaction, necessitating further data collection to accurately determine the specific efficacy of tFUS beyond placebo effects.

Conclusions: In conclusion, our interim analysis demonstrates overall improvement in anxiety symptoms in both tFUS and sham groups, highlighting a significant placebo effect. The lack of a significant interaction effect suggests that the specific efficacy of tFUS remains to be determined. Ongoing data collection and final analysis are crucial to establish the true potential of tFUS as a novel treatment for Generalized Anxiety Disorder. If the final results confirm efficacy, tFUS could represent a revolutionary non-invasive therapeutic option for patients unresponsive to conventional treatments, potentially transforming the management of GAD.

Keywords: Neuromodulation, focused ultrasound, generalized anxiety disorder

Disclosure: BrainSonix: Consultant (Self)

P53. Identification of Opposing Neurotensinergic Circuits Controlling Anxiety-Like Behavior in Mice

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Background: Novelty is an essential aspect of life that drives exploratory behavior in animals. Exploration in response to novelty is a highly conserved process and fundamental to survival. However, novelty also presents the potential for danger. Anxiety-like behaviors (i.e. latency to explore new foods or open environments) is an adaptive response that delays exploration and ensures survival. Anxiety-like behaviors is enhanced by psychological stress and is a hallmark of neuropsychiatric disorders in humans. Neural circuits that control anxiety-like behaviors via integration of stressful information and that modulate exploration remain unknown. Elucidating the behavioral function of this well-conserved phenomenon and its underlying neural mechanisms are important open-ended questions. Here we identify and characterize neurotensinergic circuits in the lateral septum (LSNT) in stress-induced anxiety like behaviors in mice.

Methods: Mice were housed according to the guidelines of MUSC's Animal Facility (DLAR), in a 12 hr light/dark cycle and with free access to standard chow diet and water. C57BL/6J background males and females were used throughout this study. All experimental protocols in animal studies were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, or by the applicable requirements of MUSC. Stereotaxic surgeries and craniotomies were performed to express viral constructs for projection

mapping, fiber photometry, tagging or chemogenetics. To simulate predator stress, mice were exposed to undiluted 2,4,5-trimethylthiazole in a filter paper for 1h acutely or 1h/day for 7, 15 or 30 days. Control mice were exposed in the same context to water. We assessed anxiety-like behaviors using novelty-suppressed feeding (NSF) and elevated plus maze (EPM). Latency to approach food, time spent in the open arms and closed arms and distance traveled were plotted using GraphPad Prism. For immunohistochemistry (ihc) and image quantification, we used free-floating slices, standard ihc protocols and quantifications were made in ImageJ. PhosphoTrap profiling experiments were performed according to Knight et al., 2012. Single cell RNA seq analysis was conducted utilizing Allen Brain Atlas repository and custom codes. Fiber photometry was performed using fiber photometry system and processor from TDT. Calcium signals and behavioral data were time-locked and peri-events calculated for each animal. Codes for data extraction and analysis were performed using custom codes in MATLAB. For chronic chemogenetics experiments, C21 (1 mg/kg dose i.p) were given daily in the afternoon for 30 days. Control animals received the same dose and number of injections as subjects. At day 31 and 32, animals were tested in the EPM and NSF tests, respectively. Cell tagging was performed using TRAP2 mice and 4-OHT (50 mg/kg i.p). Sample sizes ($n=3-16$ depending on the experiment) were calculated using power analysis. Mice with incorrect or no viral expression were excluded from the final analysis. All statistical analysis (One or two-Way ANOVA or student's t-test) were performed using GraphPad Prism.

Results: Using predator odor to increase neophobic behaviors in mice, we identified a neural population in the lateral septum (LS) that integrates predator information and modulates the latency to explore food in the NSF test or open environments in the EPM test. We observed that anxiety-like behaviors only appeared in animals chronically exposed to predator odor at days 15 and 30 ($p<0.05$), but not earlier. Calcium recordings in freely exploring mice, activity-based and single nuclei RNA-seq analysis revealed that predator-responsive LS neuronal clusters are GABAergic and express neurotensin (LSNT). Furthermore, chronic chemogenetic activation of LSNT neurons induced anxiety-like behaviors in the absence of a predator odor while synaptic silencing can completely abrogate stress-induced anxiety-like behaviors ($p<0.05$). Using genetic mouse models to indelibly tag predator odor-responsive neurons, we defined the downstream circuit that connects the encoding of predator information to the lateral hypothalamus and supramammillary nucleus. Projection-specific activation of LSNT-LH neurons increase stress-induced anxiety-like behaviors while LSNT-SUM projections alleviate these effects in mice ($p<0.05$).

Conclusions: Our findings offer two opposing neurotensinergic circuits that are genetically- and projection-defined and link psychological stress and anxiety-like behaviors in chronically stressed mice. These molecularly defined opposing circuits may serve as framework to understand the basis of foraging in predator-rich environments, where novelty may mean danger.

Keywords: neurotensin, Anxiety and stress, hypothalamus, predator stress, RNA-seq

Disclosure: Nothing to disclose.

P54. Dorsal Raphe Nucleus Projecting-CART Neurons in Edinger Westphal Nucleus Regulate 5HT Function and Anxiety

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Background: Approximately 280 million individuals globally are affected with anxiety and depression, a number that has surged by 25% since the COVID-19 pandemic. The cocaine- and amphetamine-regulated transcript (CART) peptide is widely expressed in brain areas implicated in anxiety, stress, reward, and addiction. Genetic mutations in the CART gene have been linked to a heightened risk of developing anxiety and stress in adolescents, and particularly with suicide victims, where CART levels are upregulated in the Edinger-Westphal (EW) nucleus. While the regulatory influence of CART in anxiety, depression, and stress within the mesolimbic circuitry has been characterized, its physiological role in the brainstem nuclei, particularly the dorsal raphe nucleus (DRN), remains largely unexplored. The DRN is a central hub for serotonin (5-HT) neurons, which play a substantial role in mood and stress/anxiety regulation. Given this, we aim to investigate the interplay between the CARTergic and 5-HTergic systems.

Methods: We used a combination of state-of-the-art techniques such as fiber photometry, fos-TRAP, ex vivo electrophysiology and chemogenetics to explore the physiological and functional aspects of CART neurons and their role in modulating 5HT-DRN neuronal activity and anxiogenic behavior. Behavior: Adult 2-month-old male C57BL/6J mice were micro-infused with either aCSF or different doses of CART (55-102) peptide (5 ng, 50 ng, and 100 ng) through a guide cannula in the DRN. Anxiety tests (elevated plus maze test, light-dark box exploration test, and social interaction test) were performed 15 minutes after infusion. LCMS: DRN tissue punches were processed to measure neurotransmitter levels using liquid chromatography mass spectrometry (LCMS). fos-TRAP: We used Fos2A-iCreER mice and trapped the activated neurons using 4-hydroxytamoxifen after intracerebroventricular administration of CART; and immunofluorescence was performed on DRN slices with antibody specific to 5HT. Fiber photometry: AAV1-CAG-Flex-GCaMP6s-WPRE-SV40 and AAV9-hsyn-5HT3.5 was injected in the Sert-cre and C57BL/6J mice respectively, and a fiber-cannula (OmFC) was implant into DRN. CART was micro-infused into the guide cannula and the in-vivo calcium imaging or 5HT release was monitored for 30 mins. We then computed percent fluorescent changes (% of $\Delta F/F$) between the last 5 min pre-infusion event (baseline) and 12 min post-infusion event and compared the area under the curve (AUC) in the peri-event plot for the CART versus aCSF groups. Retrograde tracing: AAVrg-hsyn-DIO-EGFP was injected in the DRN of CART-cre x Ai14 reporter mice, and the nuclei that expressed CART was screened for the GFP expression using immunofluorescence. Acute stress/CART overexpression and ex vivo electrophysiology: Acute stress was induced by restraining the mice for 30 mins and ex-vivo recordings were performed on the retrograde labeled CART neurons. In another cohort, a set of mice was injected with AAV-overexpressing CART in the EW and ex-vivo recordings were performed on DRN-5HT neurons. Chemogenetics: Pathway specific manipulation was performed by injecting AAVrg pEF1a-DIO-FLPo-WPRE-hGHPa in the DRN and AAVDJ-hsyn-fDIO-hM3D(Gq)-mCherry in the EW of CART-cre mice. CART-EW $\square$ DRN circuit was activated by injecting clozapine N oxide (CNO), and the anxiety tests were performed 40 mins later.

Results: We found that CART neurons densely innervate 5-HT neurons in the DRN, and micro-infusion of the CART (55-102) peptide into the DRN exhibited an anxiogenic effect in male mice. Central administration of the CART (55-102) peptide reduced c-fos activation in the ventral 5-HT neurons in the DRN, a region associated with reward and anti-stress circuits. Furthermore, the inhibitory effect of the CART (55-102) peptide on 5-HT-DRN function and local release was demonstrated using in vivo fiber photometry coupled with calcium and 5-HT biosensors, as well as LCMS. Using Cre-dependent retrograde tracing, we identified DRN-projecting CART neurons in the Edinger-Westphal (EW) nucleus, nucleus accumbens (NAc), amygdala, hippocampus, and

various hypothalamic nuclei. Interestingly, ex vivo electrophysiological recordings revealed that acute restraint stress increased excitability in DRN-projecting CART neurons located in the EW, but not in the NAc or hypothalamus. Additionally, pathway specific-chemogenetic manipulation of the CART-EW $\square$ DRN circuit induced anxiety in male mice. Overexpressing CART in the EW also reduced the excitability of DRN-5-HT neurons.

Conclusions: Our study establishes the neuromodulatory role of CART in regulating 5-HT neuronal function in the DRN. The findings suggest that the CART-EW $\square$ DRN circuit is a key driver of anxiety-like behavior, mediated by inhibiting DRN-5-HT transmission. In sum, the intricate dynamics of the CARTergic and 5-HTergic systems are crucial for understanding 5-HT-related dysfunctions, providing invaluable insights into both health and disease.

Keywords: Serotonin, neuropeptides, dorsal raphe, Anxiety, Acute Stress

Disclosure: Nothing to disclose.

P55. Exploring the Impact of Single Dose Losartan on Mnemonic Discrimination in Healthy Volunteers

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Background: Recently, the renin angiotensin system (RAS) has been implicated in several cognitive domains, including learning, memory, and emotional processing. Research studies using the angiotensin receptor blocker (ARB) losartan have shown that just a single 50 mg dose reduces aversive learning, improves prospective memory, and facilitates fear extinction, considered the laboratory analogue of exposure therapy, in healthy volunteers. These findings suggest critical involvement of the RAS in mental processing and psychological well-being, necessitating further research.

One unexplored cognitive mechanism in this context is pattern separation, defined as the neural process by which similar stimuli are encoded discretely in the hippocampus. Effective pattern separation is thought to underlie mnemonic discrimination, the behavioural ability to discriminate between stimuli that share perceptual similarity. Interestingly, deficits in mnemonic discrimination are associated with anxiety and overgeneralization, the phenomenon where individuals anxiously respond to stimuli that resemble the originally feared stimulus but have not been experienced as dangerous. Together, these findings suggest that impaired pattern separation may be one of the root causes of anxiety, driving poor mnemonic discrimination, overgeneralization, and the maintenance of anxiety symptoms.

Research suggests that the RAS may be involved in pattern separation, as rodent studies show that ARBs stimulate neurogenesis and separately that enhanced neurogenesis is sufficient to ameliorate pattern separation. Thus, it is plausible that RAS blockade via ARBs could foster neurogenesis and improve pattern separation in humans too, sharpening stimulus discrimination. In this experimental medicine trial, we examine for the first time the relationship between a single dose of losartan (50 mg) and mnemonic discrimination in healthy volunteers.

Methods: This study was approved by the Oxford University research ethics committee. Following informed consent and pre-screening, we recruited N=60 male and female healthy volunteers aged 18-50, with no current mental disorder, and no intake of psychoactive medication in the 6 weeks prior to testing. Eligible participants were invited to an in-person testing session at the Warneford Hospital, where they were randomized to a single dose losartan (50 mg) or placebo condition, stratifying for sex.

Following intake of the encapsulated pill, participants underwent a 1-hour waiting period to allow the drug to reach peak plasma levels and were administered the Mnemonic Similarity Task (Stark et al. 2019). The MST assessed mnemonic discrimination by taxing pattern separation through two phases. In phase 1, participants passively viewed neutral everyday objects on a computer and were asked to categorize them as 'indoor' vs. 'outdoor' items. During phase 2, participants were presented targets (objects seen during the encoding phase), lures (objects that resembled one seen during the encoding phase, but were different), and foils (novel objects not seen during the encoding phase) and were asked to categorize objects as 'old' (targets), 'similar' (lures), or 'new' (foils).

The primary outcome on the MST was the lure discrimination index (LDI; Stark et al., 2019), which evaluated participants' ability to recall details from phase 1 that were needed to reject lures in phase 2 (i.e., respond 'similar' instead of 'old'; Stark et al., 2019). The LDI was calculated as the rate of 'similar' responses to lures minus 'similar' responses to foils.

SPSS Version 29 was utilized to conduct two-tailed tests with $\alpha = .05$ and effect sizes were reported as Cohen's *d*. Mann-Whitney U tests were utilized to compare LDI performance between groups and 2 time (baseline, peak) $\times$ 2 group (placebo, losartan) mixed model ANOVAs were used to analyze potential drug-induced changes in physiological parameters (i.e., blood pressure and heart rate).

Results: Data from four participants were excluded given the presence of negative LDI scores ($N = 3$) or due to data loss ($N = 1$). Final groups ($N = 56$) consisted of $N = 27$ placebo and $N = 29$ losartan participants, who were balanced on sociodemographic, clinical, and physiological parameters.

Results revealed a significant difference in LDI performance between groups ($U = 255$, $z = -2.24$, $p = 0.025$, $d = 0.62$), with the losartan group demonstrating greater mnemonic discrimination of lures compared to the placebo group, likely driven by greater pattern separation in the hippocampus. To our knowledge, this RCT is the first to establish a potential link between the RAS and pattern separation, highlighting angiotensin receptors as a potential therapeutic target for the treatment of fear generalization and anxiety.

Conclusions: The findings of this study indicate that a single dose of losartan is enough to improve mnemonic discrimination in healthy volunteers, strongly suggesting a role of the RAS in pattern separation and hippocampal function. These findings have high relevance to anxiety and hold wider transdiagnostic applications, which remain to be explored in the future.

Keywords: Anxiety, Renin angiotensin system, Pattern separation, Losartan, Mnemonic Discrimination

Disclosure: Nothing to disclose.

P56. A Placebo Controlled Trial of Cariprazine in Social Anxiety Disorder

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Background: Social anxiety disorder is a prevalent, chronic and often disabling illness. New effective treatment for it is needed. Several lines of evidence suggested that a partial dopamine agonist like cariprazine could be effective for social anxiety disorder. To test this hypothesis a placebo controlled trial of cariprazine was conducted.

Methods: The study was a placebo controlled double blind 12 week trial of cariprazine 1.5-3mg/day conducted at a single site.

49 subjects meeting DSM 5 criteria for social anxiety disorder were screened and 41 were randomized. The primary outcome measure was change from baseline to endpoint in total score on the Liebowitz Social Anxiety Scale (LSAS). The statistical comparison used was analysis of covariance (ANCOVA) using LOCF.

Results: Cariprazine was statistically and clinically superior to placebo on the primary outcome measure $F(1,37) = 5.85$; $p = .021$. Least square means were -21.74 for placebo and -37.81 for cariprazine. CGI-I responder rates were 65% for cariprazine vs 40% for placebo, $p = .102$. Changes in the CGI-S were -1.10 for placebo and -2.10 for cariprazine ($p = .015$). The difference at endpoint for change in total LSAS between cariprazine and placebo was as great or > what was seen in the registration trials of FDA approved treatments for social anxiety disorder such as sertraline, paroxetine and venlafaxine ER.

Conclusions: Cariprazine may represent a new treatment for social anxiety disorder, a condition for which no new therapies have been approved in two decades. Cariprazine efficacy, if replicated by further trials, would also suggest that dopaminergically active drugs may have a place in treating social anxiety disorder.

Keywords: social anxiety disorder, dopamine partial agonist, cariprazine

Disclosure: Vistagen Therapeutics: Advisory Board, Consultant, Stock / Equity - Publicly Traded Company (Self)

P57. Neural Habituation to Deviant Sounds in Sleeping Neonates and Risk for Anxiety Disorders

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Background: Anxiety is associated with altered habituation to external stimuli. Recent work found that attention/arousal brain network differences associated with anxiety are present near birth, presenting the possibility of early detection. This project will characterize neural habituation to deviant sounds in a sample of infants at high risk for anxiety. In this project we will 1) characterize neural habituation to deviant sounds in neonates and 2) examine its relation to maternal anxiety diagnosis.

Methods: Our final sample will include approximately 100 neonates (ages 39-43 weeks) imaged with fMRI during natural sleep while undergoing an auditory oddball task. Each run of the task includes 56.4s of pure scanner noise, 24 white noise bursts at jittered intervals (9.6-14.4s) over the sound of the scanner, and then 77s of pure scanner noise. Activation to the deviant sounds will be characterized using finite impulse response modeling (FIR) to account for individual variation in the hemodynamic response. To measure habituation, subject level activation maps will be produced separately for the early, middle, and late deviant sounds (8 trials each). To measure habituation across the session, FIR modelling will be conducted separately for the first two runs versus the second two runs of the task. The resulting six subject-level activation maps ([early, middle, late trials] $\times$ [early vs late runs]) will then be entered into a mixed effects model to characterize effect of trial and run order on activation. For aim 2, maternal anxiety lifetime diagnosis will be added to this model as a main effect and an interaction with trial and run time variables.

Results: In a preliminary analysis of 63 neonates, we found decreased activation of the dorsolateral prefrontal cortex (DLPFC) and cingulate cortex in later runs compared to earlier runs. Across trials, we found increasing activation in the temporo-parietal junction (TPJ) and middle temporal gyrus over time and decreasing activation in the dorsal cingulate. Maternal anxiety

diagnosis was associated with decreased anterior insula and increased DLPFC activation across runs as well as increased TPJ activation across trials relative to infants with no maternal anxiety diagnosis.

Conclusions: The results of this study will provide key insights into the pathophysiology of anxiety and infant cognitive processing.

Keywords: Infant Brain, Anxiety, Psychiatric Risk, Anxiety-related circuitry

Disclosure: Nothing to disclose.

P58. Pain Anxiety as a Predictor of Healthcare Utilization Among Patients With OUD, Depression, and Chronic Pain

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Background: Opioid use disorder (OUD), depression, and chronic pain have all been shown to increase health service utilization across care settings. Individuals with co-occurring OUD, depression, and chronic pain therefore represent a group with complex medical and psychological service needs. Addressing symptoms that drive service use could improve patient outcomes and reduce strain on healthcare systems. Given that anxiety symptoms are often comorbid with depression and OUD, and that generalized anxiety is strongly associated with greater service usage, anxiety symptoms may be a useful predictor of healthcare utilization in this population. Moreover, little is known about pain anxiety as a predictor of care utilization in this chronic pain population. The goal of this study was to determine whether anxiety symptoms predicted healthcare utilization among people with co-occurring OUD, depression, and chronic pain. We hypothesized that greater anxiety would predict increased healthcare utilization across settings (emergency, inpatient, outpatient). Additionally, we predicted that pain anxiety would be a stronger predictor of service use than generalized anxiety.

Methods: Baseline data were obtained from an ongoing RCT entitled TOPPS (Treating Opioid Patients' Pain and Sadness) which compares an integrated care intervention with a health education contact-control condition. 163 people with OUD who also have depression and chronic pain were recruited from two primary care-based buprenorphine programs. Generalized anxiety and pain anxiety were assessed using the Generalized Anxiety Disorder Scale (GAD-7) and the Pain Anxiety Symptom Scale (PASS), respectively. Healthcare service use was obtained via the Treatment Services Review (TSR). Poisson regressions were used to estimate the association between GAD-7 (total index) or the PASS (total index or subscales: Cognitive, Avoidance, Fear, Physical Anxiety) with a type of healthcare service (emergency room visits, inpatient medical hospitalizations, outpatient medical visits, outpatient mental health visits). Recruitment site, age, gender, minority status, and years of education were included as covariates.

Results: Total GAD-7 index was positively and significantly associated with ED visits (IRR = 1.09, $p = .001$). Also positively and significantly associated with emergency room visits: total PASS (IRR = 1.49, $p = .013$), PASS cognitive, (IRR = 1.51, $p = .012$), PASS fear (IRR = 1.36, $p = .007$). Neither generalized anxiety nor pain anxiety predicted utilization of other inpatient or outpatient services.

Conclusions: Both general and pain-specific anxiety symptoms were significantly associated with increased healthcare service utilization, particularly emergency department visits. Addressing

anxiety symptoms in persons with opioid use disorder and chronic pain may be crucial for improving patient outcomes and reducing the burden on healthcare systems.

Keywords: opioid use disorder, Anxiety, chronic pain

Disclosure: Nothing to disclose.

P59. Five-Year Trends in the Consumption Rates of Benzodiazepine Anxiolytics in Serbia: A Comparative Analysis Across European Countries

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Background: Anxiety disorder affects about 4% of the world's population and can be treated with pharmacotherapy and psychotherapy, as well as a combination of both. The drugs of choice for the short-term treatment of anxiety are benzodiazepines (BZs); however, they are also one of the most commonly prescribed pharmacological drugs in general, increasingly used in many countries. Namely, in addition to use in clinical therapy, non-medical use and abuse of BZs is also registered, which represents a growing public health issue. In the Republic of Serbia and European countries, the most prescribed BZs are diazepam, oxazepam, lorazepam, bromazepam, prazepam and alprazolam. The objective of this study was to determine the trend of BZ anxiolytics consumption in Serbia from 2018 to 2022, as well as to compare it with the consumption of these drugs in other European countries.

Methods: Data on drug sales for human medicine from the Agency for Medicine and Medical Devices of Serbia (ALIMS) were used for the analysis of consumption in the period 2018-2022. The study followed the ATC/DDD methodology recommended by the World Health Organization (WHO). Data on drug consumption in other 12 European countries were obtained from the representative national registers, while GDP per capita data were sourced from the Eurostat database. Statistical analysis of the data involved using Student's t-test and Spearman's rank correlation test.

Results: In a period of 5 years, bromazepam was the most commonly prescribed drug in this group with a statistically significant increase ($R^2 = 0.36$, $p < 0.001$). Diazepam and lorazepam also had a very significant consumption increase ($p < 0.001$), while prazepam had a significant decrease in consumption ($p < 0.001$). Oxazepam and alprazolam had significant increase in consumption with $p = 0.016$ and $p = 0.0018$, respectively. Group consumption of BZs anxiolytics increased from 2018 to 2022 with a very significant change ($p < 0.001$). Compared to 12 other European countries, Serbia had higher consumption in every analyzed year. A moderate negative correlation was found between BZs consumption and GDP per capita.

Conclusions: Bromazepam was the most commonly prescribed BZ anxiolytic in Serbia from 2018 to 2022. BZs consumption in Serbia continues to grow and is significantly higher than in other European countries. The negative correlation between annual group BZs consumption and GDP per capita suggests that economic factors may play an important role in their usage trends. Analysis of consumption in our country indicates possible irrational prescribing and abuse; however, the impact of the Covid pandemic on this trend should also be considered. Moreover, BZs usage research are aimed on changing patients' attitude, education, and raising awareness of better control of prescribing these medicines by healthcare professionals. Therefore, further qualitative research is necessary to objectively determine the factors influencing the overuse of these drugs, as well as to

evaluate and potentially revise pharmacotherapeutic attitudes and prescribing practices.

Keywords: Anxiety, benzodiazepines, pharmacotherapy, Pharmacoepidemiology

Disclosure: Nothing to disclose.

P60. Anxiety as a Disease of White Matter Disruption: Association Between Anxiety Severity and Multiple Sclerosis Lesion Burden in the Uncinate Fasciculus

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Background: Multiple sclerosis (MS) is an immune-mediated neurological disorder that affects one million people in the United States. The rates of anxiety in MS are higher than anxiety comorbidity in other chronic illnesses, with up to 50% of patients with MS experiencing anxiety. However, the mechanisms of anxiety in MS are not well described.

Animal models of anxiety have frequently demonstrated that abnormalities in medial prefrontal cortex and amygdala function are associated with anxiety-like behaviors. In humans, a recent meta-analysis of functional connectivity in anxiety disorders identified associations between anxiety symptoms and hypo-connectivity between the amygdala and medial prefrontal cortex, suggesting that the prefrontal cortex is not able to exert adequate inhibitory control of the amygdala. The amygdala and medial prefrontal cortex are structurally connected by the uncinate fasciculus (UF). Though microstructural changes in UF have been found in anxiety disorders, to our knowledge no previous study has evaluated the relationship between UF disease and anxiety in MS. This study aims to test whether white matter lesion burden in the uncinate fasciculus is associated with anxiety severity in patients with MS.

Methods: Participants with MS were identified from the electronic medical record and stratified into three age- and sex-matched groups: 1) MS without anxiety (MS+noA); 2) MS with mild anxiety (MS+mildA), 3) MS with severe anxiety (MS+severeA). MS+noA included persons who had no psychiatric diagnosis, took no psychiatric medications, and were asymptomatic on PHQ 2/9 ($n = 99$, age (SD) = 49.4 (11.7), % female = 75). MS+mildA included persons with either a diagnosis of an anxiety disorder (F40*) or a prescription for an anti-anxiety medication ($n = 249$, age (SD) = 47.1 (11.1), % female = 82). MS+severeA included persons who had both an anxiety disorder and were taking an anti-anxiety medication ($n = 23$, age (SD) = 47.1 (12.4), % female = 78).

Structural MRI was obtained as part of routine care at 3T using a research-quality protocol. White matter lesions were automatically segmented using the algorithm Method for Inter-Modal Segmentation Analysis and individual lesion maps were normalized to MNI template space. For the right and left uncinate fasciculus, streamlines intersecting lesions at any point in their trajectory were considered injured. As in prior work, total volume occupied by injured streamlines was calculated as a measure of disease burden within the fascicle and averaged between right and left UF. Generalized additive models were used to test the relationship of mean UF lesion burden to diagnostic effects (MS+noA versus MS+severeA) as well as the parametric effect of anxiety severity across all three groups. In all models, sex and a spline of age were included as model covariates.

Results: Mean UF burden was higher in MS+severeA as compared to MS+noA ($T = 2.10$, $P = 0.04$, $R^2 = 0.16$, Cohen's $f^2 = 0.19$). Additionally, a parametric effect of anxiety burden was also found, where higher anxiety burden was associated with higher mean UF burden ($T = 2.1$, $P = 0.03$, $R^2 = 0.09$, Cohen's $f^2 = 0.10$). There were no sex differences in mean UF burden in either analysis. In both analyses, greater age was associated with higher mean UF burden ($P < 0.001$ for both).

Conclusions: We present an approach for studying the relationship of white matter lesions to anxiety using a combination of electronic medical record data and MRI acquired as part of routine care. Additionally, we extend previous animal and human research, and demonstrate that overall lesion burden in the uncinate fasciculus is associated with the presence and severity of anxiety in patients with MS. Future studies linking white matter lesion burden with prospectively assessed anxiety in MS may provide a way to understand both the pathophysiology of anxiety in MS and general network mechanisms of anxiety more broadly.

Keywords: Anxiety, multiple sclerosis, Lesions, clinical neuroimaging research, neuroimaging

Disclosure: Nothing to disclose.

P61. Impact of Choice Freedom and Time Predictability in a Decision-Making Task Involving Working Memory

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Background: Working memory is impaired in various psychiatric disorders, including schizophrenia and major depression. Moreover, current pharmacotherapy only partially, if at all, treats these symptoms, which impair the quality of life of patients. Thus, better models to study cognition are needed, in order to enable more scientifically driven drug development.

Methods: Here we developed a new cognitive task that requires working memory in mice (C57Bl/6 strain). The task relies on their ability to maintain sensory information (two distinct tones, 1 kHz and 11 kHz; 1.5 s) for several seconds, and to successfully associate them with the retrieval of rewards in the two opposite arms of a T-maze. During the training phase of the task, we evaluated the effect of guided training on task performance. Finally, in the working memory testing phase, we studied how temporal predictability affects decision making.

Results: During the training stage (20 sessions), mice were presented with different proportions (25%, 50% or 75%) of forced trials, where only the rewarded arm is available after the cue presentation, vs. free trials where both arms are available. When comparing early (first 5 sessions) and late (last 5 sessions) training, we found that mice increased the probability of going to the correct arm, in both forced and free trials (two-way ANOVA, early-late factor; $F(1, 16) = 181.4$, $****p < 0.0001$. $n = 9$, 6 females. 3 mice per group). Moreover, we found that accuracy increases with increasing free choice trials proportion (Student's t-test, 25% vs. 75% free trial proportion, $*p < 0.05$. $n = 9$, 6 females. 3 mice per group). Finally, in the working memory testing phase, we show that accuracy increases when animals are provided with multiple delay lengths compared to only one (Student's t-test, 1 vs. 3 delays, $*p < 0.05$. $n = 8$, 5 females. 4 mice per group).

Conclusions: Together, these findings support the importance of adequate training design with minimal guidance, and providing some degree of unpredictability for optimizing learning in decision-making processes.

Keywords: Cognition, Cognitive / behavioral flexibility, working memory

Disclosure: Nothing to disclose.

P62. Effects of Chemogenetic Activation of CRF Neurons in the Central Nucleus of the Amygdala on Cocaine-Related Behaviors

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Background: Cocaine use disorder is a serious health problem. Chronic cocaine misuse leads to adverse psychological and behavioral maladaptations. Acute rewarding effects of cocaine exposure act as reinforcing stimuli to sustain drug-seeking, and drug-taking behavior is further linked to aversive effects such as increased activity of stress-response systems during withdrawal.

Corticotropin releasing factor (CRF) in the central nucleus of the amygdala (CeA) is a critical stress-related neuropeptide that promotes anxiety, stress, fear, pain, motivation, and addiction-like behaviors. Cocaine withdrawal increases CRF mRNA levels, extracellular levels of CRF, and negative emotional states, leading to increased cocaine intake when access to the drug is resumed through negative reinforcement processes. However, the role of CRF neurons during intoxication is unclear. Recent studies suggest that CeA CRF neurons can promote incentive sensitization to cocaine without distress. One may also anticipate that activation of CRF neurons during intoxication would produce positive punishment through the emergence of negative emotional states. It is, therefore, unclear whether artificial activation of CRF neurons during cocaine self-administration (SA) in dependent rats would promote or prevent cocaine SA.

To test these hypotheses, we used a novel chemogenic receptor designed for control of CRF neuron activity whenever cocaine is in the brain. The ligand binding domain of the nicotinic $\alpha 7$ acetylcholine receptor was mutated to bind cocaine and fused to the ion pore domain of the 5HT3 receptor (Coca-5HT3), allowing for neuronal activation of CRF neurons when cocaine is present. We then tested the effect of passive cocaine administration on cocaine-relevant behavioral and physiological measures and tested the effect of coca-5HT3CRF on chronic cocaine SA under short (2h) and long (6h) access.

Methods: Coca-5HT3 was encoded in Cre-dependent AAV and stereotactically injected into the CeA of male and female CRF-Cre rats ($n=7/\text{sex}/\text{virus}$). Rats underwent a 3-stage battery of behavioral paradigms to evaluate CRF neuronal activation on cocaine-related behaviors. Stage 1: We measured acute responses to cocaine (10mg/kg, i.p.) versus saline on heart rate, breath rate, pulse distention, arterial O₂ saturation, anxiety-like behavior, mechanical nociception, irritability-like behavior, and locomotion. Stage 2: rats were implanted with an intravenous (IV) jugular catheter and underwent short access (ShA; 2hr) IVSA (0.5mg/kg), dose-response, progressive ratio, and contingent foot shock (0.3mA, 30%). Stage 3: rats underwent long access (LgA; 6hr) IVSA, followed by dose-response, progressive ratio, and contingent foot shock.

Results: Stage 1. Coca-5HT3 expression within the CeA of adult rats increased weight (time x virus; $p < 0.0001$) and increased heart rate (time x virus; $p = 0.0189$) following cocaine injection. Coca-5HT3 expression reduced pulse distention during both saline control and cocaine exposure (time x sex x virus; $p = 0.0449$ and 0.0499 , respectively). Coca-5HT3CRF males showed fluctuation in breath rate above and below control across time (time x virus; $p = 0.0568$) following cocaine injection, and Coca-5HT3CRF males

additionally had increased irritability-like behavior over control following cocaine injection (treatment x virus; $p = 0.0533$). Stage 2. Dose-response following ShA resulted a reduction in inactive lever presses for males only (dose x virus; $p = 0.0397$). Coca-5HT3CRF rats pressing < controls on the active lever during contingent foot shock following ShA (shock day x virus; $p = 0.0078$). Stage 3. LgA IVSA resulted in a significant increase in active lever presses (session x virus; $p = 0.0129$).

Conclusions: Activation of the CRF system within the CeA in response to cocaine exposure resulted in a wide array of alternate behavioral outcomes. Coca-5HT3 expression produced a significant increase in weight, and alterations in somatic behavior and IVSA lever pressing suggesting there are many non-specific effects independent cocaine. Coca-5HT3CRF females had increased heart rates following cocaine injection when compared to control, whereas coca-5HT3-expressing males were initially similar, then lowered below control heart rate. Pulse distention was reduced in Coca-5HT3CRF rats; however this was noted both during saline control as well as following cocaine injection. Only Coca-5HT3CRF male rats pressed the inactive lever at lower levels during dose-response following ShA, whereas all Coca-5HT3CRF rats pressed the active lever at reduced levels during contingent foot shock following ShA. Lastly, Coca-5HT3CRF rats pressed the active lever during LgA IVSA at higher levels than control during the first 5 sessions of LgA, leading to what appears to be an immediate escalation of intake during the first sessions. These results suggest that activation of CeA CRF neurons during cocaine intoxication impacts both somatic and neurologic behavioral outcomes, however, results largely demonstrated that behavior is dependent on the time course of cocaine exposure and the previous history of either ShA or LgA. While activation of CRF neurons by cocaine did not affect cocaine SA measures during ShA, it robustly increased cocaine SA during the first sessions of LgA.

Keywords: CRF, cocaine, central nucleus of the amygdala, chemogenetics, Intravenous self-administration

Disclosure: Nothing to disclose.

P63. The Effect of Orexin Receptor Antagonism on Motivated Behaviors and Gut Microbial Communities

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Background: Orexin (or hypocretin) hypothalamic peptides are involved in the regulation of food intake and may contribute to the development of maladaptive feeding behaviors (ie, over-consumption of high fat, sugar). Recent studies have also indicated that the brain-gut-microbiome axis plays a pivotal role in the pathophysiology of these behaviors. We contend that the orexin system may be involved in bidirectional communication of the brain-gut-microbiome axis and delineating its role can contribute to our understanding of mechanisms that lead to excessive food consumption. The goal of this study was to determine the effect of repeated orexin receptor-1 antagonism on palatable food preference and the impact on gut microbiota communities.

Methods: Female Sprague Dawley rats ($n = 8/\text{group}$, 250-300g) were categorized as high preferring (HP) or low preferring (LP) based upon their consumption of palatable food (PF, high fat, sugar pellets) across nine, intermittent feeding tests. After the phenotypes were identified, rats were administered SB-334867 (SB, 20mg/kg, IP) and given a feeding test to determine the impact of SB on PF consumption. Subsequently, the HP and LP rats were given only regular chow for several days followed by a no-drug, feeding test to ensure a return to baseline for PF intake before

administering another SB-challenge feeding test. This protocol was repeated until each rat received five SB-challenge feeding tests. A control group of rats with only regular chow and no PF exposure were included in the study. Fecal samples were collected from each rat before any SB administration and after each SB-challenge feeding test. DNA was extracted from fecal samples, and subjected to PCR to amplify the V4 region of the 16S rRNA genes, which were then sequenced on the Illumina MiSeq platform. Sequences were processed and analyzed using Mothur v.1.40.5. Rats were transcardially perfused and processed for orexin and c-Fos immunoreactivity in the lateral hypothalamus.

Results: On feeding test days, PF intake was significantly higher in HP versus LP rats ($p < 0.05$) and activation of orexin neurons may be directly correlated to PF intake. The LP and HP rats responded differently to orexin receptor-1 antagonism. After repeated administration of SB, there was no significant difference in PF consumption for LP rats; however, there was a significant reduction in PF intake for HP rats ($p < 0.05$) versus pre-SB consumption levels. There were also significant changes in alpha and beta diversity of the microbial communities in LP and HP rats after repeated SB20mg/kg. Alpha diversity was significantly affected by the number of days of SB20mg/kg tests. The Shannon Index was used to assess the richness component, while the inverse Simpson measured the level of biodiversity through richness and evenness. For each of these measures of alpha diversity, there was an increase in amplicon sequencing variants (ASVs) after five exposures of SB20mg/kg. The number of ASVs observed after 5 administrations of SB20mg/kg was significantly higher than for pre-SB20 ($p < 0.005$), day 1 SB20 ($p < 0.05$), and day 3 SB20 ($p < 0.005$). Beta diversity was significantly affected by the number of days administered SB20mg/kg, evidenced by significant shifts in the microbial communities in response to diet and time (Bray-Curtis ANOSIM, $p < 0.001$).

Conclusions: Preliminary analyses reveal that rats with prior exposure to PF have higher expressions of c-Fos positive, orexin-neurons versus control rats in the LH. Repeated administration of SB 20mg/kg may reduce this activation, thereby, reducing the amount of PF intake over time and leading to shifts in the microbial communities of these rats. Alpha and beta diversity were significantly affected by the number of days the SB20mg/kg was administered. These data will help to guide ongoing investigations to delineate microbial-dependent, functional pathways that impact behavior to reduce hedonic and maladaptive feeding states.

Keywords: orexin receptor antagonist, behavior, microbiome, food preference

Disclosure: Nothing to disclose.

P64. School's Out for the Summer: Quantifying Seasonal Variation in Children's Cognition Across Large-Scale Datasets

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Background: Children's cognitive abilities vary across both short and long timescales, from circadian fluctuations to year-by-year developmental changes. "Summer slide" refers to seasonal variation in children's performance on academic assessments, characterized by decreased performance during months of school vacation. While this phenomenon has been long described by teachers and caregivers and has been investigated in small-scale studies using linear models of academic test performance, no

large-scale studies have quantified cyclical variation in children's performance on laboratory-based cognitive assessments.

Methods: Using multiple, international, large-scale datasets (Adolescent Brain Cognitive Development (ABCD) Study: $n = 11,042$, 9-11y; Philadelphia Neurodevelopmental Cohort (PNC): $n = 9,416$, 8-22y; Growing Up in Singapore Towards healthy Outcomes (GUSTO): $n = 503$, 7y), we model time-of-year effects using generalized additive models with cyclic cubic splines. To determine whether declines in cognitive performance generalize beyond scholastic assessments, we leverage multiple well-validated laboratory-based cognitive task batteries each assessing multiple previously-derived cognitive subdomains. To model time-of-year as a cyclical rather than continuous measurement, we fit generalized additive models (GAMs) with cyclic cubic splines. To investigate whether declines in performance are specifically related to school vacation, we compare school-age children to young adults and compare school-age children across countries with different seasonal patterns in average temperature, climate, and timing of school vacation. Finally, we begin to investigate potential moderating factors by leveraging "exposome" scores derived by factor analysis, which capture deep phenotyping of environmental features and socioeconomic status.

Results: In school-age children from the United States, we consistently found minima in cognitive performance between July-September following school vacation that replicated across multiple cognitive domains and across both ABCD (General Cognition: partial R-squared = 0.023, $pFDR < 0.001$; Executive Functioning: partial R-squared = 0.013, $pFDR < 0.001$; Learning/Memory: partial R-squared = 0.011, $pFDR < 0.001$) and PNC samples (Overall Cognition: partial R-squared = 0.005, $pFDR < 0.001$; Executive Function: partial R-squared = 0.004, $pFDR < 0.01$; Complex Reasoning: partial R-squared = 0.003, $pFDR < 0.001$; Social Cognition: partial R-squared = 0.003, $pFDR < 0.001$; Memory: partial R-squared = 0.004, $pFDR < 0.001$). This effect was specific to school-age children and not young adults (age x time-of-year interaction: all $ps < .01$). In school-age children from Singapore, minima instead aligned with November-January school vacation (Matrix Reasoning: partial R-squared = 0.033, $pFDR = 0.037$; Block Design: n.s.). In the ABCD Study dataset, we found a large, significant main effect of general exposome scores (partial R-squared = 0.260, $p < 0.001$) but did not observe a significant interaction between time-of-year and general exposome scores. We observed a significant time-of-year interaction with one of six exposome subscore scores referred to as Dense Urban Poverty, which primarily captures environmental features related to dense urbanicity, crowding and crime, neighborhood poverty and household income (partial R-squared = 0.002 $p < 0.01$).

Conclusions: These results demonstrate a systematic, generalizable, small-magnitude decrease in children's cognitive performance aligning seasonally with school vacation. These seasonal decreases in performance are found not only on academic test scores but on a wide variety of laboratory-based cognitive assessments, suggesting that time-of-year variation in youth cognition is a more general phenomenon that may extend beyond "forgetting" of recently learned material. Our results also show that seasonal decreases in cognitive performance consistently replicate across international samples and are specific to the timing of school vacation in school-aged children, highlighting the importance of the structure of the academic school year rather than other factors (e.g. seasonal variation in temperature or weather patterns) that would presumably affect adults and children similarly and exhibit different patterns across climates. Future studies examining variation in children's cognitive performance may want to account for this cyclical variability in cognition across the year, particularly as more large-scale datasets collect cognitive measures across year-long or longer study time frames.

Keywords: Cognition, statistical methods, Children and Adolescents, exposome, Behavior

Disclosure: Nothing to disclose.

P65. From Sacred to Toxic: Decoding the Neurodevelopmental Effects of Incense Stick Smoke

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Background: Incense stick combustion (ICS) is a widespread cultural practice, especially prevalent in Southeast Asia, where it is imbued with religious and therapeutic significance. Despite its traditional benefits, modern ICS often contains a variety of synthetic chemicals, raising concerns about potential health risks. Recent analyses via Gas Chromatography-Mass Spectrometry (GCMS) have identified several compounds in ICS that violate Lipinski's rule of five, suggesting a propensity for neurotoxic effects. Key affected pathways include neuroactive ligand-receptor interaction and calcium signaling pathways, which are crucial in neurodegenerative conditions such as Parkinson's and Alzheimer's diseases. Prominent genes such as STAT3, BCL2, and MTOR have been implicated, underscoring the potential health hazards associated with ICS exposure.

Methods: To investigate the toxicity of ICS, we employed zebrafish (*Danio rerio*) embryos as an in vivo model due to their transparent development and genetic similarity to humans. Embryos were exposed to ICS at various concentrations (0.1, 0.3, 0.5, 1, 7, and 14 µg/ml) and monitored for developmental endpoints and mortality. We assessed oxidative stress by measuring reactive oxygen species (ROS) levels and lipid peroxidation via the DPPP assay. Lipid accumulation was evaluated using Nile red staining. The activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), along with lactate dehydrogenase (LDH) activity, were quantitatively analyzed. Gene expression levels of SOD1, CAT, TNF-α, IL-1β, p53, Bcl-2, and BDNF were also measured using qPCR.

Results: ICS exposure resulted in a dose-dependent increase in developmental toxicity. High concentrations (7 and 14 µg/ml) caused immediate mortality, whereas lower concentrations (0.1 to 1 µg/ml) led to various developmental defects, including yolk sac edema, skeletal malformations, and pericardial edema. Behavioral assays indicated hypoactivity, with reductions in both distance traveled and velocity. Biochemical assays showed a significant increase in ROS and lipid peroxidation in a dose-dependent manner. Enzymatic assays revealed a reduction in SOD and CAT activities, indicative of compromised antioxidant defense mechanisms, and an increase in LDH activity, suggesting cytotoxicity. Gene expression analysis supported these findings, showing downregulation of antioxidant genes, upregulation of inflammatory markers, and modulation of apoptosis-related genes.

Conclusions: Our study highlights the significant health risks associated with the use of incense sticks, demonstrating dose-dependent neurotoxicity and developmental disruptions in zebrafish embryos. The findings underscore the critical need for regulation and modification of incense compositions to mitigate health risks, especially in settings where ICS is used frequently and in closed environments. Further studies are warranted to explore the long-term effects of chronic exposure to ICS in human populations.

Keywords: Incense sticks, Toxicity, Public health, Zebrafish

Disclosure: Nothing to disclose.

P66. Sex and Estrous Cycle Dependent Effects on Cue-Triggered Food-Seeking in Obesity-Prone and Resistant Rats

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Background: The prevalence of severe obesity (BMI ≥ 40 kg/m²) is greater in woman than men and in women obesity and its comorbidities are associated with hormone imbalances that increase risk for breast cancer, infertility, and polycystic ovarian syndrome. Therefore, there is a critical need to understand how ovarian hormones influence neurobehavioral processes that promote over-eating and obesity in females. Furthermore, in people cravings triggered by sights, sounds and smells associated with food (i.e., food cues) promote over-eating and these cue-triggered urges to eat are more pronounced in people who are susceptible to diet-induced weight gain. We have established that motivational responses to food cues are more pronounced in selectively bred obesity-prone (OP) and obesity-resistant rats (ORs). However, there is very limited understanding of how ovarian hormones influence cue-triggered food-seeking, and how this may interact with susceptibility to obesity. As a first step, we examined how the expression of Pavlovian-to-Instrumental Transfer (PIT) varies across the cycle in OP and OR females.

Methods: Rats first underwent Pavlovian conditioning where one cue was always paired with food pellet delivery (CS+) and a second cue was never paired with food (CS-). Next, rats learned to lever press for this same food pellet (Bio-Serv; banana flavored pellet). During testing, the ability of the CS+ vs CS- to invigorate active lever pressing in the absence of food was measured. Using vaginal cell cytology, estrous cycle phase was monitored throughout initial training and during PIT testing.

Results: Initial results show robust expression of PIT in OP and OR females tested in the M/D phase of the cycle, but an absence of PIT when tested during P/E (three way RM ANOVA: main effect of CS F (1, 43) = 17.07, p < 0.001; main effect of cycle stage F (1, 43) = 6.105, p < 0.02; no significant interactions among factors of CS, strain, or cycle stage) that was largely driven by the absence of PIT in OPs.

Conclusions: These results provide an important foundation for examining underlying neurobiological mechanisms. Results will be discussed in light of the established roles for estradiol and progesterone in food-seeking, and the ability of ovarian hormones to influence striatal function. Additionally, preliminary results describing how machine-learning based approaches to behavioral analysis using LabGym can deepen our examination of rodent behavior in experimental settings will be presented.

Keywords: incentive motivation, Ca²⁺ -permeable AMPA- type glutamate receptors, Obesity, Ventral Striatum

Disclosure: Nothing to disclose.

P67. Prior Stress Increases Reactivation of Contextual Fear Engram in Hippocampal Dentate Gyrus

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Background: Exposure to traumatic stress can cause long-lasting pathological changes in cognition and affect sometimes leading to diagnoses such as post-traumatic stress disorder (PTSD). The stress-enhanced fear learning (SEFL) model recapitulates important and understudied components of PTSD, such as stress-induced sensitization of fear learning. The SEFL procedure entails exposing mice to footshock stress in context A followed later by a single shock in a different context (context B). When tested later for fear recall in context B, previously stressed mice exhibit enhanced levels of freezing compared to non-stressed controls. Previous studies have elucidated the influence of the amygdala on

SEFL. How stress affects hippocampal encoding of fear memory is unknown.

Methods: We used SEFL and activity-dependent neuronal tagging with FosTRAP/Ai6 mice to investigate the effects of stress on fear memory ensembles in hippocampal dentate gyrus and basolateral amygdala. Prior research shows that contextual fear acquisition activates an ensemble of fear engram cells in DG, and the later reactivation of these cells is both necessary and sufficient for behavioral expression of the fear memory. Here, FosTRAP2/Ai6 mice received shock stress or exposure to the context without shock in context A on day 1. Five days later, mice received 1-shock conditioning in context B and immediately received an intraperitoneal injection of 4-OHT (55mg/kg) to tag fear acquisition neurons with the zsGreen reporter. One day later, mice were tested for fear recall in Context B and were perfused 90 minutes after the completion of testing. We then examined 1) the number of zsGreen+ fear engram cells, 2) the number of recall-activated cells (Fos+ cells), and 3) the number/percentage of fear engram cells reactivated during recall (Fos+ and zsGreen+) in the dentate gyrus.

Results: Our results show that prior stress increases the number of fear engram cells in the dorsal dentate gyrus ($F(3, 30) = 8.265$, $p = 0.004$) and alters the percentage of reactivated fear engram cells (Fos+ and zsGreen+ cells/mm²) in the ventral dentate gyrus and basolateral amygdala ($F(3, 24) = 10.44$, $p = 0.0001$; $F(2, 13) = 4.517$, $p = 0.0324$, respectively).

Conclusions: Thus, stress has dissociable effects in the encoding and retrieval of a new fear memory in the dorsal versus dentate gyrus. Taken together, these data suggest a role for the ventral hippocampus- basolateral amygdala system in enhancing formation of new fear memories via reactivation of a fear ensemble, and may reflect distinct mechanisms of stress in the ventral hippocampus. These studies are expected to reveal novel mechanisms for the stress-induced sensitization of fear learning.

Keywords: Acute Traumatic Stress, Fear conditioning, engram

Disclosure: Nothing to disclose.

P68. Spectrotemporal Profiling of Ultrasonic Vocalizations During Neonatal Opioid Withdrawal Reveals a Kappa Opioid Receptor Component in Female FVB/NJ Mice

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Background: Opioid use during pregnancy can lead to neonatal opioid withdrawal syndrome (NOWS), comprising withdrawal symptoms that appear 24 – 72 hours after birth due to the spontaneous cessation of in utero opioid exposure, including gastrointestinal and autonomic system dysfunction, irritability, and excessive high-pitched crying. NOWS severity is assessed using subjective, complex scoring systems, that could potentially lead to inaccurate evaluations, ineffective treatment plans and increased hospital costs. Our goal was to employ a 3rd trimester-approximate mouse model for NOWS to examine severity of behaviors accompanying neonatal opioid withdrawal [e.g., reduced body weight gain, hypothermia, thermal hyperalgesia, and increased ultrasonic vocalizations (USVs)] and in particular, the classification and quantification of USV syllables comprising the spectrotemporal profile of withdrawal. Neonatal mouse USVs are emitted exclusively in isolation to signal distress and initiate maternal care and thus model the severity of the negative affective state of withdrawal. We also examined the brainstem transcriptomic profile during withdrawal to identify potential

pharmacological targets for reducing the negative affective state associated with neonatal opioid withdrawal.

Methods: All mouse experiments were conducted in strict adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committees at Boston University and Northeastern University. We used a 3rd trimester-approximate mouse model for NOWS where inbred FVB/NJ pups received twice-daily injections of morphine (10mg/kg, s.c.) or saline (0.9%, s.c.) from postnatal (P) day 1 to P15. This exposure period models the neurodevelopmental events that occur during the third trimester of human pregnancy and is both necessary and sufficient to induce robust withdrawal traits [e.g., low body weight, thermal hyperalgesia, increased ultrasonic vocalizations (USVs), and altered USV spectrotemporal profiles]. Excessive, high-pitched cries are a hallmark feature of NOWS. USVs in mice are classified into syllables based on their spectrotemporal properties. However, the contribution and significance of individual syllable types to opioid withdrawal are unknown. We developed a custom USV classifier to identify specific syllable types comprising affective withdrawal severity. On P16 during spontaneous withdrawal, we collected brainstem tissue and performed bulk RNA-seq to identify transcriptomic adaptations and potential pharmacological targets for reducing withdrawal. Finally, we tested the role of the kappa opioid receptor (KOR)/dynorphin system in mediating increased USVs and specific syllable types during withdrawal.

Results: During spontaneous morphine withdrawal (16h) on P14, we observed a Morphine Treatment x Sex interaction ($\beta = 919.6$, $SE = 180.7$; $t(33) = 5.088$, **** $p < 0.0001$), where morphine-withdrawn females emitted more USVs than saline-treated females ($\beta = 937.0$, $SE = 181$; $t(33) = 5.19$, **** $p < 0.0001$). We identified a unique USV syllable profile associated with morphine withdrawal, i.e., an increase in percentage of Complex 3 syllables driven by morphine-withdrawn females ($\beta = 0.14$, $SE = 0.044$; $t(33) = 3.13$, ** $p = 0.0036$). SAL: $n = 20$ (8F,12M); MOR: $n = 17$ (9F,8M). Brainstem transcriptomics revealed an upregulation of Oprk1 (kappa opioid receptor (KOR)); $\log_2FC = 0.35$, ** $p = 0.0049$). SAL: $n = 4$ (2F,2M); MOR: $n = 4$ (2F,2M). The dynorphin/KOR system contributes to withdrawal-induced dysphoria; thus we tested the contribution of KOR to the withdrawal-induced increase in USVs and syllable types. Pre-treatment (20h) with the KOR antagonist nor-BNI (30mg/kg, s.c.), reduced USVs in morphine-treated females ($\beta = -272.35$, $SE = 127.11$, $t(143) = -2.143$ * $p = 0.034$). There was no nor-BNI x Morphine Treatment interaction on Complex 3 emission ($\beta = 0.012$, $SE = 0.024$, $t(149) < 1$). SAL/SAL: $n = 40$ (22F,18M); SAL/norBNI: $n = 33$ (17F,16M); MOR/SAL: $n = 39$ (21F,18M); MOR/norBNI: $n = 39$ (22F,17M). The KOR agonist U50,488h (0.625mg/kg, s.c.) increased USV emission in both sexes on P10 ($\beta = 406.2$, $SE = 148.9$, $t(56) = 2.73$, ** $p = 0.0085$) and in females on P14 ($\beta = 164.85$, $SE = 67.34$, $t(65) = 2.45$, * $p = 0.017$). SAL: $n = 29-32$ (18-19F,11-13M); U50,488h: $n = 31-37$ (17-21F,12-16M).

Conclusions: Consistent with excessive crying observed in human NOWS infants, we observed increased USVs in females on P14 during spontaneous morphine withdrawal. We identified a withdrawal-associated USV syllable signature shown by an increase in the percentage of Complex 3 syllables, which we hypothesize is a novel biobehavioral marker for negative affective withdrawal in neonatal mice. We can use the USV signature as a tool to assess the efficacy of pharmacotherapeutics. Upregulation of Oprk1 in the brainstem led us to evaluate KOR as a target for alleviating the negative affective state associated with withdrawal. Blocking KOR activation during morphine withdrawal reduced USVs in females on P15, while activating KOR in morphine-naïve pups increased USVs in females on P14. Our results show a female-specific contribution of KOR to negative affective symptom severity associated with NOWS and provide a novel approach

utilizing spectrotemporal profiles of USVs for assessing affective states during neonatal opioid withdrawal.

Keywords: Kappa Opioid Receptor Antagonist, neonatal opioid withdrawal, neonatal abstinence syndrome, Ultrasonic vocalization (USV) model, machine learning classification

Disclosure: Nothing to disclose.

P69. Quantitative Profiling of the Effects of Subanesthetic Ketamine on Naturalistic Behavior and Brain Microstructure

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Background: Ketamine is an anesthetic, analgesic, and rapid-acting antidepressant drug which has also shown promise for treating chronic pain and obsessive-compulsive disorder (OCD). For evaluating the multifaceted effects of ketamine and for understanding the conditions it treats, there is a need for quantitative, translatable biomarkers which are sensitive to the drug's mechanisms of action. Prior studies have shown that while ketamine primarily antagonizes N-methyl-D-aspartate receptors, its opioidergic and dopamine-stimulating actions also play a role in its antidepressant-like effects. Additionally, preclinical studies have shown that ketamine induces structural plasticity in the medial prefrontal cortex (mPFC) and that this process is correlated to its therapeutic effects. Here, we employ computational ethology and multishell diffusion imaging to quantify ketamine's psychopharmacology and effects on brain microstructure, respectively, in healthy rodents and in rodent models of neuropathic pain and OCD.

Methods: This study consists of four experiments (I-IV).

In Experiment I, we administer ketamine and other drugs by various routes of administration to male and female Sprague-Dawley rats ($n = 6-8$ per condition) and record them behaving in an open field acutely post-administration. We employ four computational ethology tools for pose estimation and segmentation: DeepLabCut, B-SOiD, VAME, and Keypoint-MoSeq. For blood pharmacokinetics comparisons, ketamine was quantified in whole blood collected serially from the cut tail with LC-MS/MS.

In Experiment II, we surgically induce a spared nerve injury model of chronic pain or sham injury in male and female Sprague-Dawley rats ($n = 6-8$ per condition). On day 15 post-injury, we treat rats with one of five treatments: intraperitoneal (IP) saline; 10 mg/kg IP ketamine; or targeted delivery of ketamine to the rostral anterior cingulate cortex (rACC) with our group's novel ultrasound uncaging platform (intravenous (IV) saline; 1 mg/kg IV ketamine; or 1 mg/kg liposome-encapsulated ketamine, each with rACC sonication [250 kHz focused transducer, 1.1 MPa peak in situ pressure]). At baseline and days 1, 7, 14, 15, and 16 post-injury, we measure longitudinal changes in open field pose module usage, analgesia (via Von Frey), and anhedonia (via sucrose preference test).

In Experiments III-IV, we use multishell diffusion imaging in male and female C57BL6 mice ($n = 6-11$ per condition) and fit the diffusion tensor imaging (DTI) model to evaluate mean diffusivity (MD) and fractional anisotropy (FA) as well as the neurite orientation dispersion and density imaging (NODDI) model to evaluate neurite density index (NDI) and orientation dispersion index (ODI). Images were acquired on a Bruker 7T BioSpec with a CryoProbe RF coil (sequence parameters: $b = 1000, 2500 \text{ s/mm}^2$, 40 diffusion directions, $TE / TR = 21.7 / 4000 \text{ ms}$). Data were processed with FSL, Microstructure Diffusion Toolbox, and ANTs as

implemented in the MIRACL Toolbox. In Experiment III, we image wild type mice 0.5 h before and 24 h after administering 10 mg/kg IP ketamine with or without 10 mg/kg subcutaneous (SC) naltrexone pretreatment. In Experiment IV, we image SAPAP3 knockout mice 2 d before and 24 h after treatment and record their behavior in an open field at 24 h before and 0, 1, and 23 h after treatment with 30 mg/kg IP ketamine.

Results: Experiment I: 1-10 mg/kg IV and IP ketamine induced dose-dependent increases in locomotion pose modules and decreases in grooming pose modules which were modulated by opioid or dopamine blocker pretreatment. Linear discriminant analysis based on pose segmentation yielded higher classification accuracy in cross-validation than classifiers based on locomotion or center/edge preference. Delivering 10 mg/kg ketamine by IV, IP or SC routes of administration evoked behavioral changes with differing magnitude and kinetics, aligning by biexponential curve fitting with dose- and route-of-administration-matched blood pharmacokinetic data.

Experiment II: The neuropathic pain state was longitudinally characterized by increased use of stationary and ataxic gait modules and decreased use of walking modules, while ketamine administered by all tested methods (10 mg/kg IP, 1 mg/kg IV systemic, 1 mg/kg IV liposomal uncaging) altered exploratory modules at 1 h post-administration. By contrast, we did not see a significant effect of ketamine on mechanical withdrawal threshold at 1.5 h post-administration.

Experiment III: Ketamine increased NDI in the mPFC of male mice but not in females or males pretreated with naltrexone. We also observed trends toward positive Δ NDI and negative Δ ODI following ketamine treatment as compared with saline in males, but less so in females and males pretreated with naltrexone, in infralimbic, prelimbic, and orbitofrontal areas. These results recapitulate our published finding of sex- and opioid-dependence of ketamine-induced structural plasticity in rats.

Experiment IV: SAPAP3 knockout mice exhibited at baseline increased FA and decreased ODI broadly across striatum, pallidum, and cerebral cortex. Ketamine induced trends toward increased NDI in the mPFC as well as trends toward positive Δ NDI and Δ ODI relative to saline in striatum and pallidum. Analysis of behavioral data is still underway.

Conclusions: Together, these results provide an in-depth, quantitative picture of therapeutically relevant effects of ketamine on behavior and brain microstructure using noninvasive, translatable approaches.

Keywords: Ketamine, computational ethology, diffusion MRI, chronic pain, Obsessive Compulsive Disorder

Disclosure: Nothing to disclose.

P70. Role of the Prelimbic Cortex in Signaling the Aversive Properties of Ethanol

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Background: The decision to consume alcohol is regulated by a balance between the rewarding properties of ethanol, which promote drinking, and the aversive properties, which limit drinking. Individuals who experience greater reward or less aversion in response to acute ethanol exposure are more likely to develop alcohol use disorder. Although the mechanisms of ethanol reward have been extensively studied, the neurobiological mechanisms underlying sensitivity to its aversive properties are unknown. The prelimbic subdivision of the medial prefrontal cortex (PL mPFC) has been implicated in both ethanol drinking and the response to aversive stimuli, leading us to hypothesize

that activity in this region is involved in signaling the aversive properties of ethanol.

Methods: We used *in vivo* fiber photometry to record intracellular calcium activity, a proxy of neural activity, in the PL mPFC during ethanol-induced conditioned taste aversion (CTA). Adult male and female Long-Evans rats received an injection of AAV9-Syn-GCamP7s and an optic fiber implant into the right PL mPFC. Following four weeks of recovery, calcium activity was recorded in rats as they underwent CTA using a lickometer-equipped system. Rats were trained over two conditioning trials to associate the taste of a novel 0.1% saccharin solution with an intraperitoneal injection of either 1.5 g/kg ethanol or saline (volume-matched control), followed by a test day where rats received saccharin in the absence of an injection. Calcium signal was time-locked with licks during daily sessions in order to assess changes in PL mPFC population activity during drinking.

Results: Male and female rats that had saccharin paired with ethanol exhibited a significant reduction in both saccharin intake (mL) and number of licks across trials compared to saline controls (ANOVA, $n = 5-7/\text{sex}$; $*p \leq 0.004$), indicative of ethanol-induced CTA. This was associated with sex-specific changes in drinking strategies with males (unpaired t-test, $n = 5-7$; $*p \leq 0.05$), but not females (unpaired t-test, $n = 5-7$; $p > 0.05$), that received saccharin paired with ethanol exhibiting fewer drinking bouts on test day compared to saline controls. Preliminary analysis of the relationship between saccharin consumption and PL mPFC calcium activity shows significantly lower calcium activity at the onset of a drinking bout in rats expressing ethanol-induced CTA compared to saline controls (unpaired t-test, $n = 6/\text{sex}$; $*p \leq 0.05$). This was positively correlated with saccharin intake (Pearson correlation, $n = 6/\text{sex}$; $*p \leq 0.02$).

Conclusions: Together, these data suggest that the development of ethanol-induced CTA is associated with sex-specific changes to drinking microstructure, and that this may be due to distinct alterations in PL mPFC activity. Ongoing analyses are exploring the relationship between PL mPFC calcium signal and individual differences in ethanol-induced CTA. These findings have the potential to provide valuable insight into the neurobiological mechanisms driving sensitivity to the aversive properties of ethanol.

Keywords: conditioned taste aversion, prelimbic cortex, lick microstructure

Disclosure: Nothing to disclose.

P71. Brain Circuits of Cognition: A Transdiagnostic and Multidimensional Study

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Background: Cognitive function is commonly impaired across a range of psychiatric and neurological disorders. In many patients, cognition has been shown to be a strong predictor of quality of life, daily functioning, and overall prognosis of their disease. However, cognitive impairment is poorly treated by existing pharmacotherapies.

To date, few studies have focused on measuring cognitive deficits and understanding the neurocognitive mechanisms across disorders. This is important because many disorders have overlapping symptoms and comorbidities. It is unclear, though, to

what extent the underlying brain pathophysiology of transdiagnostic symptoms is common across disorders, or rather specific to a patient's diagnosis.

Methods: A 4-stage programme of work has been designed that aims to fill this gap in knowledge by: a) identifying the most robust and clinically relevant combination of tasks and physiological readouts to compare cognitive deficits and the circuits involved across disorders, and b) describing the commonalities and differences across neuropsychiatric disorders, both in terms of cognitive deficits and in their underlying brain circuit dysfunction.

Results: Here, we present data from the first 2 stages focusing on the dimensional analysis of the cognitive capabilities of a transdiagnostic cohort of patients with depression or schizophrenia. Preliminary results indicate, as expected, severe and multifaceted deficits in patients with schizophrenia, while impairments are more nuanced and heterogeneous in depressed patients. The newly developed cognitive task battery compares favourably against gold standard tools both in terms of sensitivity and ability to resolve cognitive dimensions.

Conclusions: The preliminary results lay the groundwork for the subsequent stages, both in terms of method enablement, and by resolving the dimensional deficits of the patients' cognition. The upcoming stages 3 and 4 will complement the description of cognitive deficits with the related neurophysiological underpinnings, using both electroencephalography and neuroimaging. The results of the full study will have an impact on multiple stages of research and development of new therapies: brain circuits identified as underlying cognitive dysfunction can inform drug target identification; physiological-behavioural profiles of deficits will help define patient populations susceptible to benefit from these targets' modulation; and both physiological and behavioural measures identified can inform the development of treatment response biomarkers.

Keywords: Cognition, Behavioral Tasks, Brain Circuits, Human Neuroimaging, EEG

Disclosure: Boehringer Ingelheim: Employee (Self)

P72. Sexually Divergent Behavioral and Activation Profiles of Midbrain Dopamine Neurons After Fentanyl Exposure

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Background: While men are more likely to be diagnosed with an opioid-use disorder, in the past decade, women have had a more rapid rate of opioid-related deaths than men. This highlights a need to understand how the brain may regulate individual susceptibilities to opioid seeking. Opioids, such as fentanyl, are known to act in dopaminergic circuits to modulate the reinforcing aspects of the drug and potentiate opioid seeking behaviors through the induction of associative learning mechanisms. Ventral tegmental area (VTA) dopamine neurons were once thought to be monolithic in function in both males and females. With the advancement of circuit dissecting and genetic tools, investigators have found that VTA dopamine neurons that send projections to subdivisions of the nucleus accumbens (NAc), such as the NAc core or NAc shell, have discrete roles in reward processing, reward learning, and motivation for food and for drugs. Yet, we still do not know if subpopulations of VTA dopamine neurons have sexually dimorphic function after opioid exposure.

Methods: To determine whether opioids distinctly regulate the heterogeneous midbrain dopamine system, we used combinatorial genetic strategies to isolate functionally-distinct dopamine subpopulations that project to the NAc core or NAc shell. We expressed cre-inducible fluorescent proteins (AAV-FLEX-eYFP) in

the VTA of Crhr1-Cre or Cck-Cre mice to isolate Crhr1VTA and CckVTA neurons that project to the NAc core or NAc medial shell, respectively. We then exposed opioid-naïve mice to a single injection of the opioid fentanyl at a range of doses and fixed brain tissue to capture the induction of Fos, an immediate early gene indicative of elevated neural activity. In a separate cohort of mice, we also profile the possibility of distinct rewarding properties of those doses with a fentanyl conditioned place preference assay.

Results: We found that a single injection of fentanyl selectively activates cFos in CckVTA dopamine neurons when compared to Crhr1VTA dopamine neurons. Further, male mice were able to acquire a conditioned place preference for fentanyl at much lower doses when compared to female mice.

Conclusions: Our findings suggest a sexually dimorphic regulatory process for activation of VTA dopamine neuron subpopulations, which could underlie differences in conditioned place preference for fentanyl.

Keywords: Opioid addiction, Dopamine, Sexual Dimorphism, mice

Disclosure: Nothing to disclose.

P73. Network-Wide Neural Markers of Emotional Reactivity and Regulation in Infants

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Background: Emotional reactivity and regulation undergo critical developmental during infancy, paralleling the rapid maturation of the infant brain, and can be assessed to predict future emotional behavioral outcomes. Identifying objective markers of infant emotional behavior enhances the understanding of the etiology of early psychopathology. In our previous work, we examined how emotion-related cortical structure, cortical microstructure, and white matter (WM) tract microstructure predict 3-month infant emotional reactivity and regulation in separate models. To further examine consolidated, network-wide neural markers for emotional reactivity and regulation, we now include structural and microstructural features of emotion-related cortical regions, i.e., subregions of the prefrontal cortex (PFC), as well as microstructural features of WM tracts involved in emotion functioning, i.e., the forceps minor (FM), cingulum bundle (CB), and uncinate fasciculus (UF), at 3 months, integrated into a single model to predict negative emotionality (NE), positive emotionality (PE), or soothability as a proxy for emotional regulation at 3 months. Additionally, sociodemographic and clinical variables collected from infant and their caregivers (3-month age in weeks, biological sex; caregiver age, total government assistance sum, EPDS depressed mood, PAI-BOR affective instability, and STAI state and trait anxiety) were included, as these factors can also impact the development of emotional reactivity and regulation.

Methods: Three-month-old infants ($n = 59$) underwent multi-shell diffusion and structural MRI scans. We used in-house image processing pipelines specifically designed for infant brain imaging to extract PFC subregional volume as a proxy for structural features, PFC subregional neurite density index (NDI) and orientation dispersion index (ODI) as proxies for microstructural features, and NDI and ODI of WM tracts as proxies for WM microstructure. We trained machine learning models using an artificial neural network that included all the aforementioned features, along with 3-month whole-brain developmental base-lines, and concurrent sociodemographic/clinical factors of the

infant-caregiver dyads, to predict 3-month NE, PE, and soothability in three independent models. Data were randomly partitioned into training and testing subsets with a ratio of 3:1. For each outcome, all neuroimaging-derived and sociodemographic/clinical features were first preprocessed with mutual information regressor for feature selection. Selected features were then trained in a deep artificial neural network model. A grid search with cross-validation was applied to find the best hyperparameters for the model, including hidden layer sizes, L2 regularization strengths, batch sizes, and initial learning rates. The model was subsequently trained with the optimized hyperparameters with rectified linear unit (ReLU) as the activation function and Adam optimization as the solver. The modeling accuracy was reported via root mean square error (RMSE) on the testing data.

Results: The modeling accuracy on the testing data for NE was $RMSE = 0.6875$. Predominant predictors of high NE were high microstructural complexity in cortical regions of the default mode network (DMN) and the ventral attention network (VAN). The modeling accuracy for PE was $RMSE = 1.6089$. The predominant predictor of low PE was low dispersion of WM tracts. The modeling accuracy for soothability was $RMSE = 0.7433$. The predominant predictor of low soothability was high microstructural complexity in cortical regions of the salience network (SN).

Conclusions: Higher microstructural complexity in DMN and SN cortical regions may lead to more integration with other neural networks, resulting in higher NE. Lower major WM dispersion may indicate delayed development of the emotion-related neural architecture, leading to low PE. Meanwhile, high microstructural complexity in SN cortical regions may result in greater attention and actions towards emotionally-salient external stimuli, thus leading to lower soothability. The high modeling accuracies of these consolidated models suggest that different cortical sub-regions and tracts are recruited for emotional reactivity and regulation at a network-wide level.

Keywords: Infant Brain, Emotional regulation, Multimodal Neuroimaging

Disclosure: Nothing to disclose.

P74. The Role of Neuronal TLR9 in Social Behavior

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Background: Toll Like receptor 9 (TLR9) is a key player in innate immunity. Although recent studies suggest a potential role of TLR9 in CNS plasticity, the cellular mechanisms are not clearly understood. In this study, we investigated the role of TLR9 in excitatory neurons on behavior.

Methods: Adult male mice with TLR9 deleted in excitatory neurons (CaMK2a-TLR9^{-/-}) and age matched controls were used. Social approach, social novelty, social interaction, locomotor activity, were examined. Data were analyzed using Analysis of Variance (ANOVA; for multiple-group comparisons). Post hoc analyses were carried out using Tukey's test. All the statistical analyses and graph preparation were performed using GraphPad Prism 9.0.0. $p < 0.05$ was considered significant.

Results: CaMK2a-TLR9^{-/-} mice showed deficits in social approach, social novelty and social interaction. Furthermore, TLR9-deficient mice exhibited reduced locomotor activity and anxiety-like behavior in the Open Field test. Our results show that TLR9 in excitatory neurons plays a key role in social behavior.

Conclusions: These findings provide valuable insights into the role of neuronal TLR9 in brain plasticity. Additional studies are in progress to understand the role of TLR9 in excitatory neurons on behavior.

Keywords: Toll-Like receptor 9 (TLR 9), Excitatory neuron, Social behavior

Disclosure: Nothing to disclose.

P75. Characterizing the Role of the Paraventricular Nucleus of the Thalamus During Outcome-Based Predictions

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Background: The ability to learn associations between cues and future outcomes is critical for survival. As such, individuals learn which neutral stimuli (e.g., cues) lead to rewards (e.g., food) and which cues predict harm and should be avoided. This ability to add 'meaning' or incentive value to neutral stimuli is known as "incentive value" and can elicit a strong motivational force. For example, in people suffering from substance use disorder, drug cravings or even drug relapse are often the result of exposure to cues that have been associated with drug-taking (Shaham et al., 2003). However, the neural mechanisms mediating these causal associations or incentive values are poorly understood.

Previous research has implicated the paraventricular nucleus of the thalamus as a potential brain region mediating incentive value (Khun et al., 2008). Moreover, our previous research showed that PVT neurons that express the dopamine D2 receptor (PVT2+) are critical for motivated behavior, and their activity is modulated by motivational parameters such as vigor and satiety. As such, these findings suggested that PVT2+ neurons, in particular, might be those responsible for encoding the incentive value of stimuli (Beas et al., 2024). Here, we aim to test the hypothesis that PVT2+ neurons play a critical role in the learned associations between environmental cues and the value of outcomes.

Methods: For this, we used bulk calcium imaging and fiber photometry to measure the activity of PVT2R+ neurons (using *Drd2-Cre* mice and *Cre-dependent GCaMP6s*) while hungry mice learned to perform a linear runway foraging-like task ($n = 4 - 6$). In this task, mice need to learn through trial and error to run back and forth from one of a linear maze (the trigger zone) to the other end of the maze (the reward zone) to obtain food rewards (strawberry Ensure).

Results: Quantification of area under the curve and max peak showed that at the beginning of the training in the task, PVT2+ neurons showed no significant modulation during the approach to the reward zone when compared to baseline ($t(3) = 0.94$, n.s.). However, as mice learned to associate the reward zone with the delivery of the reward, we observed a learning-dependent effect on the activity of these neurons (comparison from baseline: $t(3) = 3.35$, $p < 0.05$; comparison from session 1: $t(3) = 3.29$, $p < 0.05$). Furthermore, changing task contingencies, such as the type of outcome delivered or the relevant cues that signaled the delivery of the reward, also modulated PVT2R+ neurons.

Conclusions: Altogether, these data highlight that PVT2R+ neurons play a critical role in learned associations to make outcome-based predictions by adding subjective value to neutral stimuli.

Keywords: Reward Anticipation, Motivation, Paraventricular Nucleus of the Thalamus

Disclosure: Nothing to disclose.

P76. Neuronal Ensembles in PI Mediate Initial and Maintained Cocaine-Seeking in Male and Female Rats

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Background: Cravings, driven by strong learned associations between drugs and cues, play a crucial role in Cocaine Use Disorder. The prelimbic cortex (PL) is implicated in these drug-paired associations, but the persistence and specificity of PL neuronal ensembles in cocaine-seeking behavior remain unclear. Here, we aim to investigate whether PL neuronal ensembles formed during initial cocaine self-administration continue to drive cocaine-seeking in well-trained rats.

Methods: We used a viral Fos-TRAP (AAV-FosCreERT2 x AAV-DIO-hM4Di) approach to tag Fos-expressing PL neuronal ensembles that were active during initial acquisition of cocaine self-administration. We gave the rats 10 additional self-administration training sessions and subsequently inhibited these ensembles during a recall test with an injection of the DREADD agonist compound 21.

Results: We found that PL ensembles formed during acquisition remain behaviorally relevant in well-trained rats. Inhibiting non-relevant PL neurons did not affect cocaine-seeking, highlighting the specificity of the tagged ensembles. PL-NAc circuitry recruitment was confirmed in rats meeting acquisition criteria, supporting the role of this circuit in driving continued cocaine-seeking behavior.

Conclusions: Our findings reveal that PL neuronal ensembles formed during initial acquisition persist and mediate cocaine-seeking behavior in well-trained rats.

Keywords: Psychostimulant addiction, Learning and memory, Prelimbic Cortex

Disclosure: Nothing to disclose.

P77. Vicarious Social Defeat Stress Induces Sex-Specific Depression-Related Outcomes in Juvenile c57BL/6 Mice

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Background: Psychological stress exposure is a prevailing risk factor for the development of mood-related illnesses. Despite the growing literature indicating that affective disorders can arise after a traumatic event is vicariously experienced, this relationship remains to be thoroughly examined in juveniles at the preclinical level. Thus, the objective of the current investigation is to assess whether the vicarious defeat stress behavioral paradigm (Biol Psychiatry, 73[1], 7-14; 2013) – a model that dissociates psychological versus physical stress – induces a depression-like phenotype in adolescent male and female mice.

Methods: Adolescent (5 week old) male and female c57BL/6 mice witnessed the social defeat bout of a male conspecific, by a larger CD1 aggressor, for 10 consecutive days. Twenty-four h after the last stress exposure (postnatal day 45), separate groups of mice were tested in the social interaction, sucrose preference, or Morris water maze tests. Additionally, we evaluated body weight-gain across days of stress exposure.

Results: When compared to same-sex non-stressed controls, male mice exposed to vicarious defeat stress exhibited a depressive-like phenotype, as inferred from decreases in social behavior, lowered sucrose preference, and spatial memory deficits. In females, vicarious defeat stress decreased social behavior and impaired spatial memory performance, however, no differences in preference for a sucrose solution were noted. Lastly, both male and female mice exposed to vicarious defeat stress displayed lower body weight across days of exposure.

Conclusions: Collectively, our data suggest that the vicarious defeat stress paradigm may be used to examine the etiology of

psychological stress-induced mood-related syndromes in the juvenile population.

Keywords: Adolescence, psychological distress, sex differences, C57BL/6, Vicarious defeat stress

Disclosure: Nothing to disclose.

P78. The Choice-Wide Behavioral Association Study: Data-Driven Identification of Interpretable Behavioral Components

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Background: Behavior contains rich structure across many timescales, but there is a dearth of methods to identify relevant components, especially over the longer periods required for (self-) shaping, learning, and decision-making. Inspired by the goals and techniques of genome-wide association studies, we present a data-driven method—the choice-wide behavioral association study: CBAS—that systematically identifies such behavioral features.

Methods: CBAS uses a powerful, resampling-based, method of multiple comparisons correction to identify sequences of actions or choices that either differ significantly between groups or significantly correlate with a covariate of interest. We apply CBAS to different tasks and species (flies, rats, and humans) and find, in all instances, that it provides interpretable information about each behavioral task.

Results: For flies ($n = 1,566$), we apply CBAS to a publicly available dataset measuring left and right turns on a y-maze. CBAS identifies differences in the number of turns in a row between different outbred strains. For rats ($n = 241$), we apply CBAS to a dataset that we collected in rats performing a series of spatial alternation contingencies. CBAS identifies a phenotype consistent with restrictive and repetitive behavior when comparing wild-type rats to rats haploinsufficient for a high confidence, large effect, ASD risk gene (*Scn2a*). For humans, we apply CBAS to a publicly available dataset of subjects ($n = 1,413$) performing a two-step task to measure the interplay between model-based and model-free decision making. The subjects in the dataset also completed psychiatric symptom questionnaires, and CBAS identifies sequences of choices in the task that correlate with compulsive behavior and intrusive thought severity.

Conclusions: CBAS provides a data-driven way to extract relevant information from behavior, enabling evidence to falsify and refine hypotheses or to develop hypothesis when less is known about the behavior.

Keywords: Behavior, autism Spectrum Disorders, model-free and model-based learning, statistical methods, Compulsive behavior

Disclosure: Nothing to disclose.

P79. Identifying Neurophysiological Markers of rTMS Response in Treatment-Resistant Depression Using Long Interval Cortical Inhibition

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Background: Approximately one third of individuals with major depressive disorder (MDD) do not respond to a standard trial of antidepressants. These individuals can be treated with a form of

repetitive transcranial magnetic stimulation called intermittent theta-burst stimulation (iTBS) delivered to the left dorsolateral prefrontal cortex (DLPFC), with about a 50% response rate. Long interval cortical inhibition (LICI) is a potential biomarker for responders to iTBS, those with MDD demonstrate impaired inhibition. We hypothesize that more inhibition will be present in responders, as inhibition appears to be an indicator of plasticity and treatment response.

Methods: 111 patients with MDD underwent TMS-EEG LICI measures before and after a course of iTBS as part of a large scale therapeutic iTBS trial (NCT02729792). Responders were defined as individuals with a $\geq 50\%$ decrease in HRSD-17 scores post-treatment. LICI was elicited using a suprathreshold conditioning stimulus, followed by a suprathreshold test stimulus at 100ms intervals. EEG processing was performed by an investigator blinded to treatment arm, using EEGLAB and custom MATLAB scripts. The magnitude of LICI was determined using the ratio of peak-to-peak size of conditioned MEP over unconditioned MEP.

Results: No significant change was found in levels of inhibition overall ($t(-0.987) = 169.220$, $p = 0.324$); however, responders showed higher levels of inhibition post-treatment ($t(-2.324) = 55.345$, $p = 0.048$). Responders also had lower levels of inhibition at baseline ($t(2.865) = 41.484$, $p = 0.007$), and higher baseline LICI appears to be associated with non-response to therapeutic iTBS (GLM: $F = -0.012$, $p = 0.003$). No significant change was found in the N45 ($F(1,178) = 0.017$, $p = 0.897$) and N100 component following treatment ($F(1,178) = 2.203$, $p = 0.140$).

Conclusions: Higher baseline inhibition appears to be associated with non-response to therapeutic iTBS, inhibition also appears to increase with successful treatment. Further analysis is required to determine the predictive value of LICI; however, these results reinforce the important role of cortical inhibition as a marker of effective iTBS.

Keywords: iTBS, EEG biomarkers, TMS-EEG, Depression

Disclosure: Nothing to disclose.

P80. Distinct and Overlapping Urinary Volatile Organic Compound (VOCs) Signatures Associated With Alcohol and Concurrent Substance Use: A Population-Based Analysis of Nhanes 2013–2014

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Background: Volatile organic compounds (VOCs) are a diverse group of chemicals that can be emitted as byproducts of various metabolic processes, including those influenced by substance use. While alcohol, tobacco, and illicit drugs are known to alter VOC profiles, the specific urinary VOCs associated with the co-use of multiple substances remain underexplored. We aimed to identify and compare VOC profiles associated with concurrent use of alcohol and other substances (tobacco, illicit drugs) versus exclusive consumption of alcohol to determine unique and overlapping compounds in alcohol-only users and co-users. We hypothesized that individuals who co-use alcohol and other substances would have a unique set of urinary VOC metabolite profiles that are distinct from those who consume alcohol exclusively, reflecting the combined metabolic influences of multiple substance use. In addition, there will be overlapping VOCs between the groups that may indicate common metabolic pathways affected by substance use.

Methods: We analyzed data from 1,484 individuals aged 18 to 65 years, categorized into non-heavy/binge drinkers, consisting of

light and non-drinkers, and heavy/binge drinkers, consisting of heavy and binge drinkers, based on their responses to the NHANES Alcohol Questionnaire. Smokers and drug users were identified based on their reported last use within the past year. The primary outcome variables were derived from metabolite data (UVOC_h), including continuous and categorical data for 27 metabolites. A general linear regression model was employed, controlling for age, gender, and creatinine levels, with additional adjustments for drug use and smoking status in pairwise comparisons. The analysis was conducted using SAS 9.4, with sample weights applied to account for the NHANES complex sample design. All p-values were adjusted using the Bonferroni method.

Results: We found that N-acetyl-S-(benzyl)-L-cysteine (ng/mL) (BMA) levels were significantly higher in heavy/binge drinkers ($\beta = 0.321$, 95% CI: 0.1055 to 0.5371, $p = 0.042$) compared to non-heavy/binge drinkers, suggesting potential liver stress or damage related to alcohol use. Among individuals who used illicit drugs only, significant increases were observed in N-acetyl-S-(2-cyanoethyl)-L-cysteine (CYMA) ($\beta = 1.3517$, 95% CI: 0.8994 to 1.8039, $p = 0.0004$), N-acetyl-S-(3,4-dihydroxybutyl)-L-cysteine (DHBMA) ($\beta = 0.1776$, 95% CI: 0.04611 to 0.3091, $p = 0.046$), and phenylglyoxylic acid (PGA) ($\beta = 0.2965$, 95% CI: 0.1254 to 0.4676, $p = 0.0088$). These metabolites are known indicators of toxic effects and oxidative stress, suggesting that illicit drug use leads to significant metabolic disturbances. In smokers, we identified 11 significant metabolites, including 2-methylhippuric acid (2MHA) and various N-acetyl-S derivatives. These findings suggest that smoking contributes to a metabolic profile associated with increased risks of respiratory and cardiovascular diseases, as well as cancer.

Notably, co-users exhibited significantly higher levels of CYMA compared to single users who engaged in heavy drinking only ($p < 0.0001$, $\beta = 3.967$, 95% CI: 2.861 to 5.072), illicit drug use only ($p = 0.0029$, $\beta = 2.328$, 95% CI: 0.641 to 4.015), dual users who were heavy drinkers and illicit drug users ($p = 0.0001$, $\beta = 2.097$, 95% CI: 0.969 to 3.226), and non-users ($p < 0.0001$, $\beta = 3.856$, 95% CI: 2.699 to 5.013). This suggests that co-users experience a compounded metabolic effect, likely reflecting the additive or synergistic toxicological burden of multiple substances. The significantly elevated levels of this metabolite in co-users imply a heightened risk for adverse health effects due to the combined metabolic stress from alcohol, tobacco, and illicit drug use.

Conclusions: Our study highlights distinct and overlapping urinary VOC profiles associated with the co-use of alcohol and other substances compared to exclusive alcohol use. The significant increase of BMA in heavy/binge drinkers suggests that even exclusive alcohol consumption can lead to metabolic stress, potentially resulting in liver damage. This aligns with existing literature that associates excessive alcohol consumption with hepatic injury and oxidative stress, emphasizing the need for targeted interventions in this population. The presence of these compounds suggests ongoing exposure to harmful tobacco smoke constituents, which could serve as early indicators of disease risk, facilitating timely interventions to reduce smoking-related morbidity and mortality. The significant increase in levels of CYMA in co-users compared to single substance users suggests that the combination of alcohol, tobacco, and illicit drug use imposes an additive or synergistic metabolic burden, potentially leading to more severe health outcomes. The presence of shared VOC metabolites among different user groups points to common metabolic pathways disrupted by substance use, which may serve as biomarkers for assessing health risks. Future research should focus on elucidating the mechanisms underlying these metabolic changes and exploring the potential of VOCs as biomarkers for substance use-related health risks.

This work is supported by the National Institute on Alcohol Abuse and Alcoholism.

Keywords: Alcohol and substance use disorders, Adult smoking and alcohol traits, Volatile Organic Compounds, Biomarker
Disclosure: Nothing to disclose.

P81. Alpha-Tubulin Post Translational Modifications as Potential Translational Biomarkers in Major Depressive Disorder

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Background: Neuronal microtubules (MTs) are cytoskeletal protein elements, which are comprised of the tightly regulated polymerization of alpha- and beta-tubulin dimers, both of which are subject to post-translational modifications (PTMs). Neurons possess two compartmentalized pools of MTs, less and more dynamic, and alpha-tubulin PTMs such as tyrosination and acetylation are associated with the dynamic status. Increased levels of MT dynamics are required for structural neuronal plasticity phenomena. alpha-tubulin acetylation at Lys40, tyrosination and detyrosination at the C-terminus generate Acet-Tub, Tyr-Tub and Glu-Tub respectively. Increases in the Acet/total alpha-tubulin ratio and decreases in the Tyr/Glu-Tub ratio indicate a shift towards less dynamic MTs. Dysregulation of PTMs is associated with neuropsychiatric disorders characterized by synaptic pathology, including major depressive disorder (MDD). The current study investigates alpha-tubulin PTMs as potential translational biomarkers in an interferon-alpha (INF-alpha) animal model of depression and in the plasma of human MDD subjects compared to healthy controls.

Methods: Alpha-tubulin PTM ratios (Acet/Tot-Tub; Tyr/Glu-Tub) were measured using infrared western blot (IFWB) in plasma of subjects diagnosed with moderate (N=37) or severe MDD (N=35) and compared with healthy controls (N=40). Male Wistar rats were treated with IFN-alpha (170,000IU/kg, SC) or saline (0.9 %, SC) three times per week for four weeks and a cohort was subsequently treated with a single dose of ketamine (5 mg/kg, SC). Rats underwent a battery of behavioral and molecular testing, including forced swim test (FST) and analysis of alpha-tubulin PTM ratios, synaptic vesicle glycoprotein 2A (SV2a) and postsynaptic density protein 95 (PSD-95) by IFWB.

Results: IFN-alpha treated rats have depressive-like alterations including increase in FST immobility paralleled by central decrease in synaptic markers (SV2a and PSD-95) as well as central and peripheral increase in Acet-Tub. Acute ketamine treatment results in rescue of FST immobility, synaptic markers and Acet-Tub. Tyr/Glu-Tub was significantly decreased in the plasma of moderate and severe female MDD subjects compared with healthy controls ($p < 0.01$), but not in males. When combined, Tyr/Glu-Tub was significantly lower in the plasma of moderate and severe MDD subjects compared with healthy controls ($p < 0.01$). Tyr/Glu-Tub was significantly negatively correlated with HAM-D score clinical depression score ($r = -0.1068$, $p < 0.05$). Although Acet-Tub/Total-Tub was not significantly altered between control and MDD groups, subjects with severe MDD showed a trend towards lower plasma Acet-Tub/Total-Tub compared with moderate patients for both male ($p = 0.05$) and combined cohorts ($p = 0.07$).

Conclusions: Results indicate decreased brain microtubule dynamics in an animal model of depression which can be rescued by ketamine treatment. Recent data show decreased plasma Acet-Tub in healthy volunteers treated acutely with the antidepressant dose of ketamine (Colic et al., 2019). The decrease in plasma Tyr/Glu-Tub in MDD subjects indicates that they have less dynamic microtubules. Less dynamic MTs are associated with reduced synaptic plasticity and behavioral and cognitive impairments.

Together, the data suggest a potentially dysfunctional MT state and the association with MTs adopting a less dynamic state in MDD. Alpha-tubulin PTMs represent a potential plasma biomarker of disease progression in neuropsychiatric disease and MTs may serve as a novel therapeutic target.

Keywords: translational biomarker development, Synaptic Plasticity, Major Depressive Disorder

Disclosure: Nothing to disclose.

P82. Associations Between PTSD Symptom Severity and Resting-State Networks for Cognitive Flexibility and Emotion Regulation

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Background: Cognitive flexibility (CF) and emotion regulation (ER) are crucial for managing stressors, yet both capabilities are often compromised in individuals with post-traumatic stress disorder (PTSD). While previous research has explored the brain circuitry associated with CF and ER in PTSD populations, employing brain networks related to CF and ER identified in healthy subjects to examine their association with PTSD symptom severity in trauma-exposed individuals represents an innovative and potentially clinically useful approach. This study utilized resting-state functional magnetic resonance imaging (rs-fMRI) data from the UK Biobank (UKBB) to delineate CF and ER networks in a healthy cohort. Subsequently, we assessed how variations in these networks among participants from the trauma-exposed AURORA study correlate with the severity of PTSD symptoms.

Methods: The analysis included rs-fMRI scans from 14,047 UKBB participants and 306 trauma-exposed participants from the AURORA study. After preprocessing, constrained independent component analysis (ICA) using the NeuroMark_fmri_1.0 template yielded 53 independent components, which were organized into seven resting-state networks: subcortical network (SCN), auditory network (ADN), sensorimotor network (SMN), visual network (VSN), cognitive control network (CCN), default-mode network (DMN), and cerebellar network (CBN). Functional network connectivity was assessed by calculating Pearson correlations between each pair of components, resulting in 1,378 connectivity estimates across the entire brain. These connectivity estimates were analyzed using general linear models to explore associations with choice response times (RTs, used as a proxy for CF due to strong correlations with cognitive ability) and neuroticism (used as a proxy for ER). Covariates included in the models were age, sex, the square of age, age $\times$ sex interaction, and site. Following the identification of functional connections associated with CF and ER in the UKBB healthy cohort, we examined how these connections correlated with scores on the PTSD Checklist for DSM-5 (PCL-5) in the AURORA cohort, assessing their potential linkage with PTSD severity.

Results: In the UKBB data, we identified 29 connections that were associated with CF, primarily from the CCN, VSN and SMN, and 34 connections that were associated with ER, with the DMN playing a prominent role in ER (all corrected $p < 0.05$). Demonstrating generalization to the AURORA cohort, one of the connections associated with CF and six connections associated with ER linked with PCL-5 scores in the AURORA cohort (all corrected $p < 0.05$). Specifically, the connection between the superior frontal gyrus and middle frontal gyrus from the CF network showed significant associations with PCL-5 scores. Additionally, connections between the precuneus and middle

occipital gyrus, precuneus and inferior occipital gyrus, superior parietal lobule and inferior frontal gyrus, inferior parietal lobule and superior medial frontal gyrus, thalamus and paracentral lobule, and precuneus and medial temporal gyrus from the ER network showed significant associations with PCL-5 scores.

Conclusions: This study provides novel insight into neural networks underlying CF and ER and their relevance to PTSD symptomatology. By integrating rs-fMRI data from healthy individuals in the UKBB and trauma-exposed participants in the AURORA study (i.e., two separate cohorts with very different demographics), we identified connections that are associated with CF and ER and that linked PTSD severity. Notably, the precuneus was identified as a pivotal node in several of these connections, suggesting its potential as a target for interventions aimed at mitigating PTSD symptoms.

Keywords: Functional MRI (fMRI), PTSD, Emotional regulation, cognitive flexibility, UK Biobank/AURORA

Disclosure: Niji Corp, Consultant, Self

P83. Contribution of the Anterior Cingulate Cortex Structure to Treatment Response to rTMS Therapy in Patients With Treatment-Resistant Depression

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Background: About 30% of patients with major depressive disorder do not respond to standard antidepressant treatments, called treatment-resistant depression (TRD). Although repetitive transcranial magnetic stimulation (rTMS) is an established treatment of TRD, the individual treatment effects are highly heterogeneous. Several studies using resting-state functional magnetic resonance imaging have shown that the higher the anticorrelation between the anterior cingulate cortex (ACC) and dorsolateral prefrontal cortex, the better the therapeutic response to rTMS for TRD may be. Potential predictive biomarkers for responses to noninvasive brain stimulation therapies, regardless of modality (sMRI, fMRI, EEG), focus on the ACC and its connections (Klooster et al., 2023). However, few studies have examined the relationship between the ACC structure and response to rTMS treatment in patients with TRD, and findings across these studies are inconsistent (Boes et al., 2018; Baeken et al., 2021). Thus, this study aimed to examine whether the ACC structures could serve as predictors of rTMS response in patients with TRD.

Methods: This study was approved by the Keio University Clinical Research Review Committee (jRCTs032180188) and conducted in accordance with the Declaration of Helsinki. Pre-treatment brain MRI scans were obtained from 129 TRD patients (the Montgomery-Åsberg Depression Rating Scale (MADRS) score ≥ 18) who had not improved with at least two antidepressants. Subsequently, rTMS therapy with theta burst stimulation protocol targeting the bilateral dorsolateral prefrontal cortex was administered. Treatment efficacy was evaluated using the MADRS. Pre-treatment brain structures were analyzed using structural magnetic resonance imaging (T1 and T2-weighted images) with Freesurfer 6 to calculate the thickness of 4 subregions of the ACC defined in the Desikan-Killiany atlas (the bilateral caudal/rostral ACC). A logistic regression was performed to investigate the effects of baseline ACC structures on rTMS response, defined as a $\geq 50\%$ improvement in MADRS score with age, sex, and handedness as covariates. The predictive performance of ACC structures were evaluated by area under the curve (AUC) distribution of 500 test data acquired by 100 iterations of 5-fold

cross-validation and its comparison with that of null data with the shuffled dependent variable. As exploratory analysis, we tested all 16 classification models available in PyCaret machine learning library with the same variables and cross-validation methods to find the model with maximum AUC and repeated this with 100 random seeds.

Results: Of the TRD group, 46% were female, with a mean age (SD) of 45.0 (12.6) years. The mean $\pm$ SD pre- and post- treatment MADRS scores were 29.6 (6.5) and 14.8 (9.5), respectively. The response rate was 54%. In logistic regression, the overall model was significant ($\chi^2 = 14.9$, $p = 0.04$) and explained 14% (Nagelkerke R^2) of the variance. Of the four ACC subregions, the main effect was observed only in the right caudal ACC (Odds ratio=12.7, $p = 0.006$), with thicker right caudal ACC associated with better rTMS response. Significant main effects were not observed in the other three regions. The AUC of test data across cross-validation (CV) folds (0.60 (0.1)) were better than null CV folds (0.49 (0.12)) (Cohen's $d = 0.93$, $p < 0.001$) but still low. In exploratory analysis with PyCaret, the mean AUC of the optimal model selected for each session was 0.65.

Conclusions: Our result indicates that greater thickness of the right caudal ACC may correlate with higher responsiveness to rTMS treatment in patients with TRD. However, the predictive power of the subregions of ACC structure was not sufficiently high, even with extensive model selection and tuning. Further research is warranted to employ multimodal biometrics to predict treatment response to rTMS in this population.

Keywords: Depression, TMS, MRI

Disclosure: Nothing to disclose.

P84. Neuroactive Steroid Biosynthesis During Pregnancy Predicts Future Postpartum Depression: A Role for the 3 α and/or 3 β -HSD Neurosteroidogenic Enzymes?

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Background: Postpartum depression (PPD) affects approximately 10-15% of childbearing women and even higher proportions of individuals from marginalized communities (1-3). One promising system for biological correlates and predictors of PPD is that of the neuroactive steroids (NAS), metabolites of cholesterol that are synthesized de novo in the brain or in the gonads and adrenal glands and act on the central nervous system (CNS) to modulate neuronal excitability (4), chiefly through the GABAA receptor (GABAAR). Studies have had inconsistent findings (5-12), and in particular, it remains unclear exactly what changes in NAS levels and ratios occur prior to the onset of depressive symptoms. We hypothesized that women who were euthymic in pregnancy and went on to develop PPD would demonstrate lower ratios of NAS to their precursors in the progesterone metabolic pathway.

Methods: Samples (N=136) were collected as part of an ongoing prospective study following pregnant women with and without histories of prior mood disorders, with blood draws and psychological assessments at up to 8 visits (each trimester (T1, T2, T3) and 2 weeks (W2), 6 weeks (W6), 3 months (M3), 6 months (M6) and 9 months (M9) postpartum). The study was approved by the Johns Hopkins University School of Medicine Institutional Review Board, and every participant underwent an informed consent process. We defined PPD as an Edinburgh Postnatal Depression Scale (13) (EPDS) score of > 13 at any postpartum time point.

Participant blood was collected at each visit in four 10 ml EDTA tubes. Blood samples were non-fasting, and collection times were at the convenience of the participant. All collections occurred during the working day (9:00 a.m. to 5:00 p.m.). NAS analyses were carried out according to a previously published gas

chromatography-mass spectrometry (GC-MS) technique (14) which allows the simultaneous determination of NAS from a single sample due to high sensitivity (molar) and unsurpassed structural specificity (15). We examined absolute levels and ratios of pregnenolone, progesterone, allopregnanolone, pregnanolone, epipregnanolone, isoallopregnanolone, 5 α -dihydroprogesterone (5 α -DHP), and 5 β -dihydroprogesterone (5 β -DHP).

Generalized linear mixed-effects regression models were used to assess relationships between PPD each of the NAS biomarkers or ratios at T2 and T3, exclusively in women who remained euthymic in pregnancy. Log transformation technique was applied and adjusted odds ratios were used to quantify the relative effect of a one-unit change in a NAS biomarker on developing postpartum depression. A C-index was utilized as a measure of overall model predictive discrimination. The Benjamini-Hochberg procedure was used to reduce the probability of declaring false significances. All analyses were performed in SAS 9.4 (16).

Results: Participants were largely Caucasian (86.8%) and well educated (57% graduate or professional degree). The average age was 32.8. Slightly over half (56.9%) had a prior history of a mood disorder (either major depressive disorder or bipolar disorder), and 18.3% used psychiatric medications during pregnancy. Thirty-three participants had an EPDS > 13 at at least one time point postpartum (W2, W6, M3, M6, M9).

In euthymic women at T2, after controlling for the false discovery rate (FDR) no levels and ratios were significantly predictive of future PPD at 0.05. At T3, again in subjects not depressed in pregnancy, the covariate-adjusted mixed-effects models indicated that there were significant associations between future PPD and pregnanolone ($p = 0.015$), progesterone ($p = 0.019$), the pregnanolone/progesterone ratio ($p = 0.001$), the isoallopregnanolone/pregnanolone ratio ($p = 0.0004$), and the 5BDHP/progesterone ratio ($p = 0.0120$). FDR-adjusted p-values indicated that both the pregnanolone/progesterone ratio and the isoallopregnanolone/pregnanolone ratio remained significant at 0.05 (FDR adjusted $p = 0.008$ and 0.001 , respectively, with a one-unit increase in isoallopregnanolone/pregnanolone having higher odds (OR = 2.12, 95% CI: 1.40 to 3.20) and a one-unit increase in pregnanolone/progesterone having lower odds (OR = 0.57, 95% CI: 0.41 to 0.79) of predicting PPD. The C-index of 0.72 indicates very good discriminative power.

Conclusions: We found key differences at the third trimester between women who did and did not develop subsequent postpartum depression (EPDS > 13). Those with PPD had a lower pregnanolone/progesterone ratio and a higher isoallopregnanolone/pregnanolone ratio than those who did not develop PPD. In total, our results indicate altered progesterone metabolism to the positive (PAM) and negative (NAM) metabolites during the third trimester in women who are psychiatrically well during pregnancy but go on to develop PPD (EPDS > 13). Our findings may indicate and support the hypotheses of decreased activity of the 3 α -HSD enzyme (which metabolizes 5 α -DHP and 5 β -DHP to PAMs allopregnanolone and pregnanolone, respectively) and/or increased activity of 3 β -HSD (which metabolizes 5 α -DHP and 5 β -DHP to NAMs isoallopregnanolone and epipregnanolone, respectively), but further study will be needed to characterize whether this represents differences in expression, enzymatic activity, or both.

Keywords: Neuroactive Steroids, postpartum depression, biomarkers

Disclosure: Nothing to disclose.

P85. Microstructural Imaging in the Brain of Schizophrenia Patients: A Comparison Between Two Acquisition Protocols

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Background: Magnetic Resonance Imaging (MRI) is a powerful tool for studying the etiology and developing biomarkers for neuropsychiatric disorders. Quantitative MRI analyses, such as quantitative T1 mapping (qT1), are being increasingly used in investigating brain differences between schizophrenia patients (SCZ) and healthy control (HC). This popularity is due to its high accuracy and susceptibility to microstructural changes such as demyelination, edema, and decreased cellular density. However, it is important to identify the most sensitive, accurate, and reliable imaging protocol given the complex nature of the disorder. We compared two different qT1-acquisition protocols: a single echo, and a multi-echo fast spoiled gradient echo (FSPGR), to find the most suitable protocol for studying brain changes in SCZ. Our study aims to compare the two protocols sensitivity to brain changes in schizophrenia compared to healthy control subjects.

Methods: qT1 maps were acquired for 15 schizophrenia participants and 7 healthy controls via a single and multi-echo qT1 scans. Brain-wide qT1 scans were generated after correcting for B1 field inhomogeneity and registration to the MNI152 space. Cases vs. controls differences were calculated for each protocol in 11 regions of interests (ROIs), including the bilateral: cerebral cortex, white matter, lateral ventricles, thalamus, caudate, putamen, pallidum, hippocampus, amygdala, accumbens, and brain stem.

Results: As expected, the two methods showed very high correlation and the standardized mean group differences did not show heterogeneity, furthermore, the effect of confounders (i.e., age, sex, and education) was similar for the two sequences.

Conclusions: In conclusion, the results of this pilot study show that the multi-echo sequence presents a feasible opportunity to reduce scan time obtaining the same level of information for studying the brain changes associated with schizophrenia.

Keywords: Schizophrenia (SCZ), MR imaging, In vivo Imaging

Disclosure: Nothing to disclose.

P86. An Investigation of the Utility of a Laboratory Impulsivity Test Battery in Adult ADHD Patients Treated With Atomoxetine

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Background: Maladaptive impulse control is a transdiagnostic symptom which manifests in numerous psychiatric indications including, but not limited to, attention deficit hyperactivity disorder (ADHD), major depressive disorder, and borderline personality disorder. The underlying neurobiology of impulse control has been studied clinically and preclinically over many years and dysregulation of overlapping brain circuits is considered to mediate maladaptive impulsivity across diagnoses. Laboratory tasks for the objective measurement of impulsive behavior are thought to have utility for the discovery of novel pharmacotherapeutic approaches for the treatment of impulsivity, both in the selection of patients as well as the assessment of drug effects. The overall objective of this study was to explore the utility of an impulsivity task battery as a patient selection and efficacy assessment tool for use in a drug discovery setting. Sub-objectives were 1) to demonstrate the sensitivity of the task battery to pharmacological intervention following single and multiple administration of atomoxetine, 2) to establish an impulsivity threshold in tasks needed for measurement of a pharmacological effect and 3) to investigate the correlation between clinical scales and behavioral measures.

Methods: The impulsivity task battery comprised laboratory impulsivity tasks to assess both motor – stopping and waiting –

and cognitive impulsivity by use of the 4-choice serial reaction time task, immediate memory task, stop signal task, and the delay discounting task respectively. The Barratt Impulsivity Scale (BIS-11) and UPPS-S impulsivity questionnaires were also included. Patients diagnosed with ADHD were recruited into the study at 5 study sites in Germany. The effect of atomoxetine (40mg) compared to placebo (n=40 per treatment arm) on impulsivity readouts was assessed following a single administration and following 14 days, once daily treatment.

Results: Adult ADHD patients were recruited into the study (approximately equal males/females, average age 26 years). The effect of atomoxetine on impulsivity on days 1 and 14 and the correlation between the task performance and questionnaires will be presented. For the single behavioral measures (primary endpoints), analysis of covariance (ANCOVA) is used to compare means between groups, after single-dose and at steady state. Descriptive statistics are also calculated for all endpoints.

Conclusions: The utility of the impulsivity test battery as a novel biomarker tool for patient selection and assessment of drug efficacy will be discussed.

Keywords: impulsivity, Translational biomarker approaches to drug development, Cognitive biomarkers

Disclosure: Boehringer Ingelheim: Employee (Self)

P87. GM-1020 (NMDA Antagonist) Vs GM-2505 (5-HT2A Agonist) - Distinct Mechanisms, Same Outcome?

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Background: GM-1020 and GM-2505 are two compounds in development for the treatment of major depressive disorder (MDD).

GM-1020 is a novel non-competitive NMDA receptor (NMDAR) antagonist designed to have improved oral bioavailability and tolerability compared to the original rapid acting antidepressant, ketamine.

GM-2505 is a novel 5-HT2A receptor agonist designed to have an intermediate duration of 1-2 hr in humans, allowing for sufficient target engagement to provide antidepressant effects without requiring an extended-duration in-clinic experience like psilocybin, which requires up to 8 hr dosing session and up to one day long monitoring. While GM-1020 and GM-2505 have different mechanisms of action, they are both expected to achieve antidepressant efficacy in MDD due to their shared mechanisms with ketamine and psilocybin, respectively.

Methods: The safety, pharmacokinetics (PK), and pharmacodynamics (PD) of oral administration of GM-1020 (20-360 mg) and intravenous administration of GM-2505 (0.34-20mg) were each assessed in the same phase 1 unit under similar conditions in separate adaptive, randomized, double blind, placebo controlled first-in-human single-ascending dose studies in healthy adult volunteers. Resting state electroencephalography (rEEG), Saccadic Peak Velocity (SPV), 5-Dimensional Altered States of Consciousness (5D-ASC) and Mystical Experience Questionnaire (MEQ-30) were used to assess similarities and differences between the two compounds.

Results: The profiles of the two compounds differed on measures of subjective effects and sedation but were similar with regard to rEEG effects.

On 5D-ASC rating scale GM-2505 doses ≥ 10mg showed highest overall values at Visual Reorganization and Oceanic Boundlessness while the highest dose of GM-1020 (360 mg) dominated

the remaining dimensions. GM-2505 showed the strongest effects in all dimensions of the MEQ-30 self-rating scale. The effects of the highest dose of GM-1020 (360 mg) were comparable to an intermediate dose of GM-2505.

Saccadic Peak Velocity was reduced by GM-1020 (≥ 140 mg) reflecting a sedative effect while GM-2505 (≥ 3.3 mg) caused an increase likely due to increased arousal.

GM-1020 attenuated rsEEG power of alpha (8-13 Hz) oscillations while increasing power of gamma (> 30 Hz) oscillations in dose-related manner. Similarly, GM-2505 attenuated power in a dose-related manner predominantly in theta (4-8 Hz) but also neighboring EEG bands including delta (1-4 Hz) and alpha (8-13 Hz), while increasing power of gamma oscillations (> 30 Hz). Notably, the magnitude of GM-2505 EEG effects at the highest doses tested in both the low and high frequency EEG bands were significantly larger than those of GM-1020.

Conclusions: While GM-1020 (NMDAR antagonist) and GM-2505 (5-HT_{2A} agonist) have distinct profiles of subjective and sedative effects, they modulate similar EEG bands in comparable fashion. However, GM-2505's effects are more widespread and profound. In combination, these measures may be able to differentiate the effects of classical psychedelics (GM-2505) from dissociative (GM-1020) compounds.

Keywords: Psychedelics, EEG biomarkers, 5-HT_{2A} receptor, NMDA Receptor, Depression

Disclosure: Gilgamesh Pharmaceuticals, Inc.: Employee, Stock / Equity - Privately Held Company (Self)

P88. Pharmacodynamic Effects of Brexanolone on Resting-State and Transcranial Magnetic Stimulation Evoked Electroencephalography – a Cross-Over, placebo-Controlled, Active Comparator Study

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Background: Brexanolone (BRX), also known as SAGE-547/ZULRESSO®, is a neuroactive steroid chemically identical to endogenous allopregnanolone and a potent positive allosteric modulator (PAM) of synaptic and extra synaptic γ -aminobutyric acid-gated chloride channel receptors (GABAAR) that enhances phasic and tonic inhibition in the brain. Neuroactive steroids, e.g. BRX, have a novel mechanism of action (MoA) relative to other GABAAR modulators. Although this difference has been characterized by resting-state pharmaco-electroencephalography (pEEG) relative to placebo, the minimal exposures necessary to induce significant neurocircuitry modulation using activated/stimulated neurocircuitry, remains unclear. The aim of this study was to investigate whether resting-state and transcranial magnetic stimulation-evoked EEG (TMS-EEG) may highlight differences in the MoA of BRX compared to the classical GABAAR modulator lorazepam, and at which exposures BRX starts inducing neurocircuitry modulation.

Methods: This randomized, double-blind, placebo-controlled crossover study evaluated the effects of BRX on pEEG and TMS-EEG in healthy males (age 18-65 years). Main exclusion criteria were increased risk of TMS side effects, history of neuropsychiatric illness, and use of psychotropic medication. Subjects received single doses of BRX low dose (30 mcg/kg/hour i.v.), BRX high dose (90 mcg/kg/hour i.v.), 2 mg lorazepam p.o. and IV or PO placebo, in randomized order. The infusions lasted for 3 hours. pEEG was performed pre-dose and minute 60, 120, 180, 210, 240, 300 post-

dose, and TMS-EEG pre-dose and minute 140, 240 post-dose. pEEG was recorded per IPEG guidelines and consisted of 5 minutes of resting state per eye state (open and closed), and spectral power was calculated per frequency band. TMS was applied per current guidelines, stimulating the motor hotspot of the dominant abductor digiti minimi muscle. TMS-evoked potentials (TEP) were measured after single pulse (sp) and paired pulses (pp) at interstimulus interval (ISI) of 2, 15 and 100 msec, with conditioning (CR) and test pulses delivered at 120% intensity of resting motor threshold (rMT), except for the CR at ISI 2 and 15 msec, where 80% of rMT was used. The pEEG data were analyzed using a mixed model repeated measures and reported as log-transformed change from baseline (μ V) $\pm$ standard error (SE), p-values generated from least-square means comparisons. TMS-EEG responses were analyzed in common average montage with baseline correction, and a nonparametric cluster-based permutation analysis was used to compare the TEPs between treatments. P-values < 0.05 were considered significant.

Results: A total of 24 out of 29 enrolled subjects completed the study, and dosing was safe and well-tolerated. Numerous significant effects were observed for both pEEG and TMS-EEG following treatment with BRX and lorazepam. Here we report the most consistent significant effects (pEEG in eyes closed condition). Both broadband and low beta-power were dose-dependently increased in all leads in both eye conditions by BRX vs. placebo (low beta high dose: Fz-Cz 0.48 ± 0.06 , Pz-O1 0.23 ± 0.09 , Pz-O2 0.18 ± 0.08) and vs. lorazepam (low beta high dose: Fz-Cz 0.20 ± 0.06 , Pz-O1 0.31 ± 0.08 , Pz-O2 0.42 ± 0.08). Lorazepam vs. placebo also impacted low beta-power in both eye conditions (Fz-Cz 0.28 ± 0.06 , Pz-O2 -0.23 ± 0.08). Additionally, theta-power was significantly increased in the frontal region in both eye conditions by high-dose BRX vs. placebo (Fz-Cz 0.23 ± 0.08 and vs. lorazepam (Fz-Cz 0.93 ± 0.08) and reduced by lorazepam vs. placebo (Fz-Cz -0.70 ± 0.08). Further power bands including alpha, delta, and gamma, demonstrated unique modulation by BRX relative to both placebo and lorazepam across conditions.

For TMS-EEG BRX vs. placebo significantly increased TEP response over the first 100 msec at the contralateral centroparietal region. This was significant for low-dose spTEP (140m and 240m post-dose) and ppTEP at ISI 15 msec (140m post-dose), and high-dose spTEP (140m post-dose). Two effects were observed for lorazepam vs. placebo: 1) a significantly reduced N100 TEP component in the contralateral frontocentral region for ppTMS at ISIs 2 and 15 msec (140m and 240m post-dose); 2) a significantly reduced late TEP response between 150-300 msec including the P180 TEP component, in the contralateral centroparietal region for spTMS (240m post-dose) and ppTMS at ISI 2 and 15 msec (140m and 240m post-dose). When comparing both low- and high-dose BRX to lorazepam, many significant clusters were identified, for both spTMS and ppTMS, and early and later TEP components. Divergent effects were observed, namely that BRX mainly affected early TEP components and lorazepam later components, and BRX predominantly had parieto-occipital effects and lorazepam had frontocentral effect.

Conclusions: Here we demonstrated a dose-dependent effect of BRX, with a distinct pharmacodynamic pEEG and TMS-EEG profile compared to the active comparator lorazepam. This indicates a novel GABAAR mediated MoA for BRX compared to the classical GABA-PAMs. The results of this study, together with the TMS-EMG biomarker data (see poster 1), provide valuable insights in these different MoAs, and validates the value of pEEG and TMS-EMG-EEG biomarkers in early phase drug development for these types of compounds.

Keywords: TMS-EEG, Quantitative Electroencephalography (qEEG), GABA positive allosteric modulators neuroactive steroid

Disclosure: Nothing to disclose.

P89. Neurostimulation Approach to Evaluating the Impact of GABAA Receptor Positive Allosteric Modulators

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Background: γ -aminobutyric acid (GABA) is the primary inhibitory neurotransmitter within the central nervous system (CNS) and plays a role in regulating brain-network activity. One of the receptor classes that GABA acts through are the GABA-gated chloride channel receptors (GABAAR), which are pentameric in structure and facilitate inhibition within the CNS through ligand-gated chloride-ion conductance. Typically, these receptors contain two α , two β , and either a γ or δ subunit. γ -containing GABAARs are localized to the synapse and mediate phasic inhibition. δ -containing GABAARs are extra-synaptic and mediate tonic inhibition. There are several classes of drugs that are positive allosteric modulators (PAMs) of the GABAARs, including barbiturates, benzodiazepines, and neuroactive steroids, which differentiate based on their binding location and effect on brain-network function. For instance, benzodiazepines such as lorazepam have a binding site located on the γ -subunit of GABAARs, whereas neuroactive steroids such as brexanolone (BRX), which is approved for the treatment of postpartum depression in patients 15 years and older, bind at the α/β interface. This allows for binding of both synaptic- and extra-synaptic GABAARs. To date, pharmaco-electroencephalography (pEEG) has been a useful, translational biomarker at characterizing GABAAR activity. Neurostimulation approaches may be leveraged in addition to pEEG to explore the impact of these compounds on activated neurocircuitry. Here, following administration of two GABAAR PAMs, BRX and lorazepam, we compare 1) the impact of single-pulse transcranial magnetic stimulation (spTMS) and paired-pulse TMS (ppTMS) paradigms to explore differences in corticospinal excitability and 2) pEEG profiles (see poster 2).

Methods: This was a double-blind, double-placebo controlled, four-way cross over design in 29 (24 completers) healthy adult males to assess the impact of two doses of BRX (30 mcg/kg/hour and 90 mcg/kg/hour, i.v.) or lorazepam (2mg, p.o.) on responses to spTMS and ppTMS. Dosing was safe and well-tolerated. The BRX or placebo infusion lasted 3 hours. TMS assessments were performed at baseline (prior to dosing), 140 minutes, and approximately 4 hours after the start of dosing. These pulses were given at an intensity of 120% of the resting motor threshold (rMT) measured at the abductor digiti minimi (ADM) muscle. The single pulses were followed by paired pulse TMS, where pairs of pulses (a conditioning pulse followed by a test pulse) were given per interstimulus interval (ISI). This analysis focused on an ISI of 100 ms. Both the conditioning and the test pulses were given at an intensity of 120% of the rMT. Data was analyzed using a mixed models for repeated measures and reported as log-transformed change from baseline $\pm$ standard error (SE), with p-values generated from least-square means comparisons between conditions.

Results: Resting motor threshold (rMT) of BRX at the 90mcg dose group (1.10 ± 0.64) showed an increase from placebo (-0.78 ± 0.66) at 240 minutes, $p = 0.0443$. The 30mcg (0.65 ± 0.67) and 90mcg groups were significantly different from lorazepam (-1.79 ± 0.63) at this timepoint, $p < 0.01$ for both comparisons.

spTMS at the 30mcg (0.23 ± 0.14) and 90mcg (0.22 ± 0.14) BRX groups were significantly higher vs lorazepam (-0.30 ± 0.14) at 240 minutes, $p < 0.01$ for both. There was a trend towards a

significant decrease seen between lorazepam and placebo (0.03 ± 0.14) at this timepoint, $p = 0.0714$.

For the 100ms ISI ppTMS, the 30mcg (-0.03 ± 0.22) and 90mcg BRX groups (-0.61 ± 0.22) were both significantly reduced relative to placebo (0.60 ± 0.22), $p = 0.0247$ and $p < 0.0001$, respectively, at 140 minutes. The 90mcg group was lower than lorazepam (0.23 ± 0.21) at this timepoint, $p = 0.0026$.

Conclusions: These results here demonstrate that cortical excitability is modulated by GABAAR PAMs. While separate studies have previously observed an effect of GABAAR PAMs on TMS outputs, this is the first human comparison of neuroactive steroid versus benzodiazepine that we are aware of. BRX significantly modulated the rMT relative to placebo and lorazepam in a dose-dependent manner. Additionally, we have previously looked at the effect of CNS inhibitors including lorazepam on spTMS. This study demonstrates a similar trend of lorazepam on this measure. Finally, we show that ppTMS can be an additional, sensitive measure of target engagement for δ -containing GABAARs. This study validates the use of circuit-based approaches to capture unique pharmacodynamic properties of different GABAAR PAMs.

Keywords: GABA-A receptors, Neuroactive Steroids, TMS

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

P90. Relationships Between Early Auditory Information Processing Biomarkers and Initial Targeted Cognitive Training Performance in a Transdiagnostic Cohort

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Background: Targeted cognitive training (TCT) is an emerging, neuroplasticity-based, adaptive, computerized approach to attenuate cognitive impairment. While robust TCT gains are apparent in clinical trials at the group level, clarifying which participants eventually benefit from TCT at initiation has remained challenging. Recently, EEG biomarkers of early auditory information processing (EAIP) including mismatch negativity (MMN), P3a, auditory steady state response gamma evoked power (ASSR EP) and inter-trial coherence (ASSR ITC) measured prior to training have predicted initial TCT performance and auditory perceptual learning gains in participants with schizophrenia (SZ). Malleability in these biomarkers over the first hour of training has also predicted gains from a full 40h program in SZ. In the present analysis, we tested whether the utility of MMN, P3a, ASSR EP and ITC would transfer to a broader cohort of participants living with a range of psychiatric disorders.

Methods: MMN, P3a, ASSR EP and ITC were collected from TCT-naïve participants ($n = 70$) with a range of psychiatric diagnoses (MDD, PTSD, SZ etc.) before and after a 1 hr TCT session which focused on exercises targeting auditory fidelity. In the TCT session, subjects progressed through different levels of a "Sound Sweeps" exercise that is a typical component of a 40h TCT training suite. Participants repeated each level three times before progressing to the next level of training.

Results: Participants progressed through an average of 14.75 level attempts ($st.dev = 5.47$). TCT level attempts were correlated with both pre-TCT MMN amplitude ($r = -0.312, p = 0.01$) and ASSR ITC ($r = 0.318, p = 0.008$) but not P3a and ASSR EP. TCT was associated with malleability in MMN (pre-TCT = $-1.49 \mu V$ vs post-TCT = $-1.37 \mu V$; $p = 0.045$; $d = 0.45$) and P3a (pre-TCT = $1.02 \mu V$ vs post-TCT = $0.84 \mu V$; $p < 0.001$; $d = 0.41$).

Conclusions: EAIP biomarkers can predict initial TCT response across participants living with range of psychiatric disorders. This

report provides justification for testing EAIP biomarkers in transdiagnostic cohorts to assess predictive utility over a full course of TCT.

Keywords: EEG biomarkers, targeted cognitive training, Transdiagnostic

Disclosure: Nothing to disclose.

P91. Enhanced Neurobiological Biomarker Differentiation for Attention-Deficit/Hyperactivity Disorder Through a Risk-Informed Case-Control Design

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Background: Medical research has long sought biological markers to aid in diagnosing, prognosing, and treating mental disorders, but most biomarkers, including genetic and neural variants, still have limited clinical use. This is also true for ADHD, a common neurodevelopmental disorder. Studies have shown mean differences in candidate biomarkers between those with ADHD and unaffected individuals across various metrics, such as polygenic scores, brain structure, and neuropsychological performance. However, significant overlap between cases and controls hinders clinical application. This is partly due to the heterogeneous nature of mental disorders. Methodological improvements could include considering differential disease risk among unaffected individuals. Here, we used a validated multivariable model to estimate risk and differentiate the control group, aiming to enhance neurobiological contrasts with ADHD cases.

Methods: The Brazilian High-Risk Cohort (BHRC) consists of 2,511 children and adolescents, aged 6-14, recruited from state-funded schools in Porto Alegre and São Paulo, Brazil, in 2010. A risk model, previously validated in four independent samples with a c-statistic of 0.74 to 0.81, was used to calculate each participant's risk based on eight factors, such as sex, intelligence, and socioeconomic status. We created three groups based on the combination of diagnostic and risk status: participants with ADHD, participants without ADHD at low risk, and participants without ADHD at high risk.

These groups were compared within three candidate biomarkers:

Genotyping and Polygenic Scores: Saliva samples were genotyped, and polygenic scores (PGSs) were calculated using the PRSice V2.3.5 software, based on the latest ADHD Genome-Wide Association Study.

Subcortical Volumes: Brain MRI scans were conducted on 750 participants, analyzing age- and sex-adjusted volumes of subcortical structures such as the hippocampus and amygdala.

Executive Function: Assessed through standard neuropsychological tests, transformed into age-adjusted t-scores, and analyzed using a validated latent measure of executive function.

Statistical Analysis: We compared neurobiological differences between traditional case-control groups and risk-enriched case-control groups. Controls were split into low- and high-risk groups based on their ADHD risk percentiles ($< p_{10}$ and $> p_{90}$). Linear regression models adjusted for confounding factors, and standardized residuals were compared using t-tests. Cohen's d measured mean differences, and overlap percentages between density plots were calculated. Sensitivity analyses controlled for ADHD symptoms and compared genetic signatures across different neuropsychiatric disorders.

Results: The final sample included 274 participants with ADHD and 2,237 without ADHD (controls). The stratified control group consisted of 224 individuals each in low and high ADHD risk groups. The mean ADHD risk was 5.3% for all controls, 1.1% for low-risk, and 23.1% for high-risk individuals.

Subtle Neurobiological Differences in ADHD vs. Unaffected Peers: children with ADHD had higher PGSs for ADHD than controls ($d = .17$, $p = .009$) with 8.2% distribution overlap. ADHD participants also had smaller subcortical volumes ($d = -.25$, $p = .032$) with 13.2% overlap, and lower EF scores ($d = -.22$, $p < .001$) with 8.6% overlap.

Risk-Informed Design Shows Moderate Neurobiological Differences: low-risk controls had lower ADHD PGSs compared to high-risk controls ($d = -.20$, $p = .048$) and ADHD cases ($d = -.20$, $p = .032$). Low-risk controls also had larger subcortical volumes than high-risk ($d = .51$, $p = .003$) and ADHD groups ($d = .60$, $p < .001$), and better EF scores than high-risk ($d = .49$, $p < .001$) and ADHD groups ($d = .48$, $p < .001$). Non-overlap percentages were 11.5% (PGSs), 21.6% (SVs), and 20.5% (EF) between low- and high-risk, and 8.7%, 23.7%, and 18.9% between low-risk and ADHD cases, respectively.

High-Risk vs. ADHD Neurobiological Profiles: high-risk participants did not differ significantly from ADHD cases in PGSs ($d = .01$, $p = .933$), SVs ($d = .12$, $p = .502$), or EF ($d = .01$, $p = .912$). Non-overlap percentages were 5.4% (PGSs), 13.0% (SVs), and 4.1% (EF).

Conclusions: Employing a risk-informed case-control design powerfully revealed larger effect sizes when distinguishing key candidate biomarkers between individuals affected by ADHD and controls with different risk loadings. Our findings shed new light on the considerable overlap observed across comparison groups in traditional case/control analyses, by underscoring the importance of considering multivariable risk profiles to move beyond traditional group definitions. Moreover, the present study highlights the need for further exploration into the role of resilience factors and the dynamic nature of symptomatology over time. Future research might further integrate multivariable-defined risk information to stratify control groups according to their risk spectrum, to better understand the specific neurobiology involved in disease onset, remission and resilience throughout development.

Keywords: ADHD, Biomarker Candidates, Risk Stratification

Disclosure: Knight Therapeutics: Consultant (Self)

P92. Corticostriatal Functional Connectivity and Longitudinal Symptom Trajectory During Guaranteed Treatment Delivery in Schizophrenia

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Background: Schizophrenia is associated symptom fluctuations over the course of illness, including often relapses and progressive deterioration. The neurobiological mechanisms of these phenomena are poorly understood, but highly relevant to the long-term outcomes of this chronic condition. Various measures of striatal functional connectivity correlate with acute treatment response, but whether striatal functional connectivity is relevant to longer-term symptom fluctuations has not been studied. A major challenge to study clinical phenomena over the scale of weeks to months is that non-adherence with antipsychotic maintenance is very common yet uncertain at the individual level. Therefore, symptom trajectories are often confounded by non-adherence. To address this limitation, we study prospectively individuals who start long-acting injectable

(LAI) antipsychotics, for whom continuous treatment delivery is guaranteed. Here, we aim to study the role of striatal functional connectivity in symptom trajectories in schizophrenia while antipsychotic treatment is constant.

Methods: 54 individuals with schizophrenia or schizoaffective disorder were recruited at their onset of LAI treatment, scanned, and followed-up with monthly symptom ratings while on LAI treatment for up to one year. A second scan was obtained for each individual triggered by meeting prespecified relapse criteria or after one year of treatment. Scans included two 590 volume ($TR = 0.72$) runs of resting state fMRI, which underwent quality assurance for motion and susceptibility artifacts. We subsequently generated striatal connectivity maps which reflected the functional connectivity strength between 12 striatal regions of interest (ROIs) and 300 brain regions parcellated from the Seitzman atlas. The resulting 3600 edges (300×12) were used as features in a predictive model using cross-validated linear ridge regression for symptom severity at the time of the scan, 6 months and 12 months after baseline scan. Individual symptom trajectories were calculated using a mixed model for repeated measures, in which random intercept and slopes were fitted for the psychosis severity composite from the BPRS over time. Furthermore, in a second set of analyses, we used as predictive features the changes in the striatal connectivity maps between the baseline and endpoint scan. Accuracy of predictions were defined as the mean Pearson correlation of observed vs predicted values in the test set using the model developed with the training set. Given the cross-validated approach, the model was repeated 100 times to ensure stability. The accuracy of predictions was compared to that of a null distribution generated by shuffling the subject labels in the dataset. We also calculated feature importance for each edge using Haufe transformations to identify the striatal connectivity couplets most predictive of symptom trajectories.

Results: $n = 45$ participants fulfilled QA criteria and were entered in the analyses. $N = 29$ (64%) of participants were men, mean age 29.88 (9.56), $n = 10$ (22.2%) were of white race. Using baseline striatal connectivity maps, our predictive model had mean accuracy in the test set of $r = 0.25$, 0.23, and 0.25, for psychosis severity at time of the scan, 6 and 12 months after baseline scan. Accuracies were significantly superior compared to those of the null distribution (standard mean difference [SMD] = 1.16, 0.99, 1.16 respectively and $p < 0.001$ in all 3 comparisons). Connections arising from dorsal striatum involved 14 of the 15 most predictive edges, spanning to prefrontal cortex, insula, cingulate and cerebellum. When using change in the striatal connectome as predictive features ($n = 22$), accuracy was $r = 0.25$, 0.31, 0.28 respectively, which was statistically superior compared to the null distribution with $SMD = 0.79$, 0.97, 0.90 respectively and $p < 0.001$ in all 3 comparisons). Similarly, connections arising from dorsal striatum involved 15 out of the 15 most predictive edges, spanning also to frontal cortex, insula, cingulate and cerebellum.

Conclusions: Striatal functional connectivity is relevant to the neurobiological processes underlying mid and long-term symptom fluctuations in schizophrenia during guaranteed antipsychotic treatment. Patterns in baseline striatal connectivity predicted subsequent symptom trajectory up to one year later, and changes in the connectome also scaled with symptom change. The connectome of dorsal striatum was particularly relevant for subsequent treatment trajectory, aligning with its role in acute antipsychotic response. Overall, these data support to further study striatal functional connectivity as a putative process underlying long-term symptom trajectories, including relapse and progressive deterioration, in patients with schizophrenia. While encouraging, these data should be replicated in larger cohorts to confirm the findings.

Keywords: Dorsal striatum, Resting State Functional Connectivity, relapse

Disclosure: TEVA, Janssen, Karuna: Advisory Board, Self, Neurocrine: Contracted Research (Self), Alkermes: Grant (Self)

P93. Profiling of Metabolites for Autism Spectrum Disorder by Target-12933 Metabolome Analysis

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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. Many omics studies explored genetic and metabolic biomarkers for ASD, but none of them have been put to practical use. Unfortunately, these studies could not discover a definitive, valid, and available biomarker due to various factors depending on individual differences such as symptoms, race, sex, and age. However, metabolome analysis was carried out assuming that there must be substances that could be biomarkers under limited conditions in this study. We also hypothesized that there might be some metabolite that is related to some characteristics of ASD.

Methods: Subjects were recruited from the Hirosaki Five-year-old Children Developmental Health Check-up study between 2013 and 2018, with informed consent after approval by the Research Ethics Committee of Hirosaki University (approval number: 2013-293). Blood samples were collected from community-based 5-year-old children in Japan (healthy control: 41 males and 25 females, patients with ASD: 80 males and 38 females) and subjected to target-metabolome analysis. Target-metabolome analysis was performed using AbsoluteIDQ® Kit p180kit (Biocrates Life Sciences AG) in accordance with manufacturer's instructions. The amounts of 188 metabolites including amines, acylcarnitines, and lipids in plasma were measured by mass spectrometry and analyzed using MetaboAnalyst.

Results: Several metabolites were observed to have significant differences in blood concentration. One of the acylcarnitine, dodecanedioylcarnitine (C12-DC), was significantly higher in patients with ASD (120.0 ng/ml, 95% CI: 113.3-126.7 ng/ml) than in healthy subjects (83.1 ng/ml, 95% CI: 78.4-87.8 ng/ml) ($P = 4.2179 \times 10^{-11}$, fold change = 1.42) and have no differences between sex. Receiver operating characteristic (ROC) curve analysis determined that the sensitivity and specificity were 77.2% and 71.2%, respectively (cutoff = 82.8 ng/ml). In addition, although the average concentration of C12-DC was higher than the control group, there was a tendency to be divided into a high group and a low group. A positive correlation was found between the concentration of C12-DC and the Autism-Spectrum Quotient (AQ) score ($r^2 = 0.551$, $P < 0.001$). Especially, the score of local details was the medium correlation ($r^2 = 0.595$, $P < 0.001$). C12-DC is known to be detected in blood at high levels in oxidative stress and mitochondrial damage. Since oxidative stress and mitochondrial damage are also known to occur in patients with ASD, the increased blood levels of C12-DC may be due to these factors.

Conclusions: We showed that the blood concentration of C12-DC was significantly higher in the ASD group. A positive correlation was found between the concentration of C12-DC and the Autism-Spectrum Quotient (AQ) score. Thus, C12-DC may be a potential biomarker for diagnosing ASD.

Keywords: ASD, biomarker, metabolites

Disclosure: Nothing to disclose.

P94. Validating Potential EEG Biomarkers of Anesthesia-Induced Dream States for Future Therapeutic Application in Post-Traumatic Stress Disorder: Preliminary Results

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Background: Post-traumatic stress disorder (PTSD) has modest rates of clinically meaningful response with current first-line pharmacological and psychotherapeutic interventions, high drop-out rates, and a high risk of suicide. Given this, new, rapid-acting, and durable treatments are much needed. Altered states of consciousness induced by psychedelic-class compounds like MDMA hold great therapeutic promise, but there are multiple barriers to scaling these treatments when combined with therapy. Dreaming represents a well-known physiological sleep state that is thought to modulate memory consolidation and emotion regulation, specifically fear extinction. There is evidence that therapeutic applications of dreaming, particularly lucid dreaming therapy, may be helpful for nightmares, which often accompany PTSD. However, this type of therapy faces major obstacles. We have recently published two case reports of three patients with trauma-related disorders in which the depth of sedation in pre-emergence was controlled during surgery for the purpose of improving the post-operative experience. These anesthetic changes led to transformative dreams related to the patient's traumatic experiences and the reduction of symptoms or remission of their trauma-related disorder. Our objective for the current mechanistic study in healthy controls is to establish a precise neurophysiological marker of anesthesia-induced dreaming for use in a closed loop EEG-driven system in a non-surgical setting that can be utilized to induce therapeutic dreams for patients with PTSD.

Methods: In this ongoing single-blind, within-subject controlled procedure study, participants undergo a series of sedation stages per lab session (i.e., to induce dream and no-dream conditions) using the anesthetic propofol in a pseudorandom manner concealed from the participant. A total of 15 healthy (ASA status I/II) adults (18-70 years old) will be enrolled. Participants undergo (1) Baseline screening/questionnaire (measuring different aspects of mental health and well-being as well as typical sleep habits and dream experiences); (2) Pre-anesthesia daily home sleep and dream log as well as daily mood log (2 weeks); (3) Anesthesia session in the lab (1 day): propofol anesthesia + 32-ch EEG + post-anesthesia structured interview (modified from Brice et al., 1970), including the Mystical Experiences Questionnaire (MEQ30); (4) Post-intervention daily home sleep and dream log as well as daily mood log (2 weeks); (5) Final questionnaire (same as baseline).

Results: We currently have data for 4 participants (1F, 3M, Mage = 27.01, SDage = 2.14) who underwent 22 anesthesia sessions (6 sessions for 3 participants, 4 sessions for 1 participant). Of the 22 sessions, 12 resulted in a dream during anesthesia, 6 in no dreams, whereas 4 resulted in no loss of responsiveness (LOR). Including only the 18 LOR sessions, dreaming during anesthesia was associated with lower delta power ($B = -68.49$, $SE = 34.10$, $z = -2.01$, $p = .04$) as well as with lower alpha/beta ($B = -58.92$, $SE = 29.06$, $z = -2.03$, $p = .04$) and delta/beta ratios ($B = -75.89$, $SE = 37.67$, $z = -2.01$, $p = .04$). Moreover, lower delta ($p < .05$) and higher beta power ($p < .05$) as well as lower alpha/beta ($p < .05$), delta/beta ($p < .001$), and delta/gamma ($p = .01$) ratios were associated with higher dream vividness. Lower delta ($p < .01$), theta ($p < .05$), and alpha ($p < .05$) power were also associated with higher dream emotional intensity and a higher score on the MEQ30.

Conclusions: In the current mechanistic study in healthy human participants, we have preliminarily found that dreaming during anesthesia is associated with decreases in low-frequency brain activity and increases in high-frequency brain activity as measured by EEG. This is in line with previous findings on the neural correlates of dreaming during normal sleep. After completing the study with all 15 participants, we anticipate having a more definitive EEG biomarker to optimize dream-induction success rates in healthy participants. These results will set the stage for a planned, double-blind, sham-controlled RCT in patients with PTSD ($n = 42$) to be conducted at the VA. This study is poised to provide valuable information on the ability of our EEG biomarker to produce a stable dream state in patients with PTSD and the efficacy of such a state to induce rapid and durable PTSD symptom relief.

Keywords: Anesthesia-induced dreaming, EEG Biomarkers, PTSD

Disclosure: Roche: Advisory Board (Self)

P95. A Simple Platelet Biomarker is Associated With Symptom Severity in Major Depressive Disorder

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Background: Previous studies have shown that the heterotrimeric G protein, G α (Gsa) is ensconced predominantly in lipid rafts in acutely depressed subjects with major depressive disorder (MDD) in contrast to healthy controls, and that effective antidepressant treatment (ADT) facilitates translocation of Gsa from lipid rafts. The measurement of Gsa via prostaglandin E1 stimulation of adenylyl cyclase (PGE1 stimulation) has been proposed as a peripheral biomarker for assessing clinical status in MDD. We examined the Gsa biomarker in a new study.

Methods: PGE1 stimulation was used to assess coupling of Gsa with platelet adenylyl cyclase in depressed subjects in active treatment and healthy controls. The Quick Inventory of Depressive Symptomatology (QIDS-C16) measured thresholds of symptom severity at two study visits spaced 2 weeks apart.

Results: QIDS-C16 scores and PGE1 stimulated responses were stable between the two study visits. The QIDS-C16 was inversely correlated with PGE1 stimulated responses at each visit ($rs = -0.39$, $rs = -0.59$, respectively). MDD subjects with mild-moderate depressive symptoms (defined by QIDS-C16 ≥ 6) had significantly lower PGE1 stimulated responses than asymptomatic MDD subjects (QIDS-C16 < 6) or healthy controls ($p = 0.001$ and 0.002 respectively). MDD subjects with moderate depressive symptoms (QIDS-C16 ≥ 10) had the lowest PGE1 responses of all subjects (Fisher exact = 0.012).

Conclusions: These results extend our earlier findings that a simple, high-throughput-capable platelet assay may be a useful biomarker to assess the clinical status of depressed subjects. Larger studies are needed to evaluate the utility of this biomarker for diagnosis and treatment response.

Keywords: Biomarker, G-proteins, GPCR, Antidepressant, Major Depressive Disorder (MDD)

Disclosure: Nothing to disclose.

P96. Developing Imaging Biomarkers of Dopamine Activity: Impact of Methods for Measurement of Midbrain Neuromelanin on Test-Retest Reliability and Relationship to Symptoms

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Background: Over the past three decades the field has made slow but steady progress towards the development of imaging biomarkers related to the pathophysiology of mental disorders such as schizophrenia and related psychotic illnesses. Radio-nuclide measures of dopamine synthesis and release have largely confirmed models of excessive dopamine activity in the brain in psychosis which is related to key clinical features including positive symptoms of psychosis and response to antipsychotic treatment. Although cost and scalability have limited the clinical translation of these findings, recent studies using MRI based measures of midbrain neuromelanin as a proxy measure of dopaminergic activity have shown promise in Parkinson's disease, schizophrenia and major depression. Methods for deriving NM measurements using MRI have been evolving, and successful implementation of an imaging biomarker requires high construct validity, sensitivity and robust test-retest reliability. Here we present findings that compare two commonly used methods for NM measurement.

Methods: The sample consisted of 20 subjects (4 healthy controls, 16 individuals with early course schizophrenia) who underwent scanning twice between 2 and 10 weeks apart. NM scans were acquired using a high resolution 2D GRE sequence with Magnetization Transfer, consisting of 13 acquisitions collected on 3-Tesla Siemens Trio and Prisma scanners with slice acquisition centered over the midbrain dopaminergic nuclei. GRE images of good quality were aligned and averaged to create a mean NM image.

Method 1. Manual tracing method: Raters were trained to identify three slices (two slices if three were not visible) within the substantia nigra (SN) for each participant. Roughly defined masks were drawn in native space for each participant. Circular regions of interest were placed in the crus cerebri (CC) adjacent to each SN mask within each selected slice. The roughly defined SN mask was further refined on an individual subject basis by thresholding relative to the CC. SN contrast ratio (CNR) values were calculated according to the method of Chen et al (2014).

Method 2. Semi-automated pipeline: The NM image was co-registered to the structural scan and aligned to a template in MNI space via nonlinear warping (ANTS). Masks in template space of the SN and CC were used from a previous study (Wengler, 2020) and SN CNR values were thresholded and calculated relative to the CC reference region according to Cassidy et al., 2019.

ICCs of repeated values were computed to estimate test re-test reliability and Pearson's correlation coefficients were used to examine the predicted relationships between NM contrast measures and positive symptoms of psychosis.

Results: The manual tracing method showed poor test re-test reliability (ICC = 0.166), while the semi-automated method showed excellent test re-test reliability (ICC = 0.829).

In the patient subset (n = 16), for the manual tracing method correlations between NM estimates and reality distortion were low ($r = -.038$, $p = .89$), while for the semiautomated method the correlation showed an $r = .344$ in the expected (positive) direction as seen in previous studies by Cassidy et al and Wenger et al, albeit not statistically significant ($p = .19$) in this sample.

Conclusions: Consistent with previous studies using the semi-automated method, NM measures showed excellent test re-test reliability and the expected direction of relationship with measures of positive symptoms of psychosis in patients with schizophrenia. The reported level of reliability suggests this measure may be a promising candidate biomarker for the measurement of excessive activity in the dopamine system in this and other psychotic disorders.

Keywords: Neuromelanin-sensitive MRI, Schizophrenia (SCZ), inter-rater reliability

Disclosure: Nothing to disclose.

P97. Brain Antidepressant EXPOSURE: Fluoxetine Brain Concentrations and Neurochemicals in Adolescents With Anxiety and Depressive Symptoms

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Background: Antidepressant non-response is common in adolescents with depressive and anxiety disorders, delays recovery and increases morbidity. Identifying patients who are more likely to respond to a specific medication is the basis of precision therapeutics - choosing the right drug for the right patient at the right dose. Elucidating factors associated with response vs. non-response is vital to implementing precision therapeutics in clinical practice. This study examines the relationship between fluoxetine concentrations in the brain and neurochemicals associated with neuronal integrity, neurotransmission, cell proliferation and turnover, and implicated in the pathophysiology of anxiety and depression.

Methods: Youth aged 12-21 years who were clinically diagnosed with depressive and/or anxiety disorders or obsessive-compulsive disorder and treated with fluoxetine at steady state completed fluorine and proton magnetic resonance spectroscopy (19F/1H-MRS) at 3 Tesla to measure fluoxetine and the neurochemicals N-acetyl aspartate (NAA), glutamine (Gln), glutamate (Glu), glutamine+glutamate (Glx), myo-Inositol (ml), choline-containing compounds (Cho), and creatine (Cr) in the brain. Individual neurochemical levels were reported as the ratio of analyte to creatine. Total fluoxetine+norfluoxetine plasma concentrations were measured by UPLC-MS/MS. Participants with PHQ9 scores ≥ 11 or Promis Anxiety t-scores ≥ 60 (moderate anxiety) were considered non-responders. Correlation (Spearman's rho, ρ), independent-sample t-test, and effect size analysis (Cohen's d) were performed in SPSS.

Results: In 52 youth (mean: 16 ± 2 yr; 69% female, 19% gender diverse) with depressive (79%) or anxiety (89%) disorders or OCD (6%), strong correlations were observed between Cbrain and CPlasmaTotal ($r_s = 0.901$, $p < 0.001$), and Cbrain and Dose ($p = 0.770$, $p < 0.001$). However, no correlations were found between neurochemicals (NAA, ml, Glu, Gln, Glx, Cho) and Cbrain, CPlasmaTotal or brain:plasma ratios ($p = -0.18$ to 0.17 , n.s.). NAA was significantly lower in anxiety non-responders (n = 14) compared with responders (n = 32) (NAA:Cr mean difference -0.09, 95%CI -0.16 to -0.03, $p = 0.002$; Cohen's d -0.95, 95%CI -1.6 to -0.30). Gln was significantly lower in anxiety+depression non-responders (n = 17) compared with responders (n = 29) (Gln:Cr mean difference -0.07, 95%CI -0.13 to -0.01, $p = 0.035$; Cohen's d -0.67, 95%CI -1.3 to -0.05). Other neurochemical concentrations (ml, Glu, Glx, Cho) did not differ between responders and non-responders, nor did neurochemical levels (NAA, ml, Glu, Gln, Glx, Cho) correlate with individual depressive or anxiety symptom severity ($p = -0.26$ to 0.06 , n.s.).

Conclusions: 19F/1H-MRS is feasible in youth and facilitates simultaneous quantification of fluoxetine concentrations in the brain and neurochemicals. In this pilot study, non-responders had lower average concentrations of NAA, a marker of neuronal integrity, and Gln, which is involved in neurotransmission. However, we did not detect a relationship between brain or plasma fluoxetine concentrations and neurochemicals involved in neuronal integrity (NAA), neurotransmission (Glu, Gln, Glx), glial proliferation (ml) or cell membrane turnover (Cho). The findings

suggest that non-exposure-related factors, such as NAA and Gln levels, may predict response to fluoxetine in adolescents. Future research should further investigate these factors to enhance the precision of therapeutic approaches and improve outcomes for fluoxetine-treated adolescents. These findings suggest the need to further examine these factors and their relationship to target engagement, pharmacodynamic variables and the trajectory of response as well as fluoxetine tolerability in youth with depressive and anxiety disorders.

Keywords: neuroimaging biomarkers, Anxiety and Depression, Adolescence, Selective Serotonin Reuptake Inhibitors (SSRIs), Treatment-Response

Disclosure: Nothing to disclose.

P98. Uncovering the Risk of Alzheimer's Disease in Bipolar Disorder and its Interplay with Epigenetic Aging Acceleration in Blood and Postmortem Brains

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Background: Bipolar disorder (BD) has been associated with an elevated risk of Alzheimer's Disease (AD). However, this association is characterized by significant heterogeneity, as not all individuals with BD have a higher risk for AD or eventually develop AD. The underlying mechanisms contributing to this vulnerability remain elusive. Recent evidence suggests that epigenetic aging may offer a potential explanation for the observed variability in AD risk among individuals with BD. In this study, we aimed to assess biomarkers of AD in BD and tested whether epigenetic aging acceleration is a key mechanism driving variability of AD risk in BD.

Methods: We included N = 59 individuals with BD (69% female, mean age 34 years old) and N = 20 age- and sex-equated non-psychiatric controls (55% female, mean age 34 years old) in this analysis. Peripheral blood was collected from all participants into EDTA-containing vacutainers, followed by separation of plasma and storage at -80°C until downstream analyses. Plasma levels of amyloid beta (A β) 40, A β 42, and total Tau were measured in duplicate in all samples with the Simoa® Neurology 3-Plex A Advantage Kit for SR-X (Quanterix). We also assessed A β 42 levels in postmortem brain samples (BA9/46 area of the dorsolateral prefrontal cortex) from N = 49 individuals with BD type I from the NIH NeuroBioBank using an enzyme-linked immunoassay. Measures of epigenetic age (GrimAge and DunedinPACE in blood and DNAmClockCortical in brains) were estimated with the genome-wide DNA methylation levels assessed by the Infinium EPICBead-Chip v1.0 (Illumina). Epigenetic aging acceleration was obtained by regressing the estimated GrimAge or DNAmClockCortical on chronological age, using the residuals as aging acceleration indices (AgeAccelGrim or DNAmClockCorticalAccel). To explore the possible association between AD biomarkers and accelerated aging, individuals with BD were further split into subgroups with accelerated or slower epigenetic aging if they showed values below the 25th percentile (N = 15) or above the 75th percentile (N = 15) for AgeAccelGrim or DunedinPACE. Groups were compared for all biomarkers with the Welch Two Sample t-test, Wilcoxon rank sum test, Pearson's Chi-squared test, and linear regressions with age and sex as covariates. Pearson correlation was also used to assess the association between DNAmClockCorticalAccel and A β 42 levels assessed in the postmortem brains.

Results: We found no significant differences between individuals with BD and control participants for plasma A β 42, A β 40, or Tau levels ($p > 0.05$ for all). Individuals with BD showed a

significant decrease in the A β 42/40 ratio compared to control participants (median (IQR) for controls - 0.058 (0.008) and individuals with BD - 0.056 (0.008); $p = 0.035$), which remained statistically significant after controlling for age and sex ($p = 0.009$). When comparing individuals with BD with accelerated or slower epigenetic aging, we found a significant decrease in the A β 42/40 ratio in those with high AgeAccelGrim ($p = 0.028$), although this difference did not remain significant after controlling for age and sex ($p = 0.181$). No differences were found in any of the AD biomarkers between subgroups of individuals with BD based on DunedinPACE. Finally, in the postmortem brain samples, a significant, positive correlation was identified between A β 42 levels and DNAmClockCorticalAccel ($r = 0.327$, $p = 0.022$).

Conclusions: Our results indicate that BD is associated with a lower A β 42/40 ratio, which is a strong biomarker indicating AD risk. This underscores the heightened vulnerability of individuals with BD to developing AD. The observed heterogeneity in AD risk among BD patients suggests that factors beyond the diagnosis of BD itself influence disease trajectory. Our results provide preliminary evidence that epigenetic aging may explain this variability and suggest that targeting epigenetic aging acceleration may ultimately reduce the risk of dementia and AD in individuals with BD. Future studies with a larger sample size, particularly focusing on individuals in the mid to late-life age range, can further solidify these findings.

Keywords: bipolar disorder, Alzheimer's Disease, epigenetic aging, DNA methylation, dementia

Disclosure: Nothing to disclose.

P99. Trait-Like Associations of Lifetime Stressors with Mean Levels of—and Hormone-Related Changes in—Peripheral Expression of GABAAR Subunit Genes in Naturally-Cycling Outpatients With Suicidal Thoughts

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Background: While most individuals assigned female at birth (AFAB) do not experience significant menstrual cycle affective change (MCAC), a notable minority experience moderate-to-severe MCAC that causes distress or impairment. MCAC results from an abnormal sensitivity to normal ovarian hormonal changes, not from abnormal hormone levels or trajectories. The most extensively studied form, Premenstrual Dysphoric Disorder (PMDD), is a chronic affective disorder with symptoms confined to the luteal phase, but MCAC can occur across various psychiatric conditions, with many patients experiencing peri-menstrual exacerbation (PME) of symptoms. MCAC is also linked to suicidal ideation (SI) and behavior; psychiatric patients with SI are particularly susceptible to hormone-sensitive affective changes around menses onset, and PMDD patients report high levels of SI and attempts. Despite the prevalence and life-threatening nature of MCAC, relatively little is known about its etiology. Animal studies suggest that stressors can increase behavioral hormone sensitivity by altering the subunit composition of the GABA A receptor (GABAAR), which dictates receptor sensitivity to progesterone metabolites known to be involved in MCAC.

We have recently identified several psychosocial and biological correlates of MCAC. In a study of 114 outpatients with past-month SI ratings who provided daily ratings ($n = 6,187$) of negative affect and SI across 1-3 cycles, growth models revealed that greater lifetime stressor exposure was associated with greater perimenstrual worsening of active SI. Similarly, in PaxGene RNA blood samples from 112 participants from the CLEAR-3 Trial (75 with past-month SI, 37 controls), a greater perimenstrual increase in

GABAAR G2 subunit (GABRG2) expression predicted greater cyclical changes in anxiety and irritability, and those with greater average GABRG2 expression showed greater perimenstrual increase in SI. Here, we examine whether mean or amount of cyclical change in expression of GABAAR subunits (GABRD, GABRG2, GABRA5) correlates with the number and severity of lifetime stressor exposures in the same sample.

Methods: 85 participants (54 with past-month SI, 31 controls) completed the Stress and Adversity Inventory (STRAIN), a comprehensive measure of lifetime stressor exposure at enrollment and 5 luteinizing-hormone (LH)-timed blood samples. Peripheral blood gene expression of GABAAR subunits (GABRD, GABRG2, and GABRA5) was quantified with RT-PCR. Trait-level measures of GABAAR subunits were created: 1) person-means and 2) perimenstrual area under the curve with respect to increase (AUCi) to index degree of perimenstrual increase in expression. We conducted Kruskal-Wallis rank sum tests of differences between patients and controls and conducted Spearman rank correlations between trait-level GABAAR measures and 1) number, and 2) severity of lifetime stressors.

Results: There were no significant differences between AUCi or person-mean values in any subunit between patients and controls, so we pooled all participants in one sample. Lifetime stressor severity score significantly correlated with GABRG2 person-mean ($\rho = 0.230$, $p = 0.035$), and lifetime stressor count showed a marginally significant association with GABRG2 person-mean ($\rho = 0.197$, $p = 0.071$). There were no other significant correlations.

Conclusions: Our prior analyses have found that menstrual cycle affective changes are greater among those with higher psychosocial stress or higher mean (or perimenstrual increase in) peripheral GABRG2 expression. Here, we examined whether stress and GABRG2 expression were correlated at the between-person level. Although neither direction nor causality can be established, observed associations between stress exposure and trait-like GABRG2 expression encourage further experimental tests. Specifically, it is important for the field to understand whether and how environmental stress regulates GABAAR subunit genes and, thus, menstrual cycle affective changes.

Keywords: Menstrual Cycle, GABAA positive allosteric modulators neuroactive steroid, psychosocial stress

Disclosure: Nothing to disclose.

P100. Biomarkers of Depression Symptom Improvement Following Treatment With Cariprazine

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Background: Identification of biological factors associated with improvement in symptoms during treatment with psychiatric medications is valuable for tailoring therapies to patients. We are utilizing patient data from three Phase 3 randomized, placebo-controlled studies of adjunctive cariprazine in major depressive disorder (MDD) and bipolar I disorder (BD-I) to identify predictive biomarkers associated with the change in depression symptoms from baseline following treatment with cariprazine. Cariprazine is a dopamine D3-preferring D2/D3 receptor partial agonist and serotonin 5-HT1A receptor partial agonist that is approved for the treatment of schizophrenia, bipolar mania, bipolar depression, and as an adjunctive therapy to antidepressants for MDD.

Methods: DNA was collected from participants in two adjunctive cariprazine studies for the treatment of MDD

(NCT03738215, NCT03739203) and one monotherapy cariprazine study for prevention of relapse in BD-I (NCT03573297). DNA was genotyped using a customized combination of Illumina's Infinium™ Global Screening Array, along with 30K variants from the PsychArray that included markers previously implicated in several psychiatric disorders. Standard quality control and preprocessing were performed for directly genotyped markers including sample filtering for missing genotypes, sex mismatches, and related pairs, and variant filtering for low quality or monomorphic variants and those not conforming to the Hardy Weinberg Equilibrium expectations. For each MDD study, two Genome-Wide Association Studies (GWAS1 and GWAS2) were performed independently in patients treated for 6 weeks with cariprazine 1.5 mg QD or 3 mg QD (GWAS1: $N = 298$; GWAS2: $N = 340$). For the BD-I study, GWAS3 was performed in patients with moderate to severe depression (baseline MADRS ≥ 23) who were treated for up to 16 weeks with cariprazine 3 mg QD ($N = 310$) during an open-label period prior to randomization to a double-blind withdrawal period. All GWAS were performed using linear regression with sex and the first five principal components as covariates. Genetic association to treatment response was defined using percent change from baseline to end of treatment in Montgomery-Åsberg Depression Rating Scale (MADRS) total score. Meta-analysis of GWAS results across the three studies was performed using Genome-Wide Association Meta-Analysis (GWAMA) software with a fixed-effects model.

Results: GWAS analysis within each study did not detect any single nucleotide polymorphisms (SNPs) surpassing genome-wide significance ($p < 5 \times 10^{-8}$) for percent change in MADRS. However, GWAS meta-analysis across the three studies identified a chromosome 2 locus surpassing genome-wide significance for percent change in MADRS and a chromosome 5 locus surpassing the suggestive threshold ($p < 10^{-5}$). The chromosome 2 locus is an intergenic and regulatory region (peak SNP rs60728345; $p = 7.24 \times 10^{-9}$, $B = 6.7393$). The chromosome 5 locus is near the Fms-related tyrosine kinase 4 gene (FLT4; peak SNP rs100659; $p = 9.25 \times 10^{-6}$, $B = 9.41297$), which acts as a cell-surface receptor for vascular endothelial growth factors C and D (VEGFC and VEGFD). FLT4 is expressed in brain and other tissues, and promotes proliferation, survival and migration of endothelial cells which, in brain, are key constituents of the blood-brain barrier. These associations were not detected when GWAS analyses were performed in patients who received placebo, suggesting the associations are specific to improvement in depression symptoms following cariprazine treatment.

Conclusions: Improvement in clinical symptoms in patients with mood disorders may be influenced by genetic variation. We identified genetic associations in our GWAS analyses of change in MADRS in MDD and BD-I patients treated with cariprazine. Incorporating other types of clinical study data (eg, baseline clinical scale scores, demographic data) is expected to refine our understanding of predictive biomarkers of symptom improvement in psychiatric patients.

Keywords: Genomics, Treatment-Response, Major Depressive Disorder (MDD), bipolar depression

Disclosure: AbbVie Inc.: Employee (Self)

P101. Gaba-A Receptor Subunit Gene Expression as a Biomarker for Menstrual Cycle Affective Change

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Background: Fluctuating ovarian hormones substantially impact psychiatric symptoms in some individuals, leading to Premenstrual Syndrome, Perimenstrual Exacerbation of underlying disorders, and Premenstrual Dysphoric Disorder. Because evidence indicates these categories are likely arbitrary labels for a continuous spectrum of sensitivity to ovarian hormone fluctuations, we use the term “Menstrual Cycle Affective Change” (MCAC) to encompass any menstrual cycle-linked change in symptoms. MCAC etiology appears multifactorial, but the leading hypothesis is aberrant sensitivity of the GABA-A receptor (GABAAR) to neuroactive steroids. However, this hypothesis has never been translated to humans due to the impracticality of measuring GABAAR gene expression in CNS.

Methods: Here, we utilize PaxGene RNA blood samples in 112 participants (75 with suicidality, 37 controls) to test if 1) peripheral gene expression for GABAAR delta, gamma2, and alpha5 subunits (GABRD, GABRG2, GABRA5) fluctuates across the cycle; and 2) subunit gene expression predicts MCAC. We collected two months of daily symptom ratings and five luteinizing hormone (LH)-timed blood samples, then quantified GABRD, GABRG2, and GABRA5 with RT-PCR.

Results: We found decreased GABRG2 during the high-progesterone midluteal phase (Est = -0.01, $p = 0.05$), translating preclinical work showing progesterone suppresses GABRG2. We next predicted linear and quadratic symptom trajectories from gene expression mean and Area Under the Curve (AUC). GABRD and GABRA5 predicted luteal phase SI trajectory (GABRA5: Est = -0.27, $p < 0.05$). GABRG2 predicted follicular phase anxiety (Est = -0.10, $p < 0.05$), luteal (Est = 0.11, $p < 0.01$) and follicular (Est = -0.15, $p < 0.01$) phase irritability, and follicular phase SI (Est = -0.10, $p < 0.05$).

Conclusions: Our work suggests that GABAAR subunit gene expression, particularly GABRG2, may be a novel peripheral biomarker of MCAC.

Keywords: Menstrual Cycle, premenstrual dysphoric disorder, premenstrual syndrome, GABA-A receptors, Suicidality

Disclosure: Nothing to disclose.

P102. Towards Validation of the Neurobiological Craving Signature (NCS)

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Background: Craving – a strong urge or desire – is a core feature and diagnostic criterion of substance use disorders (SUDs). Importantly, craving – including cue-induced craving – predicts future substance use and relapse. However, self-reported cravings are limited by recall and self-presentation bias, degree of self-insight, and do not shed light on the neurobiological basis of craving. Thus, an objective, brain-based neuromarker of craving holds great potential. Such a marker could help characterize the brain basis of craving and its role in SUDs, including treatment response. Although neuromarker research has blossomed in some fields over the past 25 years (e.g., Alzheimer’s), the pace in addition neuroscience lags far behind. Recently, we used state-of-the-art methods, including machine learning algorithms and rigorous cross-validation, to develop the fMRI-based Neurobiological Craving Signature (NCS). The resulting NCS is a whole-brain multivariate pattern that shows promise as a predictive neuromarker of craving. In its development, the NCS successfully predicted self-reported craving ratings on a trial-by-trial basis. In addition, the NCS accurately classified individuals with SUDs versus individuals without SUDs, suggesting that it can serve as a diagnostic neuromarker. It also classified lowest versus highest cravings within individuals and showed sensitivity to a cognitive regulation strategy drawn from Cognitive-Behavioral treatments (CBT; e.g., considering negative

consequences of consuming drugs), suggesting promise as a response neuromarker. That said, per FDA-NIH guidelines, any putative marker requires rigorous testing in completely independent validation datasets. As a first step in this process, we applied the NCS in a novel sample of $N = 80$ individuals with tobacco use disorder, aiming to: (1) validate the NCS’s ability to predict craving in a new sample; (2) validate classification of lowest versus highest levels of craving within-persons; and (3) test whether the NCS is modulated by strategies drawn from either CBT or Mindfulness-Based treatments (MBT).

Methods: Quitting-motivated individuals who smoked daily enrolled into a three-arm randomized controlled trial consisting of six sessions of targeted regulation-of-craving skills training (CBT, MBT, or inactive control training) over a four-week intervention period. Here, we focus on pre-treatment data. Participants completed the Regulation of Craving (ROC) task during fMRI prior to initiating treatment ($N = 80$). Power analysis showed that a sample size of 67 participants would detect an association of $r = .30$ at $p < .05$ and 80% power. In the ROC task, participants viewed images of cigarettes and food. On some trials, participants experienced cue-induced cravings, whereas on the remaining trials, participants were instructed to regulate their cravings using CBT- and MBT-based strategies. After all trials, participants provided a self-reported craving rating on a 1-5 Likert scale. We modeled fMRI data at the single-trial level to assess the prediction-outcome association of the NCS with trial-by-trial craving ratings. Trials were also averaged into lowest and highest level of self-reported craving per participant for classification, as well as averaged by regulation strategy in the ROC task.

Results: The NCS predicted craving ratings across food and cigarette cues ($B = 0.01$, $STE = 0.00$, $t(79) = 4.44$, $p < .001$) with no differences between sexes. The NCS also classified lowest versus highest levels of cravings within individuals with 70% accuracy ($\pm 5.1\%$ STE, binomial test $p < .001$; sensitivity = 70%, 95% CI [59% - 80%], specificity = 70%, 95% CI [60% - 80%], AUC = 0.75). Both regulation strategies modulated NCS responses ($F(2, 158) = 11.37$, $p < .001$), such that responses decreased for CBT- ($p < .001$) and MBT-based ($p < .001$) strategies, but did not differ between the two strategies ($p = .85$), evidencing the sensitivity of the NCS to both behavioral interventions.

Conclusions: The results confirm that the NCS remains predictive of craving in novel contexts, including for new participants, stimuli, different scanners, and treatment context. In addition to replicating the effect of a cognitive regulation strategy on NCS response, we extended this finding to a novel regulation strategy derived from MBT. Importantly, these results provide preliminary evidence showing the utility of the NCS as a potential mechanistic target for interventions (response neuromarker), though extensive validation work remains. Moving forward, the NCS will need to be validated across additional cue reactivity studies, including a greater diversity of drug types, participant characteristics, and stimulus modalities. This future work should also examine how the NCS might change throughout treatment, and whether it is predictive of future drug use or relapse (prognostic neuromarker). The present findings move the NCS a step closer toward use in clinical trials.

Keywords: substance use disorder, craving, biomarkers, neuro-markers, Functional MRI (fMRI), machine learning

Disclosure: Nothing to disclose.

P103. Trajectories of Task-Based EEG Delta-Beta Coupling: Capturing Early Neural Risk for Psychopathology

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Background: Delta-beta coupling measures the correlation between simultaneous delta and beta EEG power activity and is thought to reflect the dynamic crosstalk between limbic and cortical regions underlying regulation. Stronger positive delta-beta coupling has been robustly associated with anxiety symptoms in adults, adolescents, and children (Brooker et al., 2016; Poole and Schmidt, 2019). This literature suggests that delta-beta coupling may capture over-controlling, hypervigilant states, and serve as a useful neural marker of anxiety risk. However, previous studies have mostly examined cross-sectional samples and employed resting-state EEG, which provide limited information about the functional and developmental significance of delta-beta coupling.

Methods: We leverage EEG data from a study ($N = 197$) of mother-child dyads recruited during pregnancy to examine the development of delta-beta coupling during an auditory Oddball paradigm at ages 12, 24, and 36 months, capturing early development. Trial-by-Trial, Delta, and Beta power were exported across Standard, Odd, and After-Odd trials. Delta and Beta power timeseries were correlated to compute person-specific coupling scores at each assessment, and across Frontal, Central, and Parietal regions. Cross-sectional studies of delta-beta coupling in children report significant and similar effects across these regions (Brooker et al., 2016; Phelps et al., 2016; Poole et al., 2020). However, other studies suggest that the association between delta-beta coupling and psychopathology risk may be specific to the Frontal region (Knyazev et al., 2019), and shown different developmental patterns as a function of scalp areas (Anaya et al., 2021). Based on these previous findings, we contrast task and developmental patterns of delta-beta coupling across Frontal, Central, and Parietal regions. We first examine task-level differences at each assessment by comparing delta-beta coupling across Standard, Odd, and After-Odd trials. We then characterize the development of delta-beta coupling by investigating 1) mean-changes in coupling between 12, 24, and 36 months for each trial type (modeling continuity), 2) Pearson correlations between coupling at each assessment and for each trial type (modeling rank-order stability), and 3) growth models of delta-beta coupling from 12 to 36 months (modeling within-person change).

Results: After controlling for child sex, gestational age, and child age at time of assessment, trial comparisons indicated that at 12 months, delta-beta coupling during Odd trials was weaker at Frontal ($\beta = -0.023$, $p = .004$) and Central ($\beta = -0.019$, $p = .013$) regions compared to Standard trials. No trial differences were identified at 24 or 36 months.

Developmental trajectories and group means are presented in Figure 1. Mean-comparisons indicated that delta-beta coupling during Standard and After-Odd trials was weaker at 24 and 36 months compared to 12 months across all cortical regions. In contrast, mean coupling during Odd trials was not different between 12, 24, or 36 months at Frontal or Central regions, and only showed significant mean differences (weaker at 24 and 36 months compared to 12 months) at the Parietal region.

Pearson correlations between delta-beta coupling across assessments were largely not significant, indicating no rank-order stability across any trial type.

Finally, growth models controlling for child sex, gestational age, and social disadvantage indicated that individual trajectories of delta-beta coupling significantly decreased from 12 to 36 months at Frontal ($\beta = -0.024$, $p = .001$), and Parietal regions ($\beta = -0.015$, $p = .023$), but remained stable at the Central region ($p = .083$), and this pattern did not differ across trial type.

Conclusions: These findings support developmental differences in delta-beta coupling during early childhood using task-based EEG data. Our results suggest that while discontinuity and instability was evident during Standard and After-Odd trials, delta-beta coupling during Odd trials showed a different developmental pattern at Frontal and Central regions. Specifically, there was continuity of group means juxtaposed with instability in

individuals' ranking, which may indicate that individual differences may play a larger role in explaining variance over time. We plan to follow-up these analysis with variance-decomposition analysis of coupling at each region to examine how variance is attributed to individual vs. visit-level differences.

Our growth models indicated a prototypical decrease in delta-beta coupling at the Frontal and Parietal regions, which aligns with prior resting-state EEG studies (Anaya et al., 2021) and supports neurodevelopmental accounts of parietal-to-frontal maturation (Gotgay et al., 2004) that implicate posterior regions in cognitive and regulation processing during this age period.

Of note, expected trial differences (Odd compared to Standard trials) were only evident at 12-months, indicating that delta-beta coupling only indexed attention-related differences at this earlier timepoint. Further sensitivity analyses will examine whether trial differences become evident at 24 and 36 months in the context of individual differences previously associated with the Oddball paradigm (e.g., temperament). Additionally, we will also examine trajectories of child internalizing and externalizing symptoms across 12, 24, and 36 months to contextualize adaptive of risk-related delta-beta coupling trajectories.

Keywords: EEG biomarkers, Brain development, Delta-beta coupling, Infancy

Disclosure: Nothing to disclose.

P104. Prediction of Postoperative Delirium Using Resting-State EEG Phase-Amplitude Coupling

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Background: Postoperative delirium (POD) is an acute disorder of consciousness accompanied by disturbed cognitive function that develops after a surgical intervention. POD is linked to persistent cognitive decline, frailty and mortality. Although it is well established that interventions to reduce modifiable risk factors for delirium is important, the incidence of POD remains high partially due to limited and suboptimally distributed medical resources, highlighting the need for objective predictive markers. EEG phase-amplitude coupling (PAC) reflects the efficiency of information processing through the interaction of neural activities with different frequency rhythms and has been applied to the evaluation of brain function in various psychiatric conditions. The present study examined whether the preoperative PAC could be used as a biomarker to predict POD.

Methods: We recruited 71 patients hospitalized for their surgical operations (UMIN:000029814). They underwent preoperative resting-state EEG (rs-EEG). The EEG signals were divided into the conventional five frequency bands (δ , θ , α , β and γ). PAC was assessed with the modulation index (MI) for all the 10 possible combinations of phase and amplitude frequency bands. We compared the MI between 18 (25.4%) subjects who subsequently developed delirium and 53 subjects who did not. The mean MI across the 19 electrodes was statistically tested for the group difference for each of the 10 phase- and amplitude- frequency band combinations. For the δ -phase β -amplitude MI (δ - β MI), which showed a significant group difference, the group difference was further tested for each electrode separately. For all these tests Bonferroni correction was applied. The MI of the electrodes with a significant group difference was averaged and its power to predict POD was assessed using a receiver operating characteristic (ROC) curve.

Results: We found that δ - β MI averaged across all the electrodes was lower in the delirium group than in the non-

delirium group ($p < 0.01$). The δ - β MI showed a group difference at six out of 19 electrodes (Fp1, F3, F4, F7, T3 and T4; $p < 0.01$ for each electrode). The mean δ - β MI of these six electrodes predicted POD with moderate accuracy (area under the curve = 0.84).

Conclusions: The δ - β MI may be useful to predict POD. The reduction in this index may reflect an altered brain state that exists from before a surgical intervention. Significant changes in the δ - β MI were mainly observed above the bilateral prefrontal cortices, suggesting functional change in these areas, which is in line with the finding in previous functional MRI studies. Further validation studies in larger populations are needed.

Keywords: Delirium, EEG, Phase-amplitude coupling, Biomarker Prediction, Prefrontal cortex

Disclosure: Nothing to disclose.

P105. The Association Between Amygdala Volume Changes and Depressive Symptom Improvements After Repeated Ketamine Infusion in Treatment-Resistant Depression: A Double-Blind, Randomized, Placebo-Controlled Trial

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Background: The mechanism of the antidepressant effect of repeated intravenous ketamine infusion remains unclear in patients with treatment-resistant depression (TRD). The amygdala plays an important role in emotional processing, mood state, fear conditioning and extinction, and social behaviors. Some of the functional magnetic resonance imaging (MRI) studies reported abnormal hyperactivity in the amygdala during emotion processing tasks in patients with depression in comparison with healthy controls. In addition, previous functional MRI studies noted that ketamine administration decreased overreactions in the amygdala of patients with TRD during the processing of negative emotions. Therefore, the amygdala may be involved in the pathophysiology of TRD and the antidepressant effects of ketamine. However, the relationship between the effect of ketamine and the amygdala volume changes in patients with TRD has not been well investigated. To date, there is the only study that investigated amygdala volume changes after repeated ketamine treatment in patients with depression. However, this was an open-label study and included mixed patients with either TRD, suicidality, or both. Furthermore, while the amygdala is known to comprise multiple nuclei that exhibit different connectivity and profiles, the volume of each subfield was not analyzed. Therefore, this study sought to examine whether changes in amygdala subfield volumes would correlate with clinical response to ketamine in patients with TRD in an exploratory manner.

Methods: We used data from a double-blind, randomized, placebo-controlled trial to assess the efficacy of repeated intravenous ketamine in Japanese patients with TRD (JRCTs031210124).

In this trial, 34 participants were randomly allocated to either ketamine or placebo groups in a 1:1 ratio and received either repeated intravenous ketamine or placebo. MRI scans were conducted before and after administration of these study drugs. The depressive symptom was assessed with the Montgomery Åsberg Depression Rating Scale (MADRS). All participants were imaged on a 3T Siemens scanner using a 32-channel head coil. We used three-dimensional fast spoiled-gradient T1 and T2 weighted images to measure subcortical volume with the following parameters: repetition time/echo time = 2400/2.22 ms; flip angle = 8°; field of view = 24 cm; 320 × 320 pixel matrix; and

voxel size; 0.8 × 0.8 × 0.8 mm. To measure the amygdala subfield volumes, image segmentation and the estimation of the amygdala volumes were performed using the publicly available FreeSurfer 7.0 software with the standard parameters. We focused on the volumes of 9 amygdala subfields: lateral nucleus, basal nucleus, central nucleus, medial nucleus, cortical nucleus, accessory basal nucleus, corticoamygdaloid transition, anterior amygdaloid area, paralaminar nucleus. For the analysis, we used data from the participants who underwent MRI scans both before and after the intervention. Preliminary multivariable regression analyses were conducted to explore the factors associated with the change in the MADRS total scores with explanatory variables including treatment, volume change in each region of interest, and treatment by volume change interaction. Subsequently, we used a multivariable regression analysis to examine the relationship between the MADRS total score change with the volume changes in the regions of interest that showed significant interactions in the first multivariable regression analysis. Next, we also fit separate multivariable linear regression models with changes in the MADRS total scores and amygdala volume changes in each treatment group.

Results: Of the participants who completed treatment, 11 (64.7 %) in the ketamine group (5 [45.4 %] females; mean ± standard deviation age, 43.0 ± 9.6 years) and 15 (88.2 %) in the placebo group (4 [26.7 %] females; mean ± standard deviation age, 42.9 ± 9.4 years) underwent MRI scans before and after the intervention. Preliminary multiple regression analyses on both groups found that the subregion volumes in the right cortical nucleus (rCO) and the right corticoamygdaloid transition area (rCAT) were associated with the MADRS total score change ($F(3, 22) = 6.07$ and 4.32 , $p = 0.004$ and 0.015 , $R^2 = 0.38$ and 0.29 , and treatment by volume change interaction: $p = 0.016$ and $= 0.031$, respectively). Next, a multiple regression including volume changes in the rCO and rCAT as explanatory variables demonstrated that these variables provided a prediction of the MADRS total score change in the combined group ($F(5, 20) = 5.60$, $p = 0.002$, $R^2 = 0.48$, and treatment by volume change interaction: $p = 0.016$ and $= 0.031$, respectively). Moreover, a post-hoc multiple regression found that changes in MADRS total scores were related to volume changes only in the rCO in the ketamine group ($F(2, 8) = 4.83$, $p = 0.04$, $R^2 = 0.43$, volume changes in the rCO: $p = 0.032$). However, there was no such association in the placebo group ($F(2, 12) = 2.82$, $p = 0.10$).

Conclusions: The volume reduction in the rCO correlated with a better clinical response to ketamine treatment in patients with TRD. The result suggests that the volume of the amygdala, particularly the rCO, may be related to the mechanism of the effects of ketamine for TRD.

Keywords: IV- Ketamine, Treatment-resistant depression, Structural MRI, Amygdala, Randomized Double-Blind

Disclosure: Nothing to disclose.

P106. Depression and Anxiety in Pregnancy in Association With Markers of Postpartum Parent and Infant Mental Health

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Background: Depressive and anxiety symptoms in pregnancy have been associated with poorer postpartum maternal and infant outcomes. This work investigates different types of mental distress during pregnancy in relation to biologic markers of the postpartum parent and the infant.

Methods: Individuals were given the Mood and Anxiety Symptom Questionnaire (MASQ) including general distress

depressed (GDD), general distress mixed (GDM), and general distress anxious (GDA) at two time points in pregnancy to two months postpartum ($n = 94$); with a subset of their infants also included in the study ($n = 52$). During the postpartum visit mothers underwent the Trier Social Stress Test (TSST) and infants underwent a heel stick stressor; while obtaining EKG for heart rate variability (HRV) measures of high frequency (HF), Respiratory Sinus Arrhythmia (RSA), low frequency (LF), very low frequency (VLF), Root Mean Square of the Standard Deviation (RMSSD), and the Standard Deviation of Normal R-R Intervals (SDNN). Change between baseline to stressor (reactivity), stressor and post-stress (recovery), and baseline to post-stress (normalization) were calculated. All significant p -values are after corrections. Maternal blood was assayed for metabolites with targeted LC-MS.

Results: Greater changes across pregnancy in GDD associated with VLF recovery ($p = 0.01$) and heart rate recovery ($p = 0.048$, $p = 0.049$). Net change from third trimester to postpartum positively correlated with maternal LF reactivity and normalization and LF/HF ratio reactivity ($p = 0.023$, $p = 0.027$, $p = 0.016$). Net change in GDD across pregnancy positively correlated with infant LF post-stress ($p = 0.017$).

Net change in GDM from third trimester to postpartum was negatively correlated with VLF at baseline ($p = 0.019$). Net change in GDM from third to postpartum negatively correlated with baseline and post-stress SDNN ($p = 0.015$, $p = 0.010$). Higher GDM in the postpartum correlated with higher infant heart rate in the period after the stressor ($p = 0.011$).

Net change from earlier in pregnancy to postpartum in GDA correlated with the maternal LF/HF ratio during the stressor and LF/HF ratio reactivity ($p = 0.046$, $p = 0.022$). Net change in GDA from 3rd trimester to postpartum negatively associated with the infant RSA at baseline, during the stressor, and after the stressor ($p = 0.004$, $p = 0.006$, $p = 0.011$). Maternal kynurenine in the third trimester trended towards a significant positive association with GDA postpartum ($p = 0.059$). Maternal kynurenine to tryptophan ratio in the third trimester associated with the mother assessing the baby as being colicky for more than three weeks ($p = 0.01$).

Conclusions: Different HRV patterns arose with regards to maternal anxiety, mixed symptoms, and depressive symptoms. GDM was negatively associated with maternal SDNN, a measure thought to reflect risk for cardiac morbidity. GDM was associated with infant higher heart rate; possibly indicating early transgenerational transmission of stress and cardiac risk. Maternal anxiety associated with infant vagal tone as reflected by RSA and with the kynurenine to tryptophan ratio which is thought to reflect immune activation, and associated with prolonged infant colic.

Keywords: pregnancy/postpartum, Anxiety, Depression, heart rate variability, Kynurenine pathway

Disclosure: Sage Therapeutics: Contracted Research (Self). Abbvie Laboratories: Stock / Equity - Publicly Traded Company (Spouse/Partner)

P107. Shared Biomarkers of Alcohol Use and Anxiety in a Sample Using Cannabis for Anxiety: A Multi-Omic Approach

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Background: Anxiety and alcohol use disorders are highly co-occurring. There is also a lack of objective markers of systemic and neurobiological change associated with both conditions, and a need for new therapeutic targets that are studied more equitably

across diverse groups. Thus, this study had two goals. First, to explore relationships between biomarkers of inflammation, heart rate, and the microbiome that are shared across the anxiety and alcohol literatures in the context of medical cannabis use. Second, to investigate the effects of exogenous cannabinoids among anxiety-symptomatic community members on these biomarkers.

Methods: This research used a subsample from a larger study (R01DA04413; PI: Bidwell). Participants were in one of three groups: a) non-use, b) THC dominant cannabis use, or c) CBD dominant cannabis use ($N = 172$; 62% female; 45% gay/lesbian/queer; 62% underrepresented race/ethnicity; $\text{Mage} = 30.2$). The study design included assessments at baseline and after two and four weeks of ad libitum use of a legal market cannabis product that was assigned to them. Outcomes of interest included individual pro-inflammatory cytokines, microbiome family Prevotellaceae relative abundance, and heart rate, as well as an exploratory composite biomarker risk score.

Sample size was selected to permit analysis of the primary research questions at two-tailed alpha of 0.05 and power level of 0.80. Results suggested a total $n = 132$ would allow for detection of an interaction effect as small as $f = .125$. First, microbiome data was assessed for significant flora differences. Next, correlational analyses were conducted between all target biomarkers (heart rate, inflammatory markers, and relative abundance of gut flora Prevotella) and alcohol use variables. z -scores were then calculated for values at the 4-week timepoint and combined and averaged for each participant to create a composite biomarker risk score (BRS). Once the BRS was calculated, a regression approach was taken to predict BRS with cannabis use group assignment with the covariates of study use frequency and age at the 4-week timepoint. Finally, analyses were repeated for only the groups using cannabis. Analyses were conducted in R Studio.

Results: 152 participants used cannabis for anxiety during the study (96 in the THC-dominant group and 56 in the CBD-dominant group) and 20 participants were in the no-use control group. Symptom levels of anxiety for the sample at baseline were in the mild to moderate range on the Beck Anxiety Inventory (BAI; $M = 14.3$, $SD = 8.65$). Symptoms of cannabis use disorder ($M = 2.84$, $SD = 3.98$) and drinking levels ($M = 2.26$ drinking days/14 days, $SD = 2.48$) were both at low levels. Within the microbiome data, there were no significant group differences between mean values of the Shannon Index ($F = 0.316$, $p = 0.729$) and no significant differences in beta diversity across groups ($R^2 = 0.016$, $\text{Pr}(> F) = 0.400$). Cytokine data was limited compared to other biomarkers ($n = 72$). As anticipated, the pro-inflammatory cytokines IL-1b, IL-6, and TNF-alpha were all significantly correlated with one another ($ps < 0.001$), while IL-1b was positively, significantly correlated with number of drinking days ($r = 0.237$, $p = 0.048$) and average drinks per drinking day ($r = 0.273$, $p = 0.033$). Prevotellaceae was significantly correlated with IL-1b ($p = 0.012$) and TNF alpha ($p = 0.007$), while heart rate was not significantly related to other biomarkers. In regression analyses, there were no significant main effects of group on the BRS ($ps = 0.189 - 0.891$) or on drinking-related behaviors ($ps > 0.05$). Trends suggest possible influence of exogenous cannabinoids on BRS scores such that average BRS was highest for the THC group ($M = 0.07$), followed by the CBD group ($M = -0.13$) and the non-use group ($M = -0.31$).

Conclusions: While there were no significant effects of CBD or THC on the biomarker risk score, these results may be driven by limitations in the data. Encouragingly, correlations across multiple biomarkers and use variables, and trending differences in BRS group means suggest further exploration is warranted.

Keywords: Anxiety, Alcohol, THC, CBD, biomarkers

Disclosure: Nothing to disclose.

P108. Examining the Factor Structure of the Transdiagnostic DSM-5-TR Level 1 Cross-Cutting Symptom Measure in Real World Psychiatric Outpatients

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Background: The DSM-TR Level 1 Cross-Cutting Symptom Measure (DSM-XC), developed by the American Psychiatric Association, is a dimensional transdiagnostic mental health measure. Despite its potential, few studies have examined its latent dimensionality in generalizable populations. Existing research has limitations, including utilization of translated versions for prisoners and students or online samples of participants not seeking psychiatric care. In addition, best practices for factor analyses suggest a sample size of at least 460 participants for this 23-item measure. Only one study meeting this threshold has been published and used an online convenience sample, generating 6-factor and bi-factor solutions. Although the original intent of the DSM-XC was not to measure overall psychopathology, the bi-factor solution included a general psychopathology factor modeled after Caspi et al.'s (2014) "p factor" (Gibbons et al., 2022). Notably, both solutions excluded the self-harm question and had limited psychotic symptom endorsement. To our knowledge, no factor analysis studies have been conducted on a real world psychiatric outpatient population. We addressed this gap by examining the DSM-XC's factor structure in a large sample of patients from independent U.S. psychiatric practices.

Methods: Patients (N = 919) completed the DSM-XC as a routine part of their clinical care. The sample was 66% female and 85% Caucasian, with a mean age of 38.2 years (SD = 13.7). The most common psychiatric diagnoses were major depression (59%), generalized anxiety disorder (51%), and ADHD (34%). We used exploratory factor analysis (EFA) to identify candidate solutions and confirmatory analysis (CFA) to evaluate extracted solutions. The dataset was randomly split and stratified for the EFA (n = 459) and CFA (n = 460). All models used diagonally-weighted least squares estimation with geomin rotation and test statistics scaled for ordinal items. For EFA we tested standard and bifactor approaches containing 2-8 total factors. A single solution from each approach was extracted for the CFA according to fit indices (CFI, TLI, and RMSEA) and clinical interpretation of item factor loadings. Two solutions from the largest prior factor analysis on the DSM-XC were also evaluated in the CFA subset. Analyses used R with the lavaan package.

Results: Model fit indices (CFI, TLI, and RMSEA) all surpassed target benchmarks in the EFA models that contained at least 4 factors. From the standard EFA we extracted a 6-factor solution, which had good fit in the EFA and acceptable fit in the CFA (CFI = 0.97, TLI = 0.97, RMSEA = 0.06). A 7-factor bifactor solution also had good fit in the EFA and acceptable fit in the CFA (CFI = 0.97, TLI = 0.96, RMSEA = 0.06). In CFA models testing the Gibbons et al (2022) 6-factor standard and 3-factor bifactor solution, fit was acceptable but slightly worse than for both solutions extracted from our EFAs. Of note in this clinical sample, item loadings on the general psychopathology factor in our 7-factor bifactor solution remained high (20/23 items above 0.30), while group factors mapped closely onto expected clinical domains including Mania, Obsessive-Compulsive, Psychosis, Anxiety, Substance Use, and Personality functioning. Item structure of these 6 group factors also converged to a large extent with the 13 DSM-XC domains. Several items (3, 10, 11, 14, 15, and 18) loaded only onto the general factor and no group factors. Compared to the 7-factor bifactor solution, interpretation of the 6-factor standard solution reflected more transdiagnostic factors due to

the the greater number of item cross-loadings (8 vs 2), with factors representing Mania, Ruminative Depression, Anxiety, Neurovegetative Depression, Reality Testing, and Personality.

Conclusions: Our analysis of the DSM-XC in psychiatric outpatients supports both a 6-factor solution and a 7-factor bifactor solution, with the latter demonstrating slightly better interpretability. The strong general factor reflects overall endorsed symptomatic burden, aligning with transdiagnostic approaches to psychopathology. Further research could explore if this factor has utility in clinical and research settings. Simultaneously, the structure of the group factors points to distinct areas of dysfunction beyond this general factor. Future analyses with larger sample sizes will help further refine these candidate solutions, deepening the conceptualization of psychiatric symptoms as transdiagnostic and dimensional in real-world psychiatric populations alongside existing categorical approaches.

Keywords: precision psychiatry, Transdiagnostic, DSM, Real World Data, dimensional psychopathology

Disclosure: iRX Reminder: Employee (Spouse/Partner). Magnus Medical: Contracted Research (Self). Magnus Medical: Consultant (Spouse/Partner). Videra: Stock / Equity - Privately Held Company (Spouse/Partner). Brainsway: Contracted Research (Self). Brainsway: Contracted Research (Spouse/Partner). Soneira Bio: Consultant (Spouse/Partner).

P109. Brain-Behavior Relationships in Clinical Trials: Residualized Change Scores Reduce Variability and Improve Model Fit

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Background: One of the challenges to studying mood disorders is the tendency for both brain and behavioral measures of interest, and the relationships between them, to vary across time. While this can be of interest in some contexts, in clinical trials, this variability can impact the ability to detect effects of interest. This is especially true in conditions such as bipolar disorder, where internal states can vary greatly. Unlike measures such as BMI or weight, there is often variability in neural and behavioral states across repeated baseline measurements for within subject clinical trial designs, even independent of group-related differences in baseline. In the current study, we used data from an ongoing clinical trial, where we are blinded to group (control and participants with bipolar combined) and treatment condition (within subjects design). Our aim was to test whether using residualized change scores improve the overall fit and reduce noise in brain-behavior relationship models compared to raw change scores.

Methods: Our sample to date, includes 51 adults (64.7% women; 33.3% men, 2% non-binary; mean age=26.11, SD = 5.19). The sample includes both individuals with bipolar disorder and healthy controls. The study is a within subjects' design where each participant received three theta burst stimulation conditions (active, active control, and sham; randomized order of administration) across three separate sessions. We are currently blinded to stim condition (hereby denoted as Stim A, B, and C) and group. Participants completed a baseline Internal State Scale (ISS) measure, followed by a post stimulation measure at each stim session. They also completed a pre and post stim fMRI scan at each session. For the study, we are interested in the relationship between change in brain activity in the vIPFC and change in ISS-

Activation scores, a proxy for symptoms of mania. To test the impact of controlling for baseline on model fit and variability, we conducted two types of linear regressions for each stim condition. The first included residualized change scores for both brain and behavioral measures as independent and dependent measures respectively (post-pre after removing variance associated with baseline/pre measures), while the other used a raw measure of change for each (post-pre) a linear regression for each stim condition or each of the three conditions. We compared R-squared and residual standard error (RSE) as measures of model fit and variability respectively.

Results: In two out of the three stim condition models, we observed an improvement in model fit using the residualized change scores (Res), compared to raw change scores (Raw) to when evaluating brain-behavior relationships (Stim A ResR2 = .073 vs RawR2 = .036; Stim B ResR2 = .002 vs RawR2 = .02; Stim C ResR2 = .080 vs RawR2 = .033). In all three stim condition models, we observed a reduction in residual standard error, a marker or reduced variability in the model (Stim A ResRSE = 83.66 vs RawRSE = 137.17; Stim B ResRSE = 106.46 vs RawRSE = 144.73; Stim C ResRSE = 82.96 vs RawRSE = 127.18.) The model with residualized change in left vIPFC activity predicting residualized change in ISS-Activation for Stim C was the only statistically significant effect ($F = 4.234$, $p = .045$).

Conclusions: Since both models only had one independent variable, effects observed between models are not due to differences in number of predictors. Results showing improved model fit and reduced variability were also independent of whether model statistics were significant or not. However, it is of note that the effect observed for Stim C was only in the residualized change model and not in the raw change model, suggesting that controlling for baseline using this method may also help improve the likelihood of detecting effects of interest. This work highlights the importance of data pre-processing and choices surrounding biostatistical modeling, especially in the context of clinical trials in psychiatry where within subject variability can reduce the ability to detect or accurately estimate effects.

Keywords: Bipolar Disorder, Human Clinical trial, Clinical Trial Methodology, biostatistics, Theta-burst stimulation

Disclosure: Nothing to disclose.

P110. Expectancy Effects in Psychedelic and Comparative Effectiveness Trials: Reducing Bias and Increasing Power

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Background: Expectancy effects represent a likely confounder of treatment effect estimates in placebo-controlled trials of psychedelics and in comparative effectiveness trials. The relationship between the strength of expectancy effects and the bias introduced into treatment effect estimates is unknown. Further, although treatment expectancies are increasingly being measured in such trials, how analysis approaches may best adjust for bias is not clear.

Methods: We used a causal model of clinical trial outcome in which the outcome was a function of five variables: baseline score, true treatment effect, average treatment expectancy, an interaction between differential treatment expectancy and treatment assignment (or, for placebo-controlled trials, treatment assignment guess and certainty), and residual variability. We set the effect sizes for each of these predictors to a range of plausible values and used Monte Carlo simulations to quantify the bias that would be embedded in treatment effect size estimates if

expectancy effects were not controlled for in analyses. We also quantified the impact on statistical power of measuring and adjusting for expectancy effects.

Results: The bias that expectancy effects of plausible magnitude introduced into treatment effect size estimates ranged from small to extremely large. Using models that controlled for expectancy effects reduced or eliminated this bias and had the additional benefit of increasing statistical power.

Conclusions: Our results suggest that when conducting comparative effectiveness trials, and placebo-controlled trials where there is a risk of functional unblinding, it may be appropriate to measure treatment expectancies and calculate both expectancy-unadjusted and expectancy-adjusted treatment effect sizes.

Keywords: Psychedelics, Ketamine, Comparative effectiveness, Clinical Trial Methodology, Expectancy

Disclosure: Nothing to disclose.

P111. Higher Blood Pressure is Associated With Lower Cerebral Blood Flow in Youth With and Without Bipolar Disorder

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Background: Background: Previous research has shown that youth with bipolar disorder (BD) exhibit higher blood pressure (BP). Additionally, in youth with BD, higher BP is associated with anomalous brain structure and poorer neurocognitive performance. While it has been demonstrated that higher BP is generally associated with lower cerebral blood flow (CBF) in adults, there is a notable gap in research relating to BP-CBF associations among youth, particularly in psychiatric populations. Thus, we set out to examine the BP-CBF association in youth with BD. We hypothesized that higher BP would be associated with lower CBF in youth irrespective of psychiatric diagnosis, and that there would be a significant interaction whereby higher BP would be associated with lower CBF to a greater extent in youth with BD.

Methods: Methods: The study included 144 youth aged 13–20 years ($n = 76$ BD, $n = 68$ controls). High-resolution T1-weighted and pseudo-continuous arterial spin labeling (PC-ASL) magnetic resonance images (MRI) were acquired. Resting BP measurements, including diastolic (DBP) and systolic (SBP) were collected twice, 10 mins apart in a standard fashion, and the mean scores were used in the analyses. We assessed the association of SBP and DBP with CBF in the overall sample, BD group, and control group in regions of interest (ROIs) which included the anterior cingulate cortex (ACC), prefrontal cortex (PFC), and insular cortex. A BP-by-diagnosis interaction effect was also assessed in the overall sample. ROI analyses were complemented by an exploratory whole-brain voxel-wise analysis. All analyses controlled for age and sex. Sensitivity analyses controlled for psychiatric medications (i.e., lithium, second-generation antipsychotics, and any medication), body mass index, and psychiatric symptom severity (i.e., depression and mania).

Results: Results: In ROI analyses, higher DBP was associated with lower CBF in the ACC ($\beta = -0.21$, $pFDR = 0.045$), insular cortex ($\beta = -0.25$, $pFDR = 0.016$), and PFC ($\beta = -0.27$, $pFDR = 0.009$) in the overall sample. Additionally, higher SBP was associated with lower CBF in the PFC ($\beta = -0.23$, $pFDR = 0.045$) in the overall sample. Within the BD group, higher DBP was significantly associated with lower CBF in the ACC ($\beta = -0.32$, $pFDR = 0.004$), insular cortex ($\beta = -0.4$, $pFDR < 0.001$), and PFC ($\beta = -0.39$, $pFDR < 0.001$); and higher SBP was significantly associated with lower CBF in the ACC ($\beta = -0.26$, $pFDR = 0.021$), insular cortex ($\beta = -0.24$, $pFDR = 0.024$), and

PFC ($\beta = -0.29$, $pFDR=0.01$). There were no significant interaction effects or BP-CBF associations in the control group.

Voxel-wise analysis across the entire sample and within the BD and control groups found similar associations. Notable patterns were that DBP was more diffusely associated with CBF than SBP, and that both SBP and DBP were more diffusely associated with CBF in the BD group than control group. Additionally, there was a significant DBP-by-diagnosis interaction effect whereby higher DBP was associated with lower PFC CBF to a significantly greater extent in youth with BD vs the control group.

Conclusions: Conclusion: Higher BP in youth is associated with lower regional CBF. The magnitude of the effects and the anatomical diffuseness of the findings were more pronounced among youth with BD overall, and there was some evidence for significant interaction effects. Further research is necessary to determine the direction of the observed associations and to understand the underlying mechanisms. Present findings also highlight the potential value of BP-lowering treatment as an approach to optimizing CBF.

Keywords: Bipolar Disorder, blood pressure, youth, Cerebral Blood Flow

Disclosure: Nothing to disclose.

P112. A Cross-Sectional Analysis of Accelerated Cellular Aging and Polygenic Risk for Bipolar Disorder Among ADHD Youth With and Without Familial Risk for Bipolar I Disorder

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Background: Bipolar I disorder (BD) is a highly heritable illness, with genetic factors explaining 60-85% of risk variance. Having a first-degree relative with BD robustly increases the risk for developing BD as well as other psychiatric disorders including attention-deficit/hyperactivity disorder (ADHD). Moreover, BD and ADHD have overlapping genetic liability, and the prevalence rate of ADHD in youth with a BD family history are significantly higher than youth without a BD family history. Large genome wide association studies (GWAS) have confirmed that BD liability is polygenic, with collectively single nucleotide polymorphisms (SNPs) identified to date still accounting for only 25% of the estimated heritability. BD polygenic risk scores (BD-PRS) are a weighted aggregate of multiple BD risk alleles derived from GWAS meta-analyses. Individuals with PRS at the extremes of a population distribution have substantially higher risk of developing BD, though the predictive capacity of PRS is limited by inadequate sensitivity and specificity, likely reflecting dynamic gene-environment interactions. Candidate environmental risk factors include prenatal infections and childhood psychological stress and maltreatment have a small impact on BD risk and are not disease specific. Nevertheless, environmental factors can mechanistically impact gene expression through epigenetic alterations including DNA methylation (DNAm), though to date there have been no large epigenome wide studies in BD. An alternative to identifying specific DNAm differences is to study an epigenetic signature that is associated with a phenotype present in BD including accelerated cellular aging (ACA). A growing body of evidence suggests individuals with BD exhibit several indices of accelerated aging including premature disability and mortality in conjunction with biomarkers of cellular aging and cellular resilience deficits. ACA has been studied in patients with BD using telomere length and mitochondrial DNA copy number. Another method to estimate ACA is based on patterns of changes in DNAm levels of specific sites in the genome. DNAm is modified

with age and these changes in DNAm have been validated as biomarkers of aging in several tissues including peripheral blood. Although accelerated epigenetic cellular aging has been identified in BD, it has not been assessed in conjunction with PRS or as a predictor of BD risk progression. In the present study, we explored the association between accelerated epigenetic cellular aging and familial risk for BD in a sample of ADHD youth with a first-degree relative with BD (high-risk, HR). Additionally, we explored the relationship between genetic liability to BD as indexed by the PRS and accelerated epigenetic cellular aging. Furthermore, we compare these associations between the HR group and youth with ADHD without a family history of BD (low-risk, LR), and a healthy comparison subjects with no family history of BD (HC).

Methods: Subjects aged 10-18 years were recruited at the University of Cincinnati. All subjects/parents were evaluated with Kiddie Schedule for Affective Disorders and Schizophrenia, and the Family Interview for Genetic Studies with the Structured Clinical Interview for DSM-5 on their first-degree relatives to assess for BD diagnosis. DNA was extracted from whole blood and genotyped using the Infinium Global Screening Arrays with PsychBooster. DNAm was assessed on Infinium 850k MethylationEPIC BeadChip using standard procedures. PRSs for BD, schizophrenia, major depressive disorder, and ADHD were constructed using PRS-CSx. Markers of cellular aging included mitochondrial DNA copy number (mtDNAcn) and two methylation-based epigenetic clocks, AgeAccelGrim and Dunedin PACE. Data were analyzed using linear mixed models adjusting for age, sex, and ethnicity.

Results: A total of 144 youth (mean age: 14.7 ± 2.6 , female $n = 53$; HC $n = 42$, LR $n = 43$, HR, $n = 59$) were included in the analysis. There were no significant group differences in demographic variables. HR youth displayed lower mtDNAcn and accelerated epigenetic aging in both epigenetic clocks compared with both LR ($p < 0.001$ and $p < 0.05$, respectively) and HC ($p < 0.01$ and $p < 0.05$, respectively) youth, and no differences were found between LR and HC (both $p > 0.05$) youth. PRS differences between groups were identified for BD score, ADHD, and schizophrenia. HR youth had higher PRS for BD ($p < 0.05$) and there was a trend for schizophrenia PRS ($p = 0.07$) compared to both HC and LR groups. Both HR and LR had higher ADHD PRS compared to HC ($p < 0.05$). Among HR youth, higher PRS was correlated with lower mtDNAcn and accelerated epigenetic cellular age.

Conclusions: ADHD youth with familial risk for BD exhibit ACA, as indexed by lower mtDNAcn and accelerated epigenetic clocks, and a higher BD-PRS compared with ADHD youth without familial risk for BD and healthy youth. Future longitudinal studies are warranted to determine if ACA markers in combined with BD-PRS can serve as reliable predictors of BD risk trajectories in youth with familial risk for BD.

Keywords: Bipolar I disorder, Epigenetic clock, Polygenic risk score

Disclosure: Nothing to disclose.

P113. Distinct Patterns of Reward Expectancy-Related Left Ventrolateral Prefrontal Cortical Functional Connectivity are Associated With Mania and Depression Risk in a Young Adult Sample

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Background: Mania/hypomania is the pathognomonic feature of bipolar disorder (BD), and is characterized by elevated reward

sensitivity, goal overvaluation, impulsivity and sensation seeking. Unfortunately, BD is often misdiagnosed as major depressive disorder (MDD). Replicable neural markers of mania/hypomania risk are needed for earlier BD diagnosis and pathophysiologically based treatment development. We previously showed that reward expectancy (RE) related left ventrolateral prefrontal cortex (L-vlPFC) activity is associated with higher mania risk, with this relationship being specific to mania, and not common to depression, risk. The L-vlPFC is a key reward and salience neural network region. While elevated L-vlPFC activity to RE therefore suggests elevated attention to potential reward, it is unknown whether this pattern of elevated activity is accompanied by elevated functional connectivity (FC) with regions in reward and salience neural networks. We aimed to determine whether higher mania risk was associated with elevated L-vlPFC-reward and salience (and other neural) network FC, and the next to which these patterns of FC were specific to mania, rather than common to depression, risk.

Methods: 87 (32 male and 55 females; age=23.4 ± 2.96) individuals without BD completed an RE task during 3TfMRI. Using Psychophysiological Interaction (PPI), we first identified patterns of L-vlPFC (seed region)-wholebrain FC (target regions), including positive and inverse FC. The L-vlPFC seed region was derived from an activation likelihood estimation meta-analysis of studies showing elevated reward-related activity in this region. Age, sex at birth, education and mean framewise displacement (FWD) were covariates. We then extracted FC parameter estimates from target clusters showing significant FC with the L-vlPFC ($p < 0.001$, $k > 20$). We next ran two parallel Poisson loglinear regression models for mania and depression risk, using the MOODS-SR-Lifetime (MOODS-SR-L) manic or depressive domain scores, respectively, as the dependent variable in each model. Extracted parameters estimates of significant L-vlPFC-wholebrain FC were the independent variables. Age, sex at birth, education and mean FWD were covariates in both models. MOODS-SR-L depressive domain score was an additional covariate in the mania risk model, and MOODS-SR-L manic domain score, in the depression risk model.

Results: There were significant positive correlations between mania risk and: L-vlPFC-L-visual association cortex positive FC (0.310; $qFDR < 0.001$), L-vlPFC-R-supramarginal cortex positive FC (0.143; $qFDR = 0.034$), and L-vlPFC -R-dorsal anterior cingulate cortex (dACC) inverse FC (0.336; $qFDR = 0.02$). There was a negative correlation between mania risk and: L-vlPFC-R-pre-and-supplementary motor area inverse FC (-0.447; $qFDR = 0.012$). There were significant positive correlations between depression risk and: L-vlPFC-R-primary sensory cortex positive FC (0.137; $qFDR = 0.021$), L-vlPFC-R-fusiform gyrus positive FC (0.290; $qFDR < 0.001$), L-vlPFC-L-precuneus positive FC (0.122; $qFDR < 0.001$), L-supratemporal gyrus positive FC (0.102; $qFDR = 0.040$), L-vlPFC-L-inferior temporal cortex inverse FC (0.263; $qFDR = 0.032$), and L-vlPFC-R-pre-and-supplementary motor area inverse FC (0.383; $qFDR < 0.001$). There was a negative correlation between depression risk and: L-vlPFC-R-primary sensory cortex positive FC (-0.168; $qFDR = 0.047$), L-vlPFC-L-primary sensory cortex positive FC (-0.402; $qFDR < 0.001$), and L-vlPFC-R-dACC inverse FC (-0.373; $qFDR < 0.001$) and LvlPFC-L-cerebellar hemisphere inverse FC (-0.285; $qFDR < 0.001$).

Conclusions: We show distinct patterns of RE-related L-vlPFC FC associated with mania and depression risk. Notably, higher mania risk is associated with greater, while higher depression risk is associated with lower, inverse L-vlPFC-dACC FC. Given the role of the dACC in action preparation and other regulatory processes, our findings indicate that mania, but not depression, risk is characterized by reduced integration of regulatory networks and the L-vlPFC. Additionally, higher mania risk is associated with lower, while higher depression risk is associated with greater, L-vlPFC-pre-and-supplementary motor cortex inverse FC, suggesting that depression, but not mania, risk is associated with reduced

functional integration between motor cortical networks and the L-vlPFC. Higher mania and depression risk are also both associated with greater L-vlPFC positive FC with regions in the default mode and visuospatial processing networks, subserving self-referential and visuospatial processing. Our findings increase understanding of mania risk-specific pathophysiological processes centered on salience, regulatory, motor and sensory neural networks, and provide new, targetable neural markers of mania risk.

Keywords: Bipolar Disorder, mood disorders, mania, Depression, neural marker

Disclosure: Nothing to disclose.

P114. The Endocannabinoid System in Bipolar Disorder: Investigations of Genetic Evidence and Functional Consequences

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Background: The endocannabinoid (eCB) system is comprised of lipid signaling molecules, enzymatic machinery for biosynthesis/degradation, and their G-protein coupled receptors. The most well known eCB molecules 2-arachidonoylglycerol (2-AG) and anandamide (AEA) have been shown to play a role in a number of physiological processes, including mood, hunger, sleep and more. eCB signaling also plays a role in neuroplasticity, and is an important modulator of other key neurotransmitter pathways. Targeting eCB signaling to improve psychiatric disorders remains a tantalizing prospect, and interest in the area has resurged with the development of eCB synthesis and degradation inhibitors that have been demonstrated to be safe in clinical trials.

The present study aim is to investigate genetic alterations to the proteins involved in the production of eCB neurotransmitters in bipolar disorder (BD), and the functional consequences of these genetic variants. BD is a mood disorder which is characterized by drastic swings from states of low energy (depression) to high energy (mania/hypomania), with detrimental quality of life impacts for patients. The lifetime prevalence is ~ 4.4% in the USA. While treatment options are available, these medications often come with side effects, and their mechanism of action with regards to BD is unclear. Furthering our understanding of the underlying pathophysiology of BD expands the options for the development of new therapies which remains an important priority.

Methods: Using summary statistics of BD types I (BD-I, $n = 60$ 783) and II (BD-II, $n = 21$ 154) from the Psychiatric Genomics Consortium BD wave 3 GWAS, gene-based analyses were conducted using MAGMA software. Whole genome sequencing from healthy controls and BD patient samples used for inducible pluripotent stem cell (iPSC) generation ($n = 3$ for each) was used to screen for variants in genes of the endocannabinoid system common to the BD patients for which iPSCs were generated. Small molecule protocols were used to differentiate patient-derived iPSCs into dopaminergic, striatal and cortical neurons. The generated neurons were characterized by immunofluorescent staining for neuronal markers and measurements of electrical activity using multi-electrode arrays. Gene expression of eCB-related genes and other genes related to dopaminergic and GPCR signaling pathways were conducted using RT-qPCR. Further quantification of DAGLA was conducted by immunofluorescence. An ELISA was used to measure 2-AG levels in cell culture media of neurons. Mitochondrial calcium buffering in response to 2-AG was measured using mitochondrial calcium indicator dye Rhod-2AM. An unpaired two-tailed t test was used to compare immunofluorescent quantification of DAGLA positive cells, 2-AG levels

from ELISA assay, and changes in Rhod-2AM fluorescence intensity. Welch's t test was used to compare gene expression levels of eCB-related genes. Cohen's d score and Hedges g score were used to measure effect sizes.

Results: Whole genome sequencing data revealed an abundance of common variants in genes involved with 2-AG activity in the 3 patient-derived iPSC lines. GWAS summary statistics for BD-I and BD-II suggested that DAGLA is associated with BD-I ($p = 8.56e-6$), but not BD-II ($p = 0.23$). Immunofluorescent quantification of DAGLA expression in cortical neurons derived from $n = 3$ HC and BD patients revealed no changes in BD, and gene expression revealed no differences in DAGLA expression in dopaminergic neurons, while CB1 receptor mRNA was significantly decreased in BD dopaminergic neurons ($g = 1.054$, $p < 0.05$). ELISA results indicated an elevation of 2-AG levels in dopaminergic neurons ($d = 1.055$, $p < 0.05$) derived from BD patient iPSCs compared to HC. In cortical neurons, calcium buffering in response to 2-AG stimulation differed in HC and BD patients, whereby 2-AG dependent increases in calcium in response to 200 nM 2-AG were blocked by pre-treatment with CB1 antagonist rimonabant in HC ($g = 1.13$, $p < 0.05$), while there was no change in calcium buffering in mitochondria in BD neurons.

Conclusions: Genetic evidence from PGC-BD GWAS indicates that there may be a contribution of DAGLA variants toward the BD phenotype. Results in BD patient iPSC-derived neurons indicate that there is an increase in 2-AG, which does not appear to be a result of changes in DAGLA expression. This increase in 2-AG levels is accompanied by reductions in CB1 expression and changes to mitochondrial responses to 2-AG in BD samples. 2-AG neurotransmission appears to be altered in BD, however further mechanistic studies are underway to elucidate the cause of increased 2-AG levels and further downstream effects. The identification of a defined 2-AG phenotype in BD will be followed by sex-specific genetic and patient-derived iPSC studies investigating if these changes are predominant in males or females.

Keywords: Bipolar Disorder, endocannabinoid system, Induced pluripotent stem cells (iPSCs)

Disclosure: Nothing to disclose.

P115. Continuity of Pediatric Bipolar Disorder Into Middle Adulthood and Childhood Symptoms Predictive of Adult Mood Episodes

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Background: Pediatric bipolar disorder (PBD) has been the topic of considerable research and controversy, driven by uncertainty about continuity with the adult disorder. Geller et al., followed children with mania defined by elation and/or grandiosity but often characterized by rapid-cycling. Here, we present the adult outcomes of those children and assess which childhood onset symptoms predict having mood episodes in adulthood.

Methods: The "Phenomenology and Course of Pediatric Bipolar Disorders" launched in 1995, with 268 participants: 93 (36F) PBD, 81 (17F) ADHD, and 94 (36F) Healthy Controls (HC), ages 7-16. In 2020-2022 142 participants, 53 PBD (20F), 22 ADHD (5F), 67 HC (31F), participated in an adult follow-up study. Adult diagnoses were obtained via the Structured Clinical Interview for DSM5. Rates of adult diagnoses were compared across pediatric diagnostic groups using chi-square tests and logistic regressions were used to assess the impact of each of the cardinal symptoms of mania in childhood (assessed using the WASHU K-SADS) on presence of mania in adulthood.

Results: At 25-year follow-up, PBD participants were more likely to have an episode of mania/hypomania after age 18 (27.8% vs 3.2%, $\chi^2 = 9.13$, FDR-corrected $p = 0.004$) and/or depressive episode(s) (72.2% vs 38.7%, $\chi^2 = 9.70$, FDR-corrected $p = 0.004$). The baseline severity of all sleep-related (insomnia, hypersomnia, and decreased need for sleep) as well as the severity of all cardinal symptoms (elation, grandiosity, hypersexuality, racing thoughts / flight of ideas, and impulsivity) were significantly associated with having mania in adulthood, though only grandiosity remained significant when also co-varying for presence of PBD ($\chi^2 = 7.74$, FDR-corrected $p = 0.043$). Interestingly, only baseline elation ($\chi^2 = 6.44$, FDR-corrected $p = 0.045$) and impulsivity ($\chi^2 = 11.17$, FDR-corrected $p = 0.006$) were significantly associated with depression in adulthood.

Conclusions: 28% of children with PBD defined using cardinal symptoms of mania continue to have episodes of mania in adulthood, while 72% continue to have episodes of depression. The severity of all of the "cardinal symptoms" of mania predicted mania in adulthood, though only grandiosity predicted above and beyond having a PBD diagnosis. Findings have implications for continuity of symptoms of mood dysregulation in childhood into adulthood and suggest key symptoms that may be markers of risk for later mood episodes.

Keywords: Pediatric Bipolar Disorder, longitudinal studies, Clinical Stability

Disclosure: Nothing to disclose.

P116. Efficacy and Safety of Non-Racemic Amisulpride in Bipolar Depression - The Prospective Enrichment for Canonical Symptom Structure at Baseline Increased Study Power at Endpoint

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Background: Nonracemic amisulpride (SEP-4199) is an investigational 85:15 ratio of amisulpride to esamisulpride. The 85:15 ratio was discovered in human clinical studies to enhance 5HT_{7R}-mediated antidepressant effects while retaining some D_{2R} antipsychotic benefits. In a Phase 2 study of SEP-4199 for the treatment of bipolar depression (Study -201 = NCT03543410) improvements in depression severity was observed in the Intent-To-Treat (ITT) population although the pre-specified primary analysis, conducted on a smaller analysis population than ITT, was not statistically separated from the large changes also seen in the placebo treatment group. In a retrospective analysis of Study -201, subjects having a canonical MADRS symptom structure at baseline demonstrated greater effect sizes post-baseline, suggesting that anomalous symptom presentation at baseline interferes with the confidence of the MADRS baseline assessments and the reliability of subsequent changes in total score (primary outcome). Here we describe the outcome of a Phase 3 pivotal study (Study 301 = NCT05169710) that randomized only subjects having a canonical MADRS symptom structure as an a priori enrichment strategy to reduce non-drug related variability.

Methods: Study -301 implemented an enrichment algorithm to identify canonical versus anomalous symptom presentations, at the individual subject level, based on MADRS ratings obtained at screening and baseline (Hopkins et al, 2024). Patients meeting DSM-5 criteria for bipolar I depression were randomized to 6 weeks of double-blind, placebo-controlled treatment with SEP-4199 Controlled Release (CR) 200 mg/d or 400 mg/d. The primary endpoint was change from baseline in MADRS at Week 6. The

planned sample size was $N = 522$. The study was terminated at $N = 83$ randomized subjects.

Results: Of $N = 275$ subjects screened, a total of $N = 129$ subjects were assessed with MADRS at screening and baseline. Of these, $N = 83$ subjects (64%) met the additional criteria of canonical symptom structure and were randomized. The $N = 46$ subjects with anomalous symptom structure were excluded from further participation. At baseline, the psychometric properties of the 10 items of MADRS were substantially improved in the group of patients with canonical symptom structure, compared to anomalous (fits of a MADRS 4-factor model, factor loadings coefficients, item characteristic curves, and internal consistency). Of the $N = 83$ subjects randomized, $N = 27$, 28, and 28 were randomized to placebo, 200 mg/d CR, and 400 mg/d CR, respectively. One randomized subject to 200 mg/d did not take any study drug and was excluded from the ITT population. In the primary analysis of efficacy, both dose groups show a numerical improvement in MADRS versus the placebo that was statistically significant for the 400mg/d ($p = 0.0286$) based on MMRM analysis. The LSMean (SE) changes in MADRS total score from baseline to endpoint at Week 6 were -10.33 (2.004) for placebo, -14.27 (2.055) for 200 mg/d, -16.67 (2.014) for 400 mg/d, and -15.47 (1.441) for the combined doses. The effect sizes of drug vs placebo separation were 0.39 and 0.62 at Week 6 endpoint for the 200 mg/d CR and 400 mg/d CR, respectively, and 0.51 for the combined doses. Adverse events were reported for 40.7% of placebo subjects versus 37.0% and 39.3% of 200 mg/d CR and 400 mg/d CR subjects, respectively. Adverse events reported in $\geq 5\%$ of subjects were electrocardiogram QT prolonged (0% placebo, 5.5% in SEP-4199), somnolence (7.4% placebo, 3.6% in SEP-4199), nausea (7.4% placebo, 3.6% in SEP-4199), and headache (11.1% placebo, 0% SEP-4199).

Conclusions: These results replicate and confirm the initial findings in the Phase 2 study of non-racemic amisulpride CR in the treatment of bipolar depression. Although the study was terminated prior to the planned sample size, the large effect sizes demonstrated statistical significance in the primary analysis. Taken together, the results of Study -201 and -301 demonstrate that selecting study participants with canonical MADRS symptom structure increases study power by reducing non-drug related heterogeneity at baseline.

Keywords: machine learning, Bipolar I Depression, Clinical Development, inclusion and exclusion

Disclosure: Nothing to disclose.

P117. Neural Hyperactivity during a Team-Based Task is Associated With Elevated Social Approach Motivation in Bipolar Disorder

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Background: Individuals with bipolar disorder demonstrate elevated reward responsivity and preference for high-risk, high-reward decision-making, even in euthymic states. While potentially adaptive in certain contexts, these patterns of behavior also reflect risk for mania in these individuals. There is also evidence that vulnerability to manic experiences in individuals with bipolar disorder is associated with heightened approach motivation, even in situations involving threat or aversive outcomes. These patterns of altered reward processing and approach motivation tendencies are thought to be related to emotion dysregulation and risky behavior characteristic of the disorder. Whether these alterations

extend to the social domain has not yet been established. Altered social reward processing is a potential mechanism underlying social dysfunction in the disorder and may be reflected in individual differences in social approach and social avoidance motivation tendencies.

Methods: 27 individuals with bipolar disorder (BD; 17 male, 10 female) and 41 nonpsychiatric comparison participants (NCs; 30 male, 11 female) completed a team-based dot counting task during fMRI. After each trial, performance feedback (won/lost) was provided by a teammate or opponent. The teammates' faces were happy for wins and angry for losses (i.e., congruent with trial outcome), whereas opponents' faces were angry for wins and happy for losses (i.e., incongruent with trial outcome). Voxel-wise whole-brain analysis of BOLD activity (cluster threshold $Z > 2.3$, $p < .05$ corrected for multiple comparisons) during the feedback period was analyzed for within- and between-group effects and for correlations with self-report measures of social motivation and clinical symptoms.

Results: Individuals with BD demonstrated increased BOLD activity compared to NCs when feedback was provided by opponents versus teammates in dorsal anterior cingulate cortex (Brodmann Area, BA 32), right middle frontal gyrus/frontal pole (BA 46), and right supramarginal gyrus/angular gyrus (BA 39/40). Further, in BD participants activity within the right middle frontal gyrus/frontal pole cluster was positively correlated with self-reported social approach motivation (Affiliative Tendency scale score; $r = .441$, $p = .024$) and negatively correlated with symptoms of depression (Hamilton Depression Rating Scale score; $r = -.429$, $p = .032$).

Conclusions: Individuals with BD showed hyperactivation in frontal and parietal regions in response to aversive or incongruent social feedback (i.e., from opponents), regardless of the actual trial outcome (won/lost). In addition, extending prior research, the hyperactivation in frontal regions was associated with excessive social approach motivation. While increased social approach motivation could reflect prosocial tendencies in support of social affiliation and connection, taken to extremes it could also reflect tendencies toward exaggerated self-esteem, emotion dysregulation, risk taking, and other elements of vulnerability to mania that negatively impact social relationships and social support. The current findings suggest that heightened processing of other-oriented social information is a potential neural mechanism underlying compromised social functioning frequently observed in BD.

Keywords: Bipolar Disorder, fMRI, Social Functioning, Approach/Avoidance, social motivation

Disclosure: Nothing to disclose.

P118. Impact of Polygenic Scores, Childhood Adversity and Gene-Environment Correlations on Bipolar Disorder Subphenotypes and Lithium Response

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Background: Bipolar disorder (BD) is a heritable psychiatric illness with diverse clinical presentations influenced by genetic and environmental factors, including childhood adversities. Polygenic scores (PGS) can quantitatively estimate genetic risk for BD and other psychiatric traits. While prior studies have partially explored the genetics of BD subphenotypes and lithium response, the interplay between PGS, childhood adversity, and these phenotypes remains unclear.

Methods: This cross-sectional study included participants from the Mayo Clinic Bipolar Disorder Biobank. Participants provided blood samples for genotyping and completed multiple questionnaires pertaining to demographic and clinical information. Childhood adversity was assessed using the Adverse Childhood Experiences (ACE) questionnaire. BD subphenotypes of interest were BD subtype (BD type I vs BD type II), early age of onset (≤ 19 years), history of psychosis, suicide attempt, and rapid cycling. Lithium response was measured by the Alda Scale. Potential gene-environment correlations between PGS for BD, schizophrenia (SCZ), major depressive disorder (MDD), anxiety disorder (ANX), attention-deficit/hyperactivity disorder (ADHD), and addiction factor (AF) with ACEs were tested using negative binomial regression. Logistic and linear regression models were used with each subphenotype and lithium response (Alda A score) as the outcome, respectively, to separately assess (1) PGS associations, (2) ACE associations, and (3) PGS associations after ACE adjustment. All models with PGS as a predictor were adjusted for genotyping batch and the top five genomic principal components of ancestry. Models examining lithium response as an outcome were also adjusted for the Alda B sum score. The p-value for significance was set at $0.05/6 = 0.008$ to account for multiple testing of six variables.

Results: The study sample consisted of 648 participants (female $n = 418$ [64.5%], mean age: 39.6 ± 14.7 years) with available ACE data. Lithium response data were available for only a subset of the participants ($n = 308$). Significant correlations were found between PGS and ACE scores for MDD PGS (incidence rate ratio [IRR] = 1.11, $p = 0.001$), ANX PGS (IRR = 1.10, $p = 0.003$), ADHD PGS (IRR = 1.19, $p = 2.9 \times 10^{-8}$), and AF PGS (IRR = 1.13, $p = 7.8 \times 10^{-5}$). Significant associations were identified between BD PGS and BD type I (OR = 1.32, $p = 0.002$), SCZ PGS and history of psychosis (OR = 1.42, $p = 0.003$), and ADHD PGS and rapid cycling (OR = 1.4, $p = 8.9 \times 10^{-4}$). Nominal associations were found between worse lithium response and MDD PGS (beta = -0.384, $p = 0.027$), ADHD PGS (beta = -0.426, $p = 0.018$), and AF PGS (beta = -0.435, $p = 0.010$). Higher ACE scores were significantly associated with early age of onset (OR = 1.18, $p = 2.2 \times 10^{-4}$), suicide attempt (OR = 1.23, $p = 1.2 \times 10^{-8}$), rapid cycling (OR = 1.29, $p = 9.4 \times 10^{-10}$) and worse lithium response (beta = -0.301, $p = 7.32 \times 10^{-6}$). Adjusting PGS associations for ACE scores attenuated the association between ADHD PGS and rapid cycling (OR = 1.25, $p = 0.036$) and diminished nominally significant associations between lithium response and MDD PGS (beta = -0.253, $p = 0.139$), ADHD PGS (beta = -0.197, $p = 0.285$), and AF PGS (beta = -0.254, $p = 0.135$).

Conclusions: The findings of this study provide strong support for the independent and overlapping effects of genetic risk and childhood adversity on various BD subphenotypes and lithium response. Notably, BD subtype and history of psychosis seem to be predominantly genetically driven, while early age of onset, suicide attempt, and rapid cycling were significantly associated with childhood adversities. The observed gene-environment correlations indicate that ACEs are not independent of the individual's genetic risk for certain psychiatric traits, especially for MDD, ADHD, and addiction factor. The association between ADHD PGS, ACEs, and rapid cycling warrants further exploration. This study underscores the value of incorporating both genetic and environmental factors in risk prediction and highlights the potential for personalized interventions through an integrated approach.

Keywords: Bipolar Disorder, Polygenic scores, Childhood Adversity, Clinical heterogeneity, Lithium response

Disclosure: Nothing to disclose.

P119. Preliminary Hemodynamic and Mitochondrial Findings in Bipolar Disorder Using Broadband Near-Infrared Spectroscopy

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Background: Both brain system dysfunction and mitochondrial abnormalities have been shown in bipolar disorder (BD); however, the specific mitochondrial abnormalities that underlie the pathophysiology of BD and whether they are linked to the brain dysfunction have not been elucidated. Broadband near-infrared spectroscopy (bNIRS) is a new non-invasive neuroimaging technology that can simultaneously provide measures of brain hemodynamics reflecting dysfunction and mitochondrial cytochrome c oxidase oxidation states. We are performing an ongoing study aimed to use bNIRS to investigate in vivo hemodynamic and mitochondrial measurements and compare them between BD and healthy control (HC) participants.

Methods: Preliminary data of this ongoing study include 6 participants with BD and 10 HCs, each performing a visual checkerboard task with varying right or left visual field presentation. Occipital cortex measures were performed using a 16-channel bNIRS device. Data preprocessing included motion wavelet correction and bandpass filtering of raw optical data. Then, the optical density changes were converted into changes in oxyhemoglobin (HBO), deoxyhemoglobin (HBR), and oxidative cytochrome-c-oxidase (oxCCO) concentrations using modified Beer-Lambert law. Right and left checkerboard responses were extracted using the general linear model approach, followed by calculating grand averaged responses. Statistical significance between the BD and HC groups in peak values of changes was evaluated using a two-tailed independent t-test with a 95% confidence interval. Secondary analyses were performed to explore the association in significant responses for BD and HC using Pearson's correlation.

Results: Peak values of HBR were lower and oxCCO were significantly higher in BD group than in HC group ($P < 0.05$). There was also a significant negative correlation between oxCCO and HBR values in the BD group ($r = -0.71$, $P = 0.001$).

Conclusions: Preliminary findings from this ongoing study provided initial evidence that bNIRS measures can be used to show both differences between BD and HC in hemodynamic responses and mitochondrial cytochrome-c-oxidation states, as well as associations between the simultaneously collected measures. The preliminary results suggest that individuals with BD, compared to HCs, had higher changes in cytochrome-c-oxidation states implicating cytochrome-c-oxidation pathology in the disorder. The BD individuals also had hemodynamic responses that correlated negatively with the changes in cytochrome-c-oxidation measures suggesting a relationship between the hemodynamics and oxidation states. However, due to the small sample size, these conclusions are provisional. Moving forward, expanding the participant pool will be crucial to confirm these findings and solidify our understanding of cytochrome-c-oxidation abnormalities and their association to brain dysfunction in individuals with BD.

Keywords: Bipolar Disorder, Human Neuroimaging, Mitochondria

Disclosure: Nothing to disclose.

P120. Allelic Analysis of Single Nucleotide Polymorphisms in the Interleukin 6 (IL 6) Gene May Predict Lower Depression Severity in Bipolar Disorder

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Background: The activation of the immune system and the inflammatory response have increasingly been linked to the underlying mechanisms of psychiatric disorders. Both phases of

bipolar disorder have shown activation of the innate immune system and elevated levels of pro-inflammatory cytokines, though findings have been inconsistent. Elevated levels of the pro-inflammatory cytokine IL-6 have been associated with depressive and anxiety symptoms in several studies and may be linked to symptom severity and poor treatment outcomes. Additionally, single nucleotide polymorphisms (SNPs) have been found to influence IL-6 expression, potentially serving as a risk factor for both depression and mania. We analyzed IL-6 SNP rs1800795, and specifically, its designation of carrier status for its alleles: C and G. By determining, which is the risk allele, we can further stratify genetic data of particular SNPs and their related alleles and determine the pathophysiology and treatment of bipolar depressive disorder.

Methods: This was a 10-week, double-blind, randomized, placebo-controlled trial; 43 patients diagnosed with TRBD and meeting criteria for bipolar I or II received escitalopram (ESC) with placebo (PBO) or ESC with celecoxib (CBX). Blood samples were measured at baseline and week 8. Details of the design and clinical findings were published previously (Halaris et al., 2020). Plasma IL-6 levels were determined by ELISA. Genome-wide genotyping was performed using the Infinium Multi-Ethnic Global-8 v1.0 Kit. After searching the literature and running the SNPs associated with bipolar disorder through a GWAS database of the study's patients, two SNPs were identified: rs1800795 and rs1800796, located on the promoter of the IL 6 gene on chromosome 7 and thus influencing its expression. We focused on IL6 rs1800795 as our patient cohort represented all three following genotypes with the C and G alleles: CC, CG, and GG. The allele frequencies of C versus G vary in different ethnic populations. Data were analyzed by way of multiple regression and analysis of covariance.

Results: Due to the small size of our study, we did not subgroup the subject population by demographics or BMI, but primarily sought to detect patterns which may/not have reached statistical significance. IL6 rs1800795 was statistically analyzed by changing the carrier status of its alleles. We first designated allele C as the carrier or risk allele. At baseline, mean IL-6 values were significantly elevated in the TRBD group ($p = 0.007$) compared to Healthy Controls, as we reported previously. The n-value for the carriers (CC and CG) was 35, and the n for non-carriers was GG. The mean IL-6 baseline blood levels (ng/ml) for C-allele carriers and non-carriers were 1.52 and 1.48, respectively, with SEs of 0.24 and 0.17. However, for G-carriers, the mean was notably higher at 1.82 versus 1.21 for non-carriers. The lower and upper bounds for non-carriers was 0.92 to 1.49, versus for carriers it was 1.02 to 2.63. This demonstrates that the G-allele may have some impact on the amount of IL-6 produced, as the significance of IL6 baseline blood levels for the G-stratification had a significance of 0.135, while for the C-carriers the significance was 0.938. The week 8 blood levels for the C-carriers and non-carriers were also similar, at 1.41 and 1.43 respectively. For carriers with G denoted as the risk allele (GG and CG), the mean IL-6 baseline blood levels were 1.58 and 1.25 for CC and GC, respectively, with standard errors of 0.23 and 0.21.

The pre-treatment HAMD level for both C-and G-allele carriers and non-carriers were all approximately 22. When analyzing the ESC plus PBO treatment arm for the C-allele carriers ($n = 17$), the mean was 12.18, while for non-carriers ($n = 2$) it was 15. The ESC plus CBX treatment arm, which for carriers ($n = 18$) was 8.39, while for non-carriers ($n = 5$) it was 7.2. The n-values were more evenly distributed when the G allele was designated as the carrier allele. The ESC plus PBO treatment arm for the G-carrier mean was 11.56, while for the non-carrier was 13.3, with a p-value of 0.54. The ESC plus CBX means for carriers and non-carriers were 6.92 and 9.45, respectively, with a p-value of 0.29.

Conclusions: At baseline, IL-6 levels did not significantly correlate with different designations of carrier and non-carrier status. While not statistically significant, there was a notable

finding that those with the G-allele carrier status had persistently lower HAMD scores. This was especially notable in the treatment group with CBX add-on, as well as in the baseline and 8-week IL6 blood levels. Also, we see a HAMD score drop from 12.18 to 8.39 of the C-carriers versus the non-carriers (in this instance, GG) having a much larger drop of 15 to 7.2. Because this is a C- > G polymorphism, the smaller n-value for GG is representative of Caucasian populations, where the G allele is much less common. In African and Asian populations, however, the G allele is prevailing. Because our subject population was primarily Caucasian, these allele frequencies follow this pattern. The HAMD scores were significantly lower in those with the G allele in rs1800795. To gain more definitive insights, a significantly larger sample size would be required for such analyses. However, these initial findings should encourage further research involving a broader patient population with greater ethnic diversity.

Keywords: Bipolar Disorder, SNPs, IL-6, Celecoxib

Disclosure: Nothing to disclose.

P121. Impulsivity and Emotional Regulation Behavioral Traits Mediate Relationships Between Neural Activity and Lifetime Risk During an Emotional N-Back Task

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Background: We previously demonstrated that greater precuneus activation during working memory (WM) may be a neural marker for increased lifetime depression risk, and greater dorsolateral prefrontal (dlPFC) cortex activation during emotional regulation (ER) may be a neural marker for both increased lifetime depression and mania risk. However, the extent to which behavioral traits mediate between these relationships has yet to be studied. We examined this using trait impulsivity and emotional regulation scales as mediators in two independent cohorts of young adults at risk for bipolar disorder.

Methods: Discovery sample comprised 101 young adults aged 23.54 (2.85), 63 female; validation sample comprised 96 young adults aged 21.56(sd2.10), 65 female. A causal mediation analysis was performed using the R package 'mediation'. Features of lifetime depression and mania risk derived from the Mood Spectrum-Self Report-Lifetime (MOOD_SR_L) were assessed at baseline and used as our dependent variables. Independent variables were brain regions with significant BOLD activity relationships with lifetime depression and/or mania risk following regression analyses: right precuneus during WM; left dlPFC during ER. Mediators in our model were the Urgency-Premeditation-Perseverance-Sensation Seeking-Positive Urgency (UPPSP) impulsive behavior scale, and the Difficulties in Emotion Regulation Scale (DERS).

Results: During WM depression, UPPSP-negative ($B = 0.117, B = 0.300, p < .001$) and DERS ($B = 0.043, B = 0.132, p < .001$) displayed full mediation in both samples. During ER depression, UPPSP-negative ($B = 0.653, B = 10.713, p < .001$) and UPPSP-positive ($B = 0.674, B = 9.011, p < .001$) displayed full mediation in both samples. During ER mania, UPPSP-negative displayed full mediation in both samples ($B = 0.529, B = 7.509, p < .001$).

Conclusions: Relationships between neural regions and lifetime depression and mania risk during WM and ER may be mediated by differential behavioral traits. Increased precuneus activity during WM is suggestive of interfering ruminative processes. Increased negative urgency (impulsivity) and reduced emotional regulation ability have both been associated with rumination and may

underlie the mechanism by which ruminative neural activity during WM is associated with increased lifetime depression risk. Increased dIPFC activity during WM is suggestive of overcompensation of the CEN. Both ER and impulsivity have been associated with depression, and our present findings suggest that impulsivity may underlie the mechanism by which over compensatory CEN neural activity during ER is associated with increased lifetime depression risk. Negative urgency may also underlie the mechanism by which over compensatory CEN neural activity during ER is associated with increased lifetime mania risk. These results are replicated in two independent samples of young adults at risk for bipolar disorder.

Keywords: Bipolar Disorder, fMRI, Early identification of risk, mediation analyses

Disclosure: Nothing to disclose.

P122. Brain Energy Metabolism Changes in Veterans With Bipolar Disorder: Preliminary Findings

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Background: Veterans diagnosed with bipolar disorder (BD) are at significant risk for suicide, with research indicating approximately 82.3 % of veterans with BD reporting a lifetime history of suicidal ideations and/or behaviors. Our previous study demonstrated both alterations in brain bioenergetics and differences in suicidal ideation intensity in Veterans diagnosed with BD living at moderate compared to low altitude (Hwang et al., 2019). In the current study, we aimed to explore the real-time response to acute hypoxia and hyperoxia by comparing 31P-MRS brain chemistry metabolites (PCr and ATP) at simulated sea level vs. 10,000 feet of altitude in veterans with BD and healthy control veterans (HC). We hypothesized that coping with decreased energy production because of hypoxia may be less efficient in veterans with BD relative to HC.

Methods: This cross-sectional imaging study included adult US veterans (18-65 years of age) with BD and HC veterans. During a single 31P-MRS scan session, participants were scanned under hyperoxic (high oxygen) and hypoxic conditions (24% and 16% FiO₂) using the Hypoxico Altitude Training System (ATS). Frontal brain regions including the anterior cingulate cortex (ACC) and prefrontal cortex were examined for hypoxia-associated changes in phosphocreatine (PCr) and β -adenosine triphosphate (ATP). 31P-MRS results were compared across oxygen conditions and diagnostic groups. Linear Mixed Models were fit to assess the impact of group and oxygen conditions on high-energy metabolites.

Results: A total of 43 veterans were included (13 BD and 30 HC; mean age 42.05, SD = 11.14, 86.05% male). Our findings indicate a significant oxygen condition $\times$ diagnostic group effect on PCr in the frontal lobe. Acute changes in oxygen levels from hypoxia to hyperoxia significantly decreased brain PCr levels in the frontal brain ($p = 0.03$) in subjects with BD. No significant main ($z = -0.16$, $p = 0.87$) or interactive ($z = 0.19$, $p = 0.85$) effects on β -NTP levels were found.

Conclusions: These findings suggest that the high-energy neurochemistry system responds differently in BD vs. HC following acute exposure to hypoxic stress. These findings may reflect a process whereby PCr may not be utilized to replenish ATP efficiently in BD subjects. These data are consistent with prior evidence of increased suicide rates in BD at altitude, which may be linked to hypobaric hypoxia exacerbating the altered brain bioenergetics in BD. Further studies are required to replicate

these findings and to delineate the mechanistic substrates that will aid the identification of novel treatments for targeting brain mitochondrial dysfunction in BD.

Keywords: Bipolar Disorder, Neuroimaging, Veterans

Disclosure: Nothing to disclose.

P123. Developing the Bipolar Learning Health Network to Improve Outcomes and Accelerate Research

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Background: About 7 million people with bipolar disorder (BD) in the United States could live better lives if they, their clinicians, and researchers could collaborate in a system intentionally designed to produce better and more equitable health outcomes, spawn innovation, and accelerate research. Our current system of care for people with bipolar disorder is not a system at all. The usual process is that an individual clinician meets with a person with bipolar disorder to develop a trial-and-error approach to treatments based on guidelines (in the best cases), although guidelines are often not followed. The process does not facilitate guideline-based care; the clinician usually does not measure how their patients are doing; no feedback is provided so that the clinician and person with bipolar disorder can learn what works and what doesn't, and the person with bipolar disorder depends on the individual clinician's knowledge and experience. Furthermore, bipolar disorder is a family illness affecting everyone, so creating solutions with families is critical. It also takes many resources for a family to support loved ones with bipolar disorder. Complicating efforts to improve the health of people with bipolar disorder is a culture in which inequities are systemic – racial, gender-based, and financial. Inequitable care is poor care.

The purpose of this project is to design, develop, test and optimize a continuous improvement Learning Health Network focused on bipolar disorder and to use data collected from routine clinical care, patient-generated and contributed data to simultaneously improve clinical care, redesign care delivery systems, conduct quality improvement, and undertake clinical trials, health services, outcomes, and comparative effectiveness research.

The project will take advantage of recent advances in information technology, improvement and implementation science and a Learning Health Network to develop and test methods to enable patients, families, clinicians, and scientists to work together to simultaneously improve care, create innovations in care delivery, generate and apply new knowledge. This type of research requires new levels of participation and collaboration among all participants in the network.

Methods: We designed the bipolar learning health network based on a highly successful model from the Anderson Center for Health Systems Excellence at Cincinnati Children's Hospital. Partnering with stakeholders, including clinicians and patients, we used an idealized system design approach integrating organizational design (an actor-oriented network), community-building, quality improvement techniques, and human-centered design principles. The organization is centered on the purpose of getting better outcomes; the community defines those outcomes that matter to all stakeholders (patients, families, clinicians, researchers, and data analysts). Healthcare systems that join the network use a participation and data use agreement that allows the network to share outcomes from each system with the entire

network and use variations in outcomes to promote constant learning and improvement. Building a culture of curiosity, generosity, humility, and a willingness to teach and learn from each other is central. The network trains participants in quality improvement methods so that anyone can initiate an innovation to solve any problems that arise. These activities are supported by a robust data infrastructure that tracks the community-defined outcomes and the effect of improvement efforts over time to optimize the system.

Results: We recruited 18 healthcare systems that treat children, adolescents, or adults with bipolar disorder, along with 12 experts by experience living with bipolar disorder or their family members. Three in-person design meetings were held to co-design the network, form the community, and determine what measures should be deployed for patient-reported outcomes. Each healthcare system produced system maps of care to determine areas that needed improvement. We identified and synthesized (~50) design concepts and prioritized those that could provide an initial portfolio of activities touching enough components of the system that could achieve an impact. Nine healthcare systems had advanced training in quality improvement techniques and implemented improvements, including measurement-based care, suicide assessments, expanding collaborative care and previsit planning, and establishing a method to determine concordance with guidelines. A survey showed that none of the healthcare systems currently assess guideline concordance. We also engaged and collaborated with the office of general counsel at MassGeneral Brigham to co-produce a participation and data use agreement that allows the healthcare systems to share their data and use variations in outcomes to learn what results in the best outcomes.

Conclusions: Designing and building a learning health network for bipolar disorder requires a radical level of collaboration and commitment to improve outcomes. By changing the system of care and breaking down the barriers between clinical care, quality improvement, and research, the Bipolar Learning Health Network can serve as a model for advancing the care and research of people with serious psychiatric disorders.

Keywords: Bipolar Disorder, Learning Health Network, Population Health

Disclosure: Alkermes, Altimate, Clexio, Flow, Merck, Myriad, Bristol Myers, NeuroRx, Unravel Bioscience, 4M Therapeutics: Advisory Board (Self). Eisai, Janssen, Otsuka, SAGE, Sunovion: Consultant (Self). Novartis: Other Financial or Material Support (Self).

P124. Cannabis Use in People with Bipolar Disorder is Associated With Temporal Perception Comparable to That of Healthy Comparison Participants

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Background: Temporal perception, or a person's ability to perceive time accurately and precisely, can impact behavior and cognition. Deficits in temporal processing may negatively impact cognitive functioning if an individual's perception of time deviates from the true value or the perceived relationship between thoughts and sensory events is disturbed. Many people with bipolar disorder (BD) report using cannabis to ameliorate cognitive symptoms, including cognitive domains that are in part regulated by temporal perception, such as attention and decision making. Acute cannabis use (CU) slows temporal perception in healthy adults and rodents,

however, the impact of chronic CU on temporal perception in people with BD remains unknown. Previously, our group reported that in BD participants, CU was associated with better attention and decision making relative to no CU. Here, we examined the interactions between BD and CU on temporal perception hypothesizing that: 1) BD participants would have a faster temporal perception, and that; 2) chronic CU people with BD will have temporal perception equivalent to non CU healthy participants (HC).

Methods: We recruited 48 HC and 35 participants with BD that do not use cannabis or used cannabis ≥ 4 times per week for the past 90 days (+CU). Participants completed a standardized CU survey including data on average weekly CU frequency. CU was dichotomized as moderate use (4-25x/week) and heavy use (> 25 x/week). Participants completed the Discrete-Trials Task (DTT), where participants had to judge whether time intervals (ranging from 1.25-4.25 seconds) were shorter or longer than 2.75 seconds. Psychometric curves were generated to test for group differences in perceived interval duration between the four comparison groups: HC ($n = 23$), HC + CU ($n = 25$), BD ($n = 11$), and BD + CU ($n = 24$). Non-parametric tests (Kruskal-Wallis and Mann-Whitney U tests) were used to test for differences in DTT performance between the above groups; group differences were also tested between moderate and heavy cannabis use in HC and BD participants. Primary outcome measures included Weber Fraction (WF; timing precision) and T50 (timing accuracy). Effects of gender and age on primary outcome measures were tested. Spearman's correlation analyses were used to test for associations between cognitive performance (i.e., Iowa Gambling Task Net Difference score = reward-based decision-making, and d-prime = sustained attention) and temporal perception.

Results: There were no significant differences in gender distribution, however BD participants were significantly older than HC + CU and BD + CU participants. There were no significant group differences in T50. BD participants had a significantly higher WF (i.e., less precise timing) compared to other groups ($KW = 9.37$, $p < 0.05$). Consistent with the effect on WF, the slope of the psychometric curve was reduced in BD participants. BD participants tended to classify a higher proportion of trials < 2.75 seconds as long compared to HC, irrespective to CU status ($p < 0.1$). In the BD + CU participants, only moderate CU was associated with improved timing precision ($Z = 2.9$, $p < 0.01$). Weber Fraction negatively correlated with attention ($rs = -0.325$, $p < 0.01$) and decision making ($rs = -0.19$, $p < 0.1$).

Conclusions: As we predicted, these data support that people with BD who do not use cannabis have reduced timing precision, whereas those who use cannabis exhibited similar timing precision to HC. In line with previous findings, the normalizing effect on temporal perception in participants with BD may be specific to moderate CU. Participants with BD also tended to overestimate short intervals more often than HC. Poor timing precision (but not timing accuracy) was correlated with worse performance in cognitive testing (i.e., attention and risk-based decision making). The cross-sectional study design limits any conclusions on causal effects of cannabis on temporal perception. Future research should investigate whether cannabis treatment can remediate temporal perception deficits in BD.

Keywords: cannabis use, neurocognition, circadian function

Disclosure: Nothing to disclose.

P125. Identifying Brain-Cognition Associations in Bipolar Disorder: A Systematic Review and Meta-Analysis

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Background: Bipolar disorder (BD) is a chronic mental disorder characterized by significant mood instability. Beyond these mood fluctuations, BD is increasingly recognized for its enduring cognitive impairments, which affect executive function, attention, memory, and processing speed. This review synthesizes current knowledge on the relationships between cognitive function and structural brain measures in BD.

Methods: This systematic review was conducted in accordance with PRISMA reporting guidelines. Studies were included if they were: published in a peer-reviewed journal in English, included participants > 18 years or older with bipolar disorder (BD) according to DSM or ICD criteria, assessed cognitive performance using standardized measures, acquired structural MRI data, including T1-weighted images for grey matter (GM) volume, thickness, or surface area analyses, and Diffusion Weighted Imaging (DWI) for white matter (WM) integrity, and analyzed the relationship between imaging measures and cognitive performance or compared cognitively impaired and intact subgroups within the BD population. Studies were excluded if they grouped patients but did not report BD-specific results, were meta-analyses, review articles, or other secondary research publications, included BD participants diagnosed with major neurological illnesses such as stroke, Parkinson's disease, epilepsy, multiple sclerosis, or traumatic brain injury, or were case studies, reports on non-human subjects, conference abstracts, commentaries, opinion pieces, and letters to the editor. Comprehensive searches were conducted in MEDLINE, PsycINFO, EMBASE, and the Cochrane Library from their inception to March 1st, 2024.

Studies were included in the meta-analysis if they reported a direct correlation coefficient (Pearson's or Spearman's rho) for cognition and brain structure (e.g., volume, thickness, surface area, tracts, white matter integrity) associations. A random effects meta-analysis in R was conducted to evaluate the pooled Z score for gray matter (GM) and white matter (WM) in subjects with BD. Separate meta-analyses were performed for GM and WM data to calculate pooled mean Z score, standard deviations (SD), 95% confidence intervals (CI), and heterogeneity statistics (Q). Domain-specific pooled Z scores for cognitive domains were also calculated. Meta-regression analyses were conducted to explore the effects of covariates (mean age, female percentage, mood state, and BD subtype) on the pooled Z scores.

Results: The search yielded 1802 studies, of which 94 were included in the systematic review. Among these, 54 studies had sufficient data to be included in the meta-analysis. For GM studies, the pooled mean Z score was 0.388 (SE = 0.0419) with a 95% CI of [0.3062, 0.4703], and heterogeneity (Q) of 82.3 ($p < .0001$). For WM studies, the pooled mean Z score was 0.375 (SE = 0.0518) with a 95% CI of [0.2736, 0.4766], and heterogeneity (Q) of 22.6 ($p = 0.0316$). None of the covariates had significant covariate effects in meta-regression.

Brain-cognition associations for GM were highest for social cognition, with a pooled mean Z score of 0.717 (SE = 0.1496) and a 95% CI of [0.4236, 1.0102], showing heterogeneity (Q) of 4.2 ($p = 0.1245$). The second highest association was for visual learning and memory, with a pooled mean Z score of 0.494 (SE = 0.1518) and a 95% CI of [0.1967, 0.7916], with significant heterogeneity (Q) of 29.3 ($p < .0001$).

For WM studies, the strongest brain-cognition association was for reasoning and executive function, with a pooled mean Z score of 0.391 (SE = 0.0840) and a 95% CI of [0.2265, 0.5557], showing heterogeneity (Q) of 32.4 ($p = 0.0003$). The second highest association in WM was for working memory, with a pooled mean Z score of 0.398 (SE = 0.0928) and a 95% CI of [0.2160, 0.5797], with heterogeneity (Q) of 12.7 ($p = 0.0264$).

Conclusions: This comprehensive analysis highlights the cognitive differences across gray and white matter studies in BD, with non-significant overall covariate effects in meta-regression, likely due to large heterogeneity between studies. These

findings underscore the complex neurobiological underpinnings of cognitive impairments in BD and the need for further research to elucidate underlying mechanisms. Future work should explore how specific disease-related factors contribute to these mechanisms and utilize whole-brain techniques such as networks and connectivity patterns to better comprehend the cognitive impairments associated with BD.

Keywords: Bipolar Disorder, Cortical brain structures, meta-analysis, Cognition

Disclosure: Nothing to disclose.

P126. Chronic Phytocannabinoid Administration Modulates Corticostriatal Endocannabinoid Signaling and Partially Normalizes Exploratory Behavior in a Mouse Model of Mania

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Background: Cannabis use is highly prevalent among people with bipolar disorder (BD), and represents a significant variable in long-term disease management. The impact of cannabis use on symptomatology and functional outcome is unclear, however, as it has been variously associated with both positive and negative clinical and cognitive outcomes. Directionality and causality are difficult to ascertain from such cross-sectional clinical studies, necessitating the use of animal models to delineate the effects of cannabinoid compounds on cross-species translatable behaviors. The dopamine transporter (DAT) knockdown (KD) mouse reproduces the DAT hypoexpression of people with BD, as well as their phenotypic pattern of hyperlocomotion, increased specific exploration, and straight-line path trajectories in the cross-species Behavioral Pattern Monitor (BPM). This hyperexploratory phenotype is pharmacologically sensitive, enabling assessment of the effects of the primary psychoactive and non-psychoactive compounds of cannabis, Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD), on BD-relevant behavior and endocannabinoid signaling in DAT KD mice.

Methods: The effects of acute (3 mg/kg, i.p.) and chronic THC (1 or 3 mg/kg i.p.; 15 days) and subchronic and chronic CBD (3 or 30 mg/kg; 5 and 15 days) on unconditioned exploration were assessed using male and female DAT KD and wildtype (WT) mice [acute THC (females only): DAT KD = 8, WT = 11; chronic THC: DAT KD = 44, WT = 48; acute and chronic CBD: DAT KD = 36, WT = 34; treatment groups approximately balanced]. Mice were assessed in 20- (chronic THC, chronic CBD), 30- (subchronic CBD), and 45-min BPM sessions (acute THC) 30 min after final injection (between-subjects). The BPM quantified overall activity (distance traveled), exploration (holepokes and rearing), and path trajectories (spatial d). Following termination of chronic THC and CBD studies, brains were removed and endocannabinoid levels (anandamide and 2-arachidonoylglycerol; AEA, 2-AG) were quantified in cortex (PFC) and dorsal and ventral striatum (DS, VS).

Results: As reproducibly reported by our group, BD-consistent elevations in locomotion, exploration, and path linearity were detected in DAT KD mice throughout. Acute THC (3 mg/kg) reduced distance traveled [$F(1,30) = 8.8$, $p = .006$], holepokes [$F(1,32) = 12.7$, $p = .001$], and rears [$F(1,27) = 29.7$, $p < .001$] across genotypes, while straightening WT trajectories during the final 15 minutes [Kruskal-Wallis non-parametric test: $\chi^2 = 6.0$, $p = .014$]. Chronic THC (3 mg/kg) reduced female locomotion in the latter half of testing ($p < .05$), while 1 mg/kg tended to increase early locomotion in male DAT KDs ($p = .059$) [block $\times$ sex $\times$ genotype $\times$ THC: $F(2,77) = 5.0$, $p < .01$]. 3 mg/kg THC reduced rearing

[$F(2,77) = 5.9$, $p < .01$] and made female movement less linear during the second half of testing [block $\times$ sex $\times$ THC: $F(2,77) = 5.6$, $p < .01$]. No main or interactive effects of CBD were observed at the subchronic time-point, although chronic CBD (30 mg/kg) increased distance in WT mice only [genotype $\times$ CBD: $F(2,52) = 3.4$, $p = .040$; post hoc: $p = .013$]. In both experiments, reduced AEA (but comparable 2-AG) levels were detected in vehicle-treated DAT KD PFC, VS, and DS relative to vehicle-treated controls [genotype $\times$ THC: $F(2,68)$'s > 6.4 , p 's < 0.005]. Each dose of THC and CBD increased AEA in each region in DAT KD mice only (except 3 mg/kg THC in DS) (p 's $< .05$). 1 mg/kg THC increased 2-AG levels in PFC, VS, and DS in WT mice and in PFC and DS in DAT KDs [genotype $\times$ THC: $F(2,69)$'s > 4.8 , p 's $< .05$; post hoc: p 's $< .05$]. 2-AG levels were reduced in DS and VS of 3 mg/kg CBD-treated mice and in VS of 30 mg/kg-treated mice [main effects CBD: $F(2,45)$'s > 3.3 , p 's $< .05$; post hoc: p 's $< .05$].

Conclusions: Acute and chronic THC reduced locomotion and exploration in a genotype-independent manner (primarily in females), thus partially normalizing hyperactivity of DAT KD mice, while chronic CBD increased locomotion only in WT mice. Furthermore, acute THC straightened only WT path trajectories in late stages of testing. Both THC and CBD normalized the reduced AEA levels of DAT KD mice. 2-AG was region-specifically increased by THC in WT mice and DAT KDs, and was dose- and region-specifically modulated by CBD. The genotype-independence of the locomotor and exploratory effects of THC indicate that DAT KD mice were equally affected as WT mice, partially normalizing their hyper-exploration. Meanwhile, the specificity of chronic CBD and acute THC effects on locomotion and path linearity in WT mice, respectively, suggests a partial resistance of DAT KD mice to some effects of cannabinoid exposure (although ceiling effects may be a contributing factor). While AEA mobilization was deficient in DAT KD mice at baseline and rescued by both THC and CBD, these effects appear largely separate from the behavioral effects of DAT hypoexpression and cannabinoid treatment (as does cannabinoid-mediated modulation of 2-AG levels). Altogether, cannabinoid treatment had somewhat less effect on DAT KD behavior than WT behavior, while largely restoring dysregulated endocannabinoid mobilization. These results suggest that cannabis use may be beneficial in people with BD at the behavioral and neurochemical level, although they may be no more susceptible to the behavioral effects of cannabis than are healthy populations. Follow-up studies will be necessary to evaluate these conclusions, including endocannabinoid quantification in cannabis-using versus cannabis-abstinent people with BD.

Keywords: Anandamide, 2-AG, Translational Animal Models, Bipolar Disorder

Disclosure: Nothing to disclose.

P127. Relationship Between Symptomatic and Functional Improvement During Cariprazine Treatment in Patients With Bipolar I Depression: Post Hoc Analysis of a Randomized, Placebo-Controlled Trial

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Background: In bipolar I disorder (BP-I), depressive symptoms substantially impair function, leading to personal and economic burden. Functional recovery is an overarching goal prioritized by patients with lived experience of bipolar disorder. Multiple factors affect functional outcomes, including, but not limited to, depression, anhedonia, and anxiety. To date, there is insufficient

characterization of the mediation of functional outcomes by select symptoms in BP-I depression. Cariprazine is a dopamine D3-preferring D3/D2 receptor and serotonin 5-HT1A receptor partial agonist that is approved for the treatment of adults with manic, mixed, and depressive episodes of BP-I. The efficacy of cariprazine in BP-I depression was demonstrated in 3 pivotal, randomized, double-blind, placebo-controlled trials. In the 8-week pivotal trial (NCT01396447), cariprazine was associated with significant improvement in functional outcomes at week 8. Herein, this post hoc analysis looked at the relationship of various BP-I symptom clusters to function, and to what extent improvement in these clusters mediates functional recovery.

Methods: Patients with BP-I and a current depressive episode were randomized to placebo or cariprazine 0.75, 1.5, or 3 mg/d. This post hoc analysis focused on characterizing the treatment effects of cariprazine 1.5 mg/d, as that dose group had significant improvement relative to placebo in the prespecified Functioning Assessment Short Test (FAST) total score outcome. Symptom clusters were assessed using 5 measures: Montgomery-Åsberg Depression Rating Scale (MADRS) total score, MADRS concentration item, MADRS anhedonia factor score (sum of apparent sadness, reported sadness, concentration, lassitude, and inability to feel items), 17-item Hamilton Depression Rating Scale (HAM-D) anxiety/somatization subscale (sum of anxiety [psychic], anxiety [somatic], somatic symptoms [gastrointestinal], somatic symptoms [general], hypochondriasis, and insight), and Young Mania Rating Scale (YMRS) irritability/hostility items (combined irritability and disruptive-aggressive behavior items). For each symptom measure, least-squares (LS) mean change from baseline to week 8 was analyzed using a mixed-effects model for repeated measures. Pearson correlation coefficients were calculated between change in FAST total score and change in each symptom measure. In a path analysis approach, a covariance structure analysis was used to quantify the direct effect of cariprazine on FAST total score and indirect effects through changes in each symptom measure at week 8. All analyses were based on the modified intent-to-treat (mITT) population (randomized patients who took ≥ 1 dose of study drug and had baseline and week 8 FAST assessments).

Results: A total of 220 patients were included in the mITT population (placebo = 103; cariprazine 1.5 mg/d = 117). LS mean change from baseline to week 8 was significantly greater for patients treated with cariprazine versus placebo for MADRS total (cariprazine = -16.6 , placebo = -13.6 ; $P = .0198$), concentration item (cariprazine = -1.8 , placebo = -1.3 ; $P = .0051$), and anhedonia factor (cariprazine = -10.4 , placebo = -8.2 ; $P = .0067$) scores. Cariprazine-treated patients demonstrated numerical reduction from baseline to week 8 in HAM-D anxiety/somatization subscale score (LS mean change: cariprazine = -3.8 , placebo = -3.2) and YMRS irritability/hostility items (cariprazine = -0.5 , placebo = -0.5); differences versus placebo were not significant. For the cariprazine-treated group, moderate correlations were found between improvement in FAST and improvements in most symptom clusters (Pearson r : MADRS total = 0.54; MADRS concentration = 0.45; MADRS anhedonia factor = 0.53; HAM-D anxiety/somatization subscale = 0.40; $P < .001$ for all). A weak but statistically significant correlation was seen between improvements in FAST and YMRS irritability/hostility items ($r = 0.24$; $P = .009$). Results of the path analysis showed that the effect of cariprazine on functioning was driven by indirect effects through symptom clusters (percentage of total effect: MADRS total = 54.4%; MADRS concentration item = 18.2%; MADRS anhedonia factor = 12.8%; HAM-D anxiety/somatization subscale = 12.3%; YMRS irritability/hostility items = 4.1%) rather than direct treatment effect (-1.7% of total).

Conclusions: In patients with BP-I depression, cariprazine demonstrated a significant treatment effect for overall depression, concentration, and anhedonia. Improvement in most symptom clusters was moderately correlated with functional improvements.

In the path analysis, indirect effects through overall depression and concentration improvement accounted for nearly 75% of cariprazine's effect on function. Improvements in anhedonia and anxiety accounted for most of the remaining treatment effect. Nearly all functional improvement was attributable to core and associated symptom clusters, suggesting that a combination of symptoms mediate the effect of cariprazine on function.

Keywords: Bipolar I Depression, Functioning, Predictor analysis, cariprazine

Disclosure: AbbVie: Employee (Self)

P128. The Role of the Mesolimbic Dopamine System in Healthy and Pathological Arousal States

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Background: Aberrant circadian rhythms, the internal regulation of bodily functions across a 24 h period, are frequently used as a diagnostic tool in many psychiatric disorders. Individuals with psychiatric conditions often display heightened sensitivity to circadian disruptions, which may in turn exacerbate symptoms. Identifying the mechanisms within neurobiological pathways that link abnormalities in circadian rhythms and psychiatric disorders is imperative for the development of targeted treatments that alleviate symptoms. The mesolimbic dopamine (DA) pathway, specifically dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), is a critical overlapping node in emotion and arousal circuitries. The HYPER rat, a spontaneous genetic variant that naturally exhibits extreme circadian psychomotor variations in response to stress, serves as a unique model for investigating the underlying mechanisms within the DA VTA-NAc circuit that may be involved in psychiatric disorders characterized by disturbances in circadian regulation of arousal. We hypothesize that disruption of the mesolimbic DA system is responsible for stress-induced hyperactivity periods in HYPER rats. We probed mesolimbic DA function in HYPER and wild-type Sprague-Dawley rats (WT) using amphetamine-induced locomotor activity and c-fos immunohistochemistry. We also used RNAscope probes targeting DA- and circadian-related genes within the mesolimbic system during baseline and stress-induced hyperactivity periods in HYPER rats and time-matched WT rats, as well as bulk RNA-sequencing of the VTA and whole genome sequencing to characterize the genomic and transcriptomic landscape of HYPER rats. Results from these experiments will enhance our understanding of how fluctuations in mesolimbic transmission contribute to persistent changes in arousal rhythmicity, measured by daily locomotor activity. This research bridges the existing gap in our understanding of circadian disturbances in mood disorders and offers a foundation for developing targeted therapeutic interventions.

Methods: Four-month-old HYPER and WT rats were used in these experiments. Locomotor activity during the dark cycle was analyzed after a mild stressor (cage change) for up to 1 week. All tissue was collected after the first 3 h of the dark cycle. Upon return to baseline activity, a subset of rats was administered amphetamine (2 mg/kg, i.p.). Locomotor activity was recorded, and rats were euthanized 90 min later for assessment of c-fos.

IHC: 30 µm sectioned tissue from HYPER (n = 8) and WT rats (n = 8) were placed in 2 ml of PBS-Triton Block solution for 1 h. 2 µl of Ch c-fos and Rb TH antibody were pipetted directly into each well (1:1000 dilution). Sections remained in primary antibodies for 24 h at 4°C. Sections were washed 3 times in 1X PBS and placed in 2 ml of fresh block solution with 4 µl of goat anti-Ch488 and anti-Rb568 (1:500 dilution). Sections were incubated in secondary

antibody for 1.5 h then mounted and coverslipped. The number of c-fos+ and Th+ cells were quantified using HALO software.

RNA experiments: Brain tissue was collected 3 days (peak hyperactivity) or ~1 week (return to baseline) after cage change. These time points consist of 10 (5 male, 5 female) HYPER and WT rats (2 genotypes x 2 experiments x 2 time points x 10 animals/group = 80 rats). Brains were flash-frozen and stored at -80°C.

RNAscope was performed using the RNAscope Fluorescent Multiplex Assay (Advanced Cell Diagnostics). The VTA and NAc were sectioned at 16 µm and slides were stored at -80°C. Tissue was pretreated using the RNAscope Sample Preparation Guide. The RNAscope assay was performed using probes for DAT, TH, VMAT2, D2, and Clock in the VTA, and D1, D2, and D3 in the NAc shell. Slides were imaged on a Keyence microscope at 20x, 40x, or 60x. Probe signal (puncta per cell as well as total number of positive cells) was quantified using HALO software.

Chilled brains for bulk RNA-sequencing were sectioned until reaching the VTA and punched unilaterally (1 mm) (AP: ranging from -4.92 to -6.46). VTA tissue was stored at -80°C until processing for bulk RNA-seq analysis by the Emory Genomics Core.

Whole Genome Sequencing: Liver samples from 4 (2 male, 2 female) animals were acquired from HYPER and WT rats 3 days after cage change (n = 8). DNA was extracted and processed for WGS by Novogene.

Results: Our results show that in comparison to WT rats, HYPER rats had blunted locomotor response to amphetamine, but paradoxically exhibited increased amphetamine-induced c-fos in the NAc. These data indicate that dysregulated mesolimbic DA transmission may contribute to the HYPER rat phenotype. RNAscope, RNA-seq, and WGS analyses are currently ongoing, and preliminary data will be presented.

Conclusions: Our study aims to elucidate the role of the mesolimbic DA pathway in regulating circadian rhythms and its potential link to psychiatric disorders. The HYPER rat model, which naturally exhibits significant circadian psychomotor variations, serves as an ideal subject for investigating these mechanisms. While data analysis is still ongoing, our results from IHC experiments suggests that there may be supersensitive DA receptor signaling following DA depletion in the NAc and supports our hypothesis that fluctuations in DA mesolimbic system may be responsible for variability in psychomotor activity. Future directions will include functional studies aimed at establishing causal relationships between identified variants and aspects of the HYPER phenotype.

Keywords: circadian rhythm, mesolimbic reward circuitry, Hyperactivity, mood disorders, locomotor activity

Disclosure: Nothing to disclose.

P129. Relationship Between Severity of Suicide Attempt, Family History of Psychiatric Disorders, and Age of Onset in Bipolar Disorder

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Background: It is estimated that one in five individuals with bipolar disorder (BD) will complete suicide, a rate up to 20 times that of the general population. Previously reported risk factors for suicide behaviours in BD include an earlier age of onset, family history of BD or major depressive disorder, and family history of suicide attempts or completed suicide. However, the relationship between age of onset, family history, and suicide behaviours is not clear. Furthermore, the majority of studies tend to use one definition of suicide behaviours (i.e. suicide attempts or completed suicide). The aim of the present study was to examine the risk

factors of three categories of suicide attempts, with a focus on family history of relevant psychiatric illnesses and suicide, age of onset, and sex differences.

Methods: A sample of adults ($N = 339$; 62.5% female) meeting DSM-IV criteria for BD (type I or II), with available information for one or more first-degree relatives (FDRs; mean = 6.5 relatives per subject), was recruited from Canadian sites as part of a larger, longitudinal study. Dependent variables were history of any suicide attempt, history of potentially lethal suicide attempts, and history of only non-lethal suicide attempts. Family history in FDRs included BD, major depressive disorder, psychotic disorder (schizophrenia or schizoaffective disorder), suicide attempts, and completed suicide. Additionally, age of onset for BD (standardized), sex, diagnostic subtype, and if subjects ever received lithium treatment were considered. Logistic regression was performed for each of the dependent variables in a preliminary analysis. Since the sample used for the analysis is followed as part of a larger study, there were three subjects who had completed suicide since their last interview. These participants were combined with the potentially lethal suicide attempt group, as there were too few to analyze separately.

Results: Distinct sets of predictors for each variable of interest were revealed from the regression analyses. Significant predictors of any type of suicide attempt were sex, family history of BD, age of onset, and family history of suicide attempt. In particular, males (OR: 0.40, CI 95% [0.23, 0.67], $p < 0.001$) and those with family history of BD (0.47 [0.26, 0.84], $p = 0.012$) had lower rates of suicide attempts. As expected, an earlier age of onset (0.55 [0.40, 0.73], $p < 0.001$) and family history of suicide attempts (2.35 [1.24, 4.47], $p = 0.009$) were both related to increased risk of any suicide attempt in participants. Family history of completed suicide was included in the best fit model, but was not significant (2.04 [0.80, 5.19], $p = 0.129$). Similar results were found for potentially lethal suicide attempts, with male sex and family history of BD being inversely related (male sex: 0.43 [0.22, 0.80], $p = 0.010$; BD: 0.50 [0.24, 0.96], $p = 0.047$), and a family history of suicide attempts positively related to lethal suicide attempts in the sample (2.11 [1.05, 4.12], $p = 0.032$). However, age of onset and family history of completed suicide were not predictors in the selected model for lethal suicide attempts. Lastly, non-lethal suicide attempts were analyzed, and the best fit model included only sex and age of onset as predictors. In this case, the only significant effect was negative relationship between age of onset and non-lethal suicide attempts (0.38 [0.22, 0.61], $p < 0.001$; male sex: 0.57 [0.28, 1.11], $p = 0.109$). Diagnostic subtype, family history of major depressive disorder, family history of psychotic disorder, and ever receiving lithium treatment were not predictors in any best fit models.

Conclusions: Our preliminary results raise interesting questions regarding the association of earlier age of onset in BD with increased risk of suicide attempts. Once stratified by the lethality of the suicide attempts, the relationship of attempts with age of onset was only present for subjects who did not have a potentially lethal attempt. Additionally, family history of completed suicide in FDRs was not associated with any suicide behaviours in this sample, contrary to prior literature. Family history of BD having an inverse relationship with suicide attempts and lethal attempts was an unexpected result. It may be that individuals who have FDRs with BD are being diagnosed and treated more quickly. As such, we are exploring potential differences in the length of time from illness onset to first suicide attempt and first treatment, and those results will be reported in the final presentation.

Considering the high prevalence of suicide attempts and completed suicide in individuals with BD, understanding and predicting the risk of these outcomes is pertinent for effective suicide prevention and clinical management of the illness.

Keywords: Bipolar Disorder, Suicide, Family History, Mood Disorders, Age of Onset

Disclosure: Nothing to disclose.

P130. The Role of BDNF Genetic Polymorphisms in Cytokine Profiles, Tryptophan-Kynurenine Pathway, and Treatment Outcomes in Bipolar Depression

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Background: Neuroinflammation and dysregulated expression of Brain-derived Neurotrophic Factor (BDNF) are reported in numerous neuropsychiatric disorders. Patients with depression show increased blood concentrations of pro-inflammatory cytokines. The Kynurenine Pathway (KP), activated by pro-inflammatory cytokines, diverts tryptophan (TRP) from serotonin (5-HT) synthesis to neuroactive KP metabolites, which have been linked to depression. BDNF plays a crucial role in the structural plasticity and function of neurons, directly regulating microglial cytokine responses and potentially interacting with tryptophan metabolites. Adding a COX-2 inhibitor to antidepressant therapy may benefit treatment-resistant depression, which often fails to respond to antidepressant treatment. This study aimed to explore the relationship between BDNF genetic polymorphisms, cytokine profiles, tryptophan metabolism, and clinical outcomes in treatment-resistant bipolar depression.

Methods: This study included 41 patients diagnosed with bipolar disorder (BD) experiencing treatment-resistant depression (TRBD), aged 21 to 65. Participants were randomly assigned to receive either Escitalopram + Placebo (ESC + PBO) or Escitalopram + Celecoxib (ESC + CBX) over 8 weeks. Treatment resistance was evaluated using the Maudsley Staging Method (MSM). Serum BDNF levels were measured using enzyme-linked immunosorbent assay (ELISA). Blood cells were used to obtain DNA for genome-wide genotyping with the Infinium Multi-Ethnic Global-8 v1.0 Kit. Three SNPs (rs1519480, rs6265, and rs10835210) were selected based on their associations with affective disorders. Carriers were defined as those possessing at least one minor allele (rs1519480G, rs6265A, and rs10835210A). Statistical analyses compared responders with non-responders and remitters with non-remitters based on BDNF SNP carrier status and explored the influence of genetic polymorphisms on cytokine levels and tryptophan metabolism.

Results: Pearson correlation analyses revealed significant correlations between baseline levels of BDNF and 5-HTP ($r = 0.445$, $p = 0.005$), TNF- α ($r = 0.398$, $p = 0.011$), and IL-8 ($r = 0.372$, $p = 0.018$). For rs1519480, t-tests demonstrated differences in baseline means between non-carriers and carriers in MSM scores: Total ($t = -4.38$, $p < 0.001$), Severity ($t = -2.50$, $p = 0.017$), Duration of Depressive Episode ($t = -2.42$, $p = 0.021$), as well as Quin/3-HAA ($t = 2.42$, $p = 0.021$) and Quin/3-HK ($t = 2.24$, $p = 0.031$) ratios. For rs10835210, significant differences in IL-1A baseline levels were observed between non-carriers and carriers ($t = -2.34$, $p = 0.024$), while rs6265 non-carriers had higher Maudsley Augmentation ($t = 2.18$, $p = 0.036$) and Total ($t = 2.15$, $p = 0.039$) baseline scores compared to carriers.

Additionally, significant differences were found between non-carriers and carriers in each SNP when comparing treatment response and remission. For rs1519480, treatment response did not differ significantly between the groups, but remission rates were higher in non-carriers: 75% in ESC + CBX vs. 0% in ESC + PBO ($\chi^2(1) = 6.857$, $p = 0.009$). In carriers, remission rates were 61.5% in ESC + CBX vs. 16.7% in ESC + PBO ($\chi^2(1) = 5.235$, $p = 0.022$).

For rs6265, treatment response was significantly different in non-carriers: 27.3% in ESC + PBO vs. 81.3% in ESC + CBX

($\chi^2(1) = 7.867$, $p = 0.005$), but not in carriers: 80% in ESC + PBO vs. 77.8% in ESC + CBX ($\chi^2(1) = 0.009$, $p = 0.923$). Remission rates in non-carriers were 9.1% in ESC + PBO vs. 62.5% in ESC + CBX ($\chi^2(1) = 7.702$, $p = 0.006$), and in carriers: 20% in ESC + PBO vs. 77.8% in ESC + CBX ($\chi^2(1) = 4.381$, $p = 0.036$).

For rs10835210, treatment response in non-carriers was 62.5% in ESC + PBO vs. 77.8% in ESC + CBX ($\chi^2(1) = 0.476$, $p = 0.490$), and in carriers: 25% in ESC + PBO vs. 81.3% in ESC + CBX ($\chi^2(1) = 7.200$, $p = 0.007$). Remission rates in non-carriers were 12.5% in ESC + PBO vs. 77.8% in ESC + CBX ($\chi^2(1) = 7.244$, $p = 0.007$), and in carriers: 12.5% in ESC + PBO vs. 62.5% in ESC + CBX ($\chi^2(1) = 5.371$, $p = 0.020$).

Conclusions: Our findings indicate a significant influence of BDNF genetic polymorphisms on treatment outcomes in bipolar disorder patients with treatment-resistant depression. Our analyses revealed significant associations between baseline BDNF levels, genetic polymorphisms, and key biomarkers, supporting BDNF's role in modulating inflammatory and metabolic pathways. Moreover, significant differences in baseline MSM scores between non-carriers and carriers of rs1519480G and rs6265A suggest that these BDNF polymorphisms may contribute to the severity and chronicity of depressive symptoms. Specifically, rs6265A non-carriers had higher Maudsley Augmentation scores, indicating a greater need for supplementary treatments, and showed a significant treatment response with the addition of Celecoxib to Escitalopram compared to non-carriers. Conversely, for rs10835210, A allele carriers demonstrated a significant response to Celecoxib compared to non-carriers. These results suggest that personalized treatment strategies considering BDNF polymorphisms could enhance therapeutic efficacy in treatment-resistant bipolar depression. Further research is warranted to validate these findings and explore the underlying mechanisms of BDNF's role in modulating cytokine profiles and tryptophan metabolism.

Keywords: Bipolar Depression, COX-2 Inhibitor, BDNF, SNPs, Kynurenine Pathway

Disclosure: Nothing to disclose.

P131. Enhancing Access to Care as Well as Patient Education for Bipolar Disorder With Group Medical Visits / Shared Medical Appointments

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Background: Group Medical Visits (GMVs), also known as Shared Medical Appointments, is a newer model of care that usually involves two clinicians providing individual patient care within a group consisting of patients with a similar disorder, together with group education and discussion of illness self-management skills. We adapted a model created by Remick and colleagues for mood and anxiety disorders to a specific model for bipolar disorder, utilizing a staff psychiatrist and resident psychiatrist treatment team. Here, we report on patient evaluation of the model that we launched several years ago.

Methods: The Bipolar Group Medical Visits (GMVs) program includes approximately 50 patients who obtain psychiatric medical care exclusively within this model. The 39 individuals who attended a GMV in the past two years were invited to participate in this study. Patients completed a questionnaire with 25 quantitative items and 3 qualitative items covering patient satisfaction (using the Client Satisfaction Questionnaire), clinical value of GMVs model, challenges/benefits for program users, and the current state of their bipolar treatment.

Results: 24/39 (61.5%) patients responded, reporting high satisfaction on the Client Satisfaction Questionnaire (an average

score of 30.29 out of 32). Patients reported (1) feeling supported by other group members (70.8%), (2) receiving additional value from receiving care in the group (83%), (3) easier access to care due to the group (70.8%), and (4) learning new strategies/information from other group members (70.8%). The most common challenge reported was the time and length of the group appointment (2 and 5 mentions, respectively).

Conclusions: Patients rated Group Medical Visits as a valuable method of receiving care, having unique benefits compared to the traditional one-on-one format, and provided avenues to explore for improving the program. Such pilot data, as well as our earlier reported favorable evaluation of GMVs as an educational modality by residents, support additional expansion and evaluation of GMVs. Widespread adoption of GMVs offers the possibility of substantial improvement in access to care.

Keywords: Bipolar Disorder, Health services, Long-term treatment, Clinical practice

Disclosure: Janssen, Compass, Aifred, Sage, Merck: Contracted Research (Self). Boehringer Ingelheim, Mensante, Myriad: x Consultant (Self) Otsuka: Advisory Board (Self)

P132. The Effect of Gender on Patterns of Frontostriatal Brain Wiring in Early Psychosis Affective Subjects and in Healthy Controls: An MRI Diffusion Imaging Tractography Study

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Background: Disrupted brain connectivity may underlie neuropsychiatric disorders such as affective and non-affective psychoses. We assessed frontostriatal wiring organization using diffusion MRI tractography from the Human Connectome Project in 56 healthy controls, and 46 Early Psychosis Affective patients; Mean age: 23.9 years; sex: 44 females; 58 males; 25 EP-AFF females and 21 EP-AFF males; 19 HC females and 37 HC males. We used our novel method of fiber cluster analysis of whole brain diffusion Magnetic Resonance Imaging (dMRI) tractography to assess brain wiring. This method allows us to quantify the degree of deviation from a topographic, parallel, arrangement in brain wiring connectivity between the frontal cortex (FCtx) and the caudate (Cd), a component of the associative striatum.

Methods: The data used in this study come from the shared data set from the Human Connectome Project for Early Psychosis (HCP-EP) study (MPI: Shenton, Breier). Diffusion MRI Data from 3 HCP sites (University of Indiana, Massachusetts General Hospital and McLean Hospital) were harmonized (Cetin-Karayumak S et al, 2019). From this harmonized data set we generated whole brain tractography using our unscented Kalman filter (UKF) 2-tensor tractography methodology (Malcolm JG et al, 2010). We used a data-driven fiber clustering atlas that allows for a whole brain tractography parcellation into 2000 white matter fiber clusters according to white matter (WM) fiber geometric trajectory (Zhang et al., 2018). Then, fiber clusters of interest (i.e., from FCtx to Cd) based on FreeSurfer parcellation from the whole brain WM were identified for each subject. We identified 17 WM fiber clusters connecting FCtx and Cd in both each hemisphere in each subject group. To determine the pattern of frontostriatal connectivity in both groups both combining gender and analyzing separately, first, we generated scatter plots for each hemisphere (not shown) based on the 17 fiber clusters (with 136 pairs of fiber clusters, yielding 136 data points), showing the relationship between the cortical distances and the corresponding caudate distances of fiber cluster pairs connecting the FCtx and the caudate. Second, in both

groups, we generated scatter plots (not shown) for each of the 17 clusters. For each cluster in each group, we performed paired t-tests of the distance between that cluster to the other clusters in the hemisphere, comparing the mean inter-cluster streamline endpoint distances at the levels of the frontal cortex and caudate, per hemisphere, using a Bonferroni adjustment. In addition, we assessed the between-group difference for each cluster pair in each cluster in the degree of convergence, reflected by a convergence quotient (CQ). Our CQ was calculated as: (Cortex Distance - Caudate Distance)/(Cortex Distance + Caudate Distance). For each cluster, we used a mixed model regression analysis of CQ in each hemisphere, separately. For each of 17 clusters in each hemisphere, we assessed the between-group difference in CQ for the 16 pairings with all other clusters in the same hemisphere. We fit a mixed-model regression for each of 17 clusters in a hemisphere, with CQ as response, and subject as a random effect. Fixed effects in these models were all 16 cluster pairings, group (EP-AFF vs HC), and the interaction of pairing with group. Where appropriate, we covaried for gender as the groups significantly differed in gender proportion ($p = 0.038$).

Results: First, in both groups, in both genders, we found bilateral non-linear relationships, yielding convex curves (not shown), between FCtx and Cd distances for FCtx-Cd connecting fiber clusters, coming from similar prefrontal cortical (PFC) subregions. Similar clusters across both groups in both genders demonstrated significant patterns of convergence. These subregions included orbitofrontal and ventrolateral PFC. Of note, in the left hemisphere (LH), in female EP-AFFs, the convex curve was more flattened compared with all the other curves in both groups across gender. Second, in a RH frontal pole cluster, across genders, we found a significant diagnosis by cluster pair interaction ($p = 0.013$). Lastly, third, in a LH inferior frontal gyrus (IFG), pars triangularis cluster, we found a gender x diagnosis x cluster pair interaction ($p < 0.0085$), with the diagnosis x cluster pair interaction much greater in females ($p < 0.0001$) than in males ($p = 0.98$).

Conclusions: In both groups, the FCtx-Cd wiring pattern deviated from a strictly topographic relationship with similar clusters in both groups across genders demonstrating significant patterns of convergence. Further, in a RH frontal pole cluster we found a significant diagnostic effect between early affective psychosis subjects and healthy controls, in wiring pattern, across genders, whereas, in a LH IFG cluster we found a gender effect, i.e., an effect was seen in females but not in males, was responsible for driving the significant diagnostic effect between groups in their FCtx-Cd connectivity patterns. Of note, our data support the importance of including gender as a variable of interest in the assessment of brain wiring patterns in early affective psychosis. We believe the importance of our long-tract brain wiring measures is that they reflect trait biomarkers which may help identify early affective psychosis subjects, early in their developmental course, who could benefit from early, more targeted, treatment intervention.

Keywords: early psychosis, affective disorders, diffusion tractography

Disclosure: Nothing to disclose.

P133. Mobile Sensing Proxy for Sleep as an Indicator of Mood Symptoms in Youth With Bipolar-I/II Disorder: A Pilot Study

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Background: Bipolar disorder (BD) is characterized by temporal instability of mood and energy, including recurrent periods of hypomania and depression. These mood fluctuations are one of

the challenges of treating BD, as mood can quickly change and require prompt assessment and possibly medication adjustment. It would be clinically advantageous to have an "early warning" sign that mood symptoms are increasing, but other than frequently administered self-report mood questionnaires (which can be burdensome), the field lacks objective and temporally sensitive indicators of worsening mood. One candidate "early warning" sign of mood changes, both for mania and depression, are changes in sleep, for example, total sleep time and sleep variability. Sleep can be measured objectively in the sleep lab or via actigraphy, but this is not feasible for longitudinally monitoring changes in sleep that might portend a mood recurrence. However, there is growing evidence that mobile sensing data (i.e., passively collected smartphone measures of physical activity and phone usage) may yield proxy indicators of sleep. Here, we construct a novel mobile sensing proxy measure of sleep duration and assess the degree to which sleep quantity and variability correlate with self-reported symptoms of mania and depression, both across the sample and on the individual level.

Methods: Young people with symptomatic BD-I/II (at least one hypo/manic and depressive episode in the past year) were longitudinally assessed via weekly self-report assessments of depression (Patient Health Questionnaire-9; PHQ-9) and mania (Altman Self-Rating Mania Scale; ASRM). Data regarding smartphone usage, motion, and battery consumption (91 variables from eight sensors) were passively and continuously collected via the AWARE app. To identify which variables were most sensitive to potential sleep/wake periods, we first screened all variables for diurnal variation across the sample, identifying those variables that showed the most marked day-to-night fluctuation. These variables included (1) physical activity classification (active vs. not) and (2) number of screen unlocks. We next utilized hourly mobile sensing data to identify the longest time period in a 24-hour window where these two variables were both equal to 0, indicating a lack of phone motion or usage (Total Continuous Offline Time; TCOT), as a proxy measure for sleep duration. Weekly measures of average TCOT (TCOT-mean) and day-to-day variability (coefficient of variation, calculated as the standard deviation divided by the mean; TCOT-CoV) were calculated. Using linear mixed models, the relationships between estimates of sleep duration (TCOT-mean and TCOT-CoV) and the corresponding weekly mood ratings (PHQ-9 and ASRM scores) were assessed. Subject-level mean-centered TCOT values were utilized to assess within-person relationships between sleep proxy measures and mood symptoms.

Results: Twelve participants (8 female; median age 20, range 17-23 years old) contributed a total of 336 weeks (median 33; range 2-41 weeks) of usable data (i.e., high-quality smartphone data and completed questionnaires). Depressive and manic symptoms showed variability over the follow-up (PHQ-9 inter-quartile range = [2,9]; ASRM interquartile range = [0,4]), allowing for the assessment of correlation between these symptoms and mobile sensing measures. The median TCOT in this sample was 6 hours, with a range from 0 to 15 hours. Weekly TCOT-mean was inversely associated with concurrent manic symptoms, both across the sample ($\beta = -.19$, $p = .004$) and within-person ($\beta = -.16$, $p = .002$). Weekly TCOT-CoV was also positively associated with concurrent manic symptoms both across the sample ($\beta = .23$, $p = .0003$) and within-person ($\beta = .21$, $p = .0002$). However, neither TCOT measure was associated with depressive symptoms either across the sample or within-person ($\beta = -.05$ to 0.04 , p -values = .26 to 0.40).

Conclusions: In a sample of 12 young people with BD, contributing a total of 336 weeks of usable data, we found that a mobile sensing-based proxy for sleep (TCOT) fluctuated with manic (but not depressive) symptoms, both across the sample and within-person. The significant relationship between this mobile sensing marker and within-person mood changes provides initial

evidence that such a marker could be used as a real-time indicator of mood worsening and/or recurrence and may be particularly relevant to manic symptoms. Important limitations that should be considered when interpreting this work are that: (1) While extensive data are collected on each participant, only 12 young people are included; thus, findings are preliminary and warrant a larger study. (2) Although we hypothesize that TCOT may be an indicator of sleep duration, particularly in young people with high rates of smartphone usage, we do not have an objective marker of sleep to validate this proxy measure in this sample. Future studies should assess the relationship between TCOT and actigraphy-assessed Total Sleep Time. Despite these limitations, this analysis is, to our knowledge, the first to demonstrate a within-person relationship between a mobile sensing-derived proxy for sleep and manic symptoms, pointing to potential for clinical utility as an “early warning” detector of mania.

Keywords: Bipolar Disorder, mobile sensing, Digital phenotyping, sleep

Disclosure: Nothing to disclose.

P134. Differential Contributions of Circadian Clock Genes to Cell Survival in Bipolar Disorder Patient Derived Neuronal Progenitor Cells Distinguishes Lithium Responders and Non-Responders

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Background: Bipolar disorder (BD) is characterized by disrupted circadian rhythms and neuronal loss. Lithium is neuroprotective and used to treat BD, but outcomes are variable. Past research identified that circadian rhythms in BD patient neurons are associated with lithium response (Li-R) or non-response (Li-NR). However, the underlying cellular mechanisms remain unknown.

Methods: To study interactions among circadian clock genes and cell survival, and their role in BD and predicting lithium response, we tested selected genes (PER1, BMAL1 and REV-ERBa) and small molecule modulators of ROR/REV-ERB nuclear receptors in models of cell survival using mouse neurons and stem-cell derived neuronal progenitor cells (NPC) from BD patients and controls. Bioluminescent survival assays were used to measure cell death following treatment of iPSC-derived neuronal progenitor cells (NPC) with staurosporine (STS). NPC were grown from lithium responders (Li-R, $n = 2$), lithium non-responders ($n = 3$) and controls ($n = 3$). Knockdown experiments were conducted with siRNA to reduce gene expression by ~75%.

Results: In apoptosis assays using STS, lithium was neuroprotective. Knockdown of PER1, BMAL1 and REV-ERBa modified cell survival across models. In NPCs, reduced expression of PER1 and BMAL1 led to more extensive cell death in Li-NR vs. Li-R. Reduced REV-ERBa expression caused more extensive cell death in BD vs. control NPCs, without distinguishing Li-R and Li-NR. In IMHN, The REV-ERB agonist GSK4112 had strong effects on circadian rhythm amplitude, and was neuroprotective in mouse neurons and control NPCs, but not in BD NPCs. Expression of cell survival genes following STS and GSK4112 treatments revealed BD-associated, and Li-R associated differences in expression profiles.

Conclusions: We conclude that the neuroprotective response to lithium is similar in NPCs from Li-R and Li-NR. However, knockdown of circadian clock genes or stimulation of REV-ERBs reveal distinct contributions to cell death in BD patient NPCs, some of which distinguish Li-R and Li-NR.

Keywords: Bipolar Disorder, circadian rhythm, Apoptosis, neuroprotection

Disclosure: Alkermes Pharmaceuticals: Consultant (Self)

P135. Regional Cerebellar Structural Deficits Distinguish Psychostimulant-Free ADHD Youth With and Without Familial Risk for Bipolar I Disorder: A Cross-Sectional Morphometric Study

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Background: Attention deficit/hyperactivity disorder (ADHD) commonly precedes the onset of bipolar I disorder (BD), and prospective studies have found that antecedent ADHD significantly increases the risk of developing BD. Additionally, having a first-degree relative with BD robustly increases the risk of developing BD, and youth with a first-degree BD relative exhibit higher rates of ADHD and more severe ADHD symptoms. While these findings suggest that ADHD in conjunction with familial risk for BD may represent a different and more severe illness that confers greater risk for developing BD, associated neurostructural substrates remain poorly understood. In addition to its role in modulating motor functions, the cerebellum (CBM) has been implicated in cognitive and affective processing and has connections with regions implicated in mood including the amygdala and prefrontal cortex. Although reductions in CBM total and subregional volumes are among the most consistently reported features associated with the pathophysiology of ADHD, prior studies did not control for BD familial risk in ADHD youth and the contribution of psychostimulant medications remains poorly understood. Prior results regarding CBM structural abnormalities in BD have been inconsistent, with both increased and decreased regional volumes being reported. Studies in healthy first-degree relatives of BD patients suggest that increased CBM vermal volume may confer resilience to BD, though another study found that BD patients as well as their healthy siblings exhibited right CBM volume deficits which may represent a marker of genetic vulnerability. To our knowledge, there have been no studies that have directly compared CBM morphology in unaffected ADHD youth with and without a first-degree BD relative. In the present cross-sectional MRI study, we investigated regional CBM volumes in psychostimulant-free ADHD youth with and without a first-degree relative with BD and a typically developing healthy control (HC) group. Exploratory analyses evaluated relationships among regional CBM volumes and relevant symptom measures.

Methods: We enrolled ADHD youth (ages 10-18 years) with at least one biological parent or sibling with BD (“high-risk”, HR) or no first- or second-degree relative with a mood or psychotic disorder (“low-risk”, LR), and healthy controls (HC) with no personal or family history of a DSM-5 Axis I psychiatric disorder. All ADHD youth met DSM-5 criteria for ADHD (any type), were stimulant-naïve or had no exposure to psychostimulants for at least 3 months prior to enrollment, and had no current DSM-5 mood, conduct, eating, or psychotic disorders. High-resolution 3-dimensional T1-weighted brain images were collected using a Philips Ingenia 3T MRI scanner. Region-of-interest (ROI) and voxel-based morphometry (VBM) analyses were performed using the Spatially Unbiased Infratentorial toolbox implemented in Statistical Parametric Mapping software (SPM V.12). A total of 28 hemispheric lobules were included in the ROI analysis (FDR-corrected $p < 0.05$). Clinician ratings of ADHD (ADHD-rating scale), mania (Young Mania Rating Scale, YMRS), depression (Children’s Depression Rating Scale-Revised, CDRS-R), global functioning (Children’s

Global Assessment Scale), and global symptom severity (Clinical Global Impression-Severity Scale) were performed, and parents completed the Child Behavior Checklist (CBCL).

Results: A total of 151 youth (mean age 14.1 ± 2.5 years; HC, $n = 49$; low-risk, $n = 50$; high-risk, $n = 52$) were included in the analysis. There were no significant group differences in age, sex, BMI, handedness, or prior psychostimulant exposure in the ADHD groups. Both HR and LR ADHD groups differed from HC on all ratings ($P \leq 0.001$). Compared with LR, HR had higher hyperactivity/impulsivity subscale scores ($P = 0.018$), CDRS-R total scores ($P = 0.018$), YMRS total scores ($P < 0.001$), CBCL total ($P < 0.001$), internalization ($P = 0.001$), externalization ($P < 0.001$), and dysregulation scores ($P = 0.019$). For the ROI analysis, significant group differences were observed in 4 of 28 CBM lobules (bilateral lobules VIIIa, Right VIIb, and left X). Post hoc comparisons found that the HR group exhibited volume deficits in bilateral lobules VIIIa and Right VIIb compared with HC and LR groups, whereas both the HR and LR groups exhibited left X volume deficits compared to HC. Similar results were obtained using VBM. Among all ADHD youth, significant inverse correlations were observed between these ROI volumes and CBCL total and subscale scores including dysregulation.

Conclusions: Psychostimulant-free ADHD youth with familial risk for BD exhibit regional CBM volume deficits compared with ADHD youth without familial risk for BD and healthy youth. Inverse associations between regional CBM volumes and CBCL total and subscale scores among ADHD youth suggests clinical relevance and may represent a potential BD risk biomarker warranting additional investigation in prospective studies.

Keywords: ADHD, Familial risk, MRI

Disclosure: Nothing to disclose.

P136. Linking Neurophysiological, Behavioral, and Digital Biomarkers of Emotion-Based Impulsivity in Bipolar Disorder

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Background: Emotion-based impulsivity (EBI), engaging in rash action in response to intense emotions, is a transdiagnostic risk factor for psychopathology, in particular, bipolar disorder (BD). EBI represents an if-then dynamic process, yet, it has been largely studied using trait self-report measures, limiting our ability to identify mechanistic targets and develop interventions to reduce EBI. The goal of this study is to identify neurophysiological biomarkers of EBI that are associated with dynamic indices of EBI measured using behavioral tasks and digital assessments in real-world contexts

Methods: Individuals with BD I or II (ongoing recruitment, expected $n = 45$) completed a novel Emotion Go/NoGo task to assess behavioral indices of EBI (e.g., d' a measure of behavioral sensitivity) while undergoing electroencephalogram (EEG) recording. The P300 Event Related Potential and frontal Theta oscillatory power (4 – 12 Hz) are primary EEG biomarker targets as they are hypothesized to reflect higher order cognitive control and response inhibition. Following this in-lab assessment, participants completed a 28-day digital phenotyping protocol with smartphone assessments measuring impulsive urges and behaviors 4 times per day.

Results: Thus far, 18 participants with BD have complete and usable data. Participants completed a median of 95 digital assessments of EBI (Range = 38 – 112; 34 – 100% compliance). Initial analyses examined within- and between-person correlations between the average P300 amplitude during NoGo relative to Go conditions, d' , and real-world EBI. At the within-person level,

individuals with higher NoGo relative to Go P300 amplitudes (index of greater effort required to engage in response inhibition) were more likely to show reduced behavioral sensitivity on the Emotion Go/No task ($\rho = -0.11$, $p = .011$) and had higher real-world EBI ($\rho = 0.30$, $p < .001$). Between-person associations showed similar patterns but were not significant likely due to the small sample size. Higher NoGo relative to Go P300 amplitudes were associated with lower behavioral sensitivity ($\rho = -0.23$, $p = 0.26$) and had higher real-world EBI ($\rho = 0.40$, $p = 0.09$).

Conclusions: These results show preliminary support that neurophysiological biomarkers of response inhibition are associated with dynamic measures of EBI in behavioral tasks and real-world EBI as measured using digital assessments. Results in the full sample could provide compelling evidence that neuromodulation should be tested as an adjunctive intervention to reduce EBI in individuals with BD.

Keywords: Bipolar Disorder, EEG biomarkers, Digital phenotyping, impulsivity

Disclosure: Nothing to disclose.

P137. Spatial Expression of Select Williams Syndrome-Dup7 Genes in Neurons and Non-Neuronal Cell of Human Cerebral Cortex

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Background: Individuals with Williams syndrome (WS) who have 1 copy of ~26 genes at chromosomal locus 7q11.23 and individuals with 3 copies of these same genes (Dup7) have distinct, often contrasting neurobehavioral phenotypes. The specific neuronal expression and co-expression patterns in cortex may be important determinants of the behavioral and cognitive phenotypes. One distinguishing characteristic of individuals with Dup7 is a profound pain insensitivity compared to diploid or WS individuals, including cutaneous nociception and pain from injury to hard and soft tissues. In an earlier study we attributed this insensitivity to multiple copies of the synaptic vesicle docking protein Syntaxin 1A in nerve terminals of primary afferent nociceptors. This suggested an exploitable mechanism for analgesia; however, determining the regional cellular expression of the STX1A gene (one of the ~26 duplicated genes in Dup7) is important for considering potential side effects of new analgesics with systemic administration. In the present study, we investigate postmortem samples of human cerebral cortex for expression of several WS-Dup7 genes including STX1A, LIMK1, GTF2I, CLIP2, and VSP37D using multiplex fluorescence in situ hybridization. We determined whether these genes were expressed in excitatory or inhibitory neurons by hybridization for, respectively, VGLUT1, a vesicular glutamate transporter, or GAD1, a glutamic acid decarboxylase paralog. Sensory-motor cortices were examined to explore sensory processes in relationship to STX1A, as was inferior parietal sulcus, an area implicated in the visuo-constructive defect that characterizes WS.

Methods: Tissue collection and processing. Human cortex samples were provided by the Human Brain Collection Core of the National Institutes of Mental Health (Bethesda, MD). Tissue from 3 healthy males was analyzed; females will be included subsequent to this pilot. Donors were aged 53 to 56 years old, with post-mortem interval 15 to 28 hours.

Tissues were immersion fixed in neutral buffered formalin for 24 hours, embedded in paraffin, and sectioned at 6 microns. Slide-mounted sections were photobleached for 24 to 72 hours using a custom device to reduce auto- and lipofuscin fluorescence. RNAs

were detected using the RNAScope® Version 2 kit from Advanced Cell Diagnostics. Standard procedures were followed, including target retrieval for 15 minutes at 100°C and treatment with a protease-free reagent prior to multiplex imaging with Akoya Opal 520, Opal 570, Opal 620, and Opal 690 dyes. Hybridized tissue was scanned using a Zeiss Axio Imager.Z2 fluorescence microscope. Single fluorescent channel microscopy images were captured at each emission wavelength and compiled to generate multi-colored composites.

Results: Single gene localization showed enriched expression of LIMK1 in cortical pyramidal neurons of layer 5. LIMK1 was also present in nearly every cell type including endothelial cells of intra-parenchymal blood vessels. GTF2I was widely expressed in both neurons and many types of non-neural cells (e.g., oligodendrocytes and endothelial cells). CLIP2 displayed a more discrete, but widespread, punctate expression pattern. VGLUT1 was expressed in multiple layers and prominently in pyramidal neurons where the hybridization signal filled not only the neuronal perikarya but also the proximal portion (~200 µ) of the apical dendritic process. GAD1 was seen in neurons in multiple cortical layers. The GAD1-positive neurons often had large perikarya and were clearly distinguishable from the excitatory populations. Colocalization within glutamatergic neurons was evident for STX1A, LIMK1, GTF2I, CLIP2. Expression in glutamatergic neurons was most prominent for LIMK1 in pyramidal neurons, and signal for this message, like VGLUT1, filled the proximal apical dendritic shaft. STX1A was observed in all cortical layers and expressed in both excitatory and inhibitory neocortical neurons.

Conclusions: Multiple expression patterns for the various WS-Dup7 genes were observed. GTF2I was seen in neurons and cells in both gray and white matter, respectively, consistent with single nucleus (SN) sequencing, as well as the general and widespread function of this gene in transcriptional control. LIMK1 was strongly expressed in pyramidal neurons, but also in nearly every cell identified in the sections, a finding also consistent with SN sequencing. LIMK1 codes for a kinase that modifies the actin cytoskeleton. As such it is well placed to modulate activity dependent plasticity in pyramidal neurons which are known to alter spine density in a dynamic fashion. GAD+ neurons were found in most layers and coexpressed STX1A, LIMK1, GTF2I, and CLIP2. STX1A showed a broad expression profile in both glutamatergic and GABAergic neurons; this broad spectrum of expression indicates a critical role for STX1A in cortical presynaptic transmission mechanisms. The expression pattern also suggests that adaptation of the STX1A overexpression mechanism for analgesia using a systemic administration approach might be associated with cognitive side effects. However, we have not yet examined cortical expression of STX1B which may provide compensatory function at this level. These data provide a detailed analysis of the neuronal expression architecture in neocortex that have implications for regulation of neuronal plasticity and modulation of synaptic function.

Keywords: Pyramidal neurons, Neocortex, Multiplex fluorescent in situ hybridization, Gene expression, Pain

Disclosure: Nothing to disclose.

P138. Beyond D2: Exploring the Function of Nucleus Accumbens D3 Receptors in Antipsychotic Action

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Background: Second generation antipsychotics (SGAs) are widely used treatments for people with serious mental illnesses and other neurological conditions. Research into antipsychotic mechanisms

of action have predominantly focused on their actions at D2 dopamine receptors given our more involved understanding of these G-protein coupled receptors (GPCRs). Many of these SGAs also bind D3 dopamine receptors (D3R), a subset of the Gi-coupled D2R family, with some binding at higher affinity compared to D2Rs. Given recent advances in structural biology and pharmacology, we now have the tools to dissect out the function of D3R independent of D2R using selective agonists/antagonists and genetic deletion in mice. A convergent area of interest lies in the nucleus accumbens where D3Rs are enriched in contrast to areas in the dorsal striatum where D2Rs are predominant. Our understanding of the role of the nucleus accumbens in reward learning and motivation makes it an attractive target relevant for the treatment of many neuropsychiatric disorders.

Methods: For slice electrophysiology, whole-cell current- and voltage-clamp recordings will be made from nucleus accumbens medium spiny neurons in acute coronal slices prepared from p25-60 mice. D3R-expressing neurons will be targeted by crossing D3-Cre mice with Ai14 reporter mice, which carry a floxed td-Tomato fluorophore that can be visualized with 880nm 2-photon excitation. These cells will be compared to Cre lines that report expression of D1 and D2. To assess D3R function, we will measure intrinsic excitability properties, evoked EPSCs/IPSCs as well as miniEPSCs/IPSCs in conditions with wash-on of D3R selective agonists/antagonists (PD128907, GR103691) and D3R-binding SGAs (cariprazine, quetiapine, clozapine). We will also utilize constitutive D3-KO mice as well as conditional D3-cKO mice after stereotaxic injection into the nucleus accumbens with AAV-Cre-GFP. Confirmation of D1R and D3R expression will be confirmed with confocal imaging as well as RNA-in situ hybridization (HCR).

Results: Data from ex vivo slice electrophysiological experiments analyzing spontaneous mini events in D3R-expressing medium spiny neurons (MSNs) of the NAC revealed a decrease in miniature IPSC (mIPSC) amplitude following the addition of a selective D3R agonist ($p < 0.05$) in a paired t-test. No significant change was observed in miniature EPSC (mEPSC) amplitude or frequency after D3R agonist addition. These findings suggest that D3R activation in the NAC modulates inhibitory activity in MSNs, likely through downstream regulation of GABAA receptors. In evoked experiments, a similar reduction in IPSC amplitudes was observed following local electrical stimulation, with the paired pulse ratio (PPR) unchanged after D3R agonist administration, suggestive of a postsynaptic response for D3R agonist. Additionally, cariprazine, a partial D3 agonist, mirrored the effect of the D3R full agonist.

In contrast, quetiapine, which acts as a D3R G-protein antagonist and beta-arrestin agonist, demonstrated an increase in the magnitude of evoked post-synaptic inhibitory currents in D3R-expressing neurons. Further investigation revealed that this effect is observed in D1+/D3+ double-positive cells but not in D1+ or D3+ single-positive cells. These results suggest that quetiapine's effects may involve combinatorial interactions between D1 and D3 receptors, and potentially non-G protein pathways, such as beta-arrestin signaling.

Conclusions: By examining the specific function of D3R in the nucleus accumbens in mice we are able to better understand the action of currently prescribed SGAs and promote development of more efficacious treatments with less significant side effect burdens. Combining pharmacology and electrophysiological tools to study the local circuitry of the nucleus accumbens, we can begin to tease apart the physiological function of D3Rs in the ventral striatum and identify potential new targets for future therapeutics. Our current results show that D3R agonism results in decreased inhibitory activity in medium spiny neurons in the mouse nucleus accumbens. This suggests that the D3R-associated activity of currently prescribed SGAs such as cariprazine leads to modulation of downstream inhibitory tone.

Keywords: Neuropsychopharmacology (NPP), neurophysiology, slice electrophysiology, Antipsychotic drugs, Dopamine3 receptors
Disclosure: Nothing to disclose.

P139. Sex Differences in Mitochondrial Metabolism in Subjects With Cognitive Impairments

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Background: Many individuals develop debilitating cognitive impairments (CI) among other neuropsychiatric symptoms at some point in life. There is a dearth of new mechanistic models for the development of objective tools to diagnose and monitor CI. In rodent models, boosting mitochondrial metabolism of acetylcarnitine (LAC) leads to the regulation of cognitive function and hippocampal plasticity by epigenetic modulation of glutamatergic function. This mechanistic model in rodents provided the basis for the current study.

Methods: We used ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and computational approaches to determine the levels of LAC and its main derivative free carnitine in relation to cognitive dysfunction leveraging available plasma samples ($n=61$) from a well-characterized cohort, including subjects with CI and age- and sex-matched cognitively healthy controls (HC).

Results: Our data showed decreased levels of carnitine in relation to cognitive function as assessed by using the Mini Mental Status Exam (MMSE) in women but not men with CI as compared to age- and sex-matched HC. Furthermore, the magnitude of carnitine deficiency reflected the severity of cognitive dysfunction in a sex-specific manner (women: $p=0.015$; men: $p=0.441$). Our data also replicated the prior finding of decreased LAC levels in both women and men with AD, supporting the robustness of the study samples assayed in our new study. Using computational approaches, we found that the integration of these mitochondrial measures with canonical CSF biomarkers improves diagnostic accuracy.

Conclusions: Together with prior mechanistic studies in rodents, the current findings support future research on the development of individualized treatment models targeting sex-specific changes in mitochondrial metabolism.

Keywords: Epigenetics, glutamate, Hippocampus, Acetyl-L-carnitine LAC, Mitochondria

Disclosure: Nothing to disclose.

P140. Mast Cell Density in the Human Brain

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Background: Changes in the immune system, specifically inflammatory processes, are involved in the pathophysiology of depression. Indeed, accumulating evidence links elevated levels of inflammation with increased symptoms of depression. Mast cells (MCs), conserved immune cells, develop from hematopoietic stem cells in the bone marrow, mature and develop in all tissues, and help regulate the immune system through the release of pro- and

anti-inflammatory mediators. Interestingly, mast cell diseases, including systemic mastocytosis—a rare but chronic disease involving the clonal expansion and hyperactivity of mast cells—and mast cell activation syndrome (MCAS), a non-clonal mast cell disease, are associated with elevated levels of depression and cognitive dysfunction. Recently, acute or post-acute COVID-19 has also been associated with greater neuropsychiatric symptoms and shares a similar symptom profile with MCAS. However, there are significant gaps in our understanding of the biological pathways, including the role of mast cells in contributing to neuropsychiatric symptoms in these conditions. Currently, it is unknown if the release of mast cell mediators in peripheral tissues, which subsequently travel to the brain, is contributing to neuropsychiatric symptoms, or if local mast cell activation in the brain is the cause. Numerous MCs have been found in the brains of rodents, but reliable estimates of the quantity and location of MCs in the human brain are lacking. Understanding the mechanisms driving depression and cognitive dysfunction in systemic mastocytosis, MCAS, and COVID-19 can enhance our broader understanding of depression and cognitive functioning, particularly in other chronic inflammatory conditions where mast cells play a crucial role, such as asthma, inflammatory bowel disease, and cancer. It is uncommon for autopsies of patients with a mast cell disease to include the brain, despite the increased prevalence of neuropsychiatric symptoms. Therefore, comparing the density of mast cells in the brains of individuals with SARS-CoV-2 infection to those without is a first step in our understanding and could provide valuable insights.

Methods: We evaluated the quantity of MCs in human brain tissue from 5 pts who died of SARS-CoV-2 infection and 5 healthy controls (HCs). MCs were identified by KIT (CD117), tryptase, and chymase immunohistochemistry in brain tissue sections from the frontal cortex and medulla (areas thought to be involved in neuro-COVID) of each patient (total slides: $N=60$).

Results: Few MCs cells were found in the brain (e.g., choroid plexus ($n=17$), leptomeninges ($n=9$), Virchow-Robin space ($n=2$), and perivasculature of the cortex ($n=7$), and the ependyma ($n=1$) with the majority found in perivascular areas of the medulla (e.g., choroid plexus). Among all 10 pts, only 36 MCs in total were found in the brain (median IQR HCs=8, pts=4.5).

Conclusions: This is the first study to examine the density and location of mast cells in the human brain of individuals with COVID-19. Although underpowered to detect statistically significant group differences, quantitatively, HCs had more MCs in the brain compared to COVID-19 pts. Our findings revealed more mast cells in the brains of healthy controls, all of whom exhibited evidence of neurovascular atherosclerosis. This may be an important area for future investigation as mast cells are considered important players in the pathophysiology of neurovascular atherosclerosis, where they can impact cognitive function by influencing blood flow and plaque stability in cerebral arteries. This work is an important first step in understanding the role of mast cells in neuropsychiatric symptoms by identifying the density and location of mast cells in HCs and individuals with COVID-19. While MCs typically reside near blood vessels, and their products can affect blood vessel permeability and leakage, the absence of MCs in some COVID-19 pts suggests that localized mast cell activation may not be a factor in the development of neuropsychiatric symptoms in COVID-19. Corticosteroid therapy in pts with severe SARS-CoV-2 infection may have impacted MC numbers. Future work will involve evaluating the quantity and location of MCs in the brain of individuals with systemic mastocytosis and mast cell activation syndrome, measurement of local and systemic mediators (e.g., histamine and prostaglandins), characterizing the depression and cognitive profiles in these patients, along with their associations. Gaining insight into the mechanisms underlying depression in mast cell diseases will help advance our understanding of depression in the context of

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chronic immune activation and identify modifiable treatment targets to reduce the suffering of individuals with depression and comorbid chronic immune diseases.

Keywords: Depression, mast cells, depressive symptoms, inflammation, COVID-19

Disclosure: Blueprint Medicines, Cogent Biosciences: Consultant (Self). The Mast Cell Disease Society: Board Member (Self)

P141. Oligodendrocyte Myelin Glycoprotein Impairs Dendritic Arbors via Schizophrenia Risk Gene Trio

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Background: During adolescence, a critical developmental epoch coincident with the emergence of clinical symptoms of schizophrenia, cerebral cortical dendritic growth shifts from a rapid phase to reaching equilibrium. Understanding the pathways involved in this dynamic homeostasis is crucial to developing interventions targeting dendritic regression in Sz. Oligodendrocyte Myelin Glycoprotein (OMGp) expression peaks during adolescence and has a known role in regulating dendritic stabilization. However, the precise signaling pathways transduced by OMGp are unknown. We hypothesized that activation of OMGp signaling leads to alterations in the phosphorylation of downstream mediators of dendritic structure, some of which are Sz-risk genes.

Methods: To identify these pathways, we performed unbiased phospho-proteomic analysis after OMGp stimulation, revealing 2,991 phosphorylated proteins. Phosphomimetic and phosphonull constructs were employed to assess the functional role of OMGp-mediated phosphorylation at a novel phosphosite in a newly identified target protein of interest, Trio.

Results: Phosphorylation is a post-translational modification capable of regulating a protein's function, either activating or inactivating it depending on the site of phosphorylation. Interestingly, several schizophrenia risk genes were identified as phospho-targets, including the potent risk factor Trio, which has a known role in regulating neurite outgrowth and the cytoskeleton through its dual Rac/RhoA catalytic domains. Phosphomimetic Trio9 was deficient in Rac1 catalytic activity and induced loss of dendritic length and complexity compared to wild type protein. Moreover, phosphonull constructs blocked the OMGp-induced impairments in dendritic length and complexity. Together, these results highlight the ability of OMGp to regulate dendritic architecture by potentially inhibiting the Rac1 catalytic activity of Trio through phosphorylation.

Conclusions: We identified an overlap between the adolescent-onset OMGp signaling pathway and genetic risk for Sz (ie Trio). These results provide a potential mechanism contributing to the emergence of neuronal structural dysfunction and schizophrenia symptomatology during adolescence.

Keywords: dendrites, Adolescence- Critical Period, Schizophrenia (SCZ)

Disclosure: Nothing to disclose.

P142. Design of G Protein Selective Allosteric Modulators for the Neurotensin Receptor 1

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Background: The neurotensin receptor 1 (NTSR1) is a G protein-coupled receptor (GPCR) that modulates brain dopamine signaling and is a promising anti-addiction therapeutic target. NTSR1 signals through G protein- and β -arrestin-mediated pathways. Development of balanced NTSR1 agonists that activate the full spectrum of NTSR1 signaling is precluded by their on-target side effects. Biased ligands provide a path forward for NTSR1 drug development. SBI-553 is an allosteric molecule that binds NTSR1 intracellularly at the receptor-transducer interface and prevents NTSR1-Gq coupling while promoting NTSR1- β -arrestin activation. This compound reduces cocaine self-administration in rodents without the thermal and hemodynamic side effects characteristic of balanced NTSR1 agonism. Neuronal GPCRs can signal through the activation of one or more of 16 G α proteins (e.g., Gq, Gi, Go, Gs) as well as through β -arrestins. While NTSR1's calcium-dependent physiological effects implicate Gq/11 as preferred signaling mediators, NTSR1 is promiscuous, activating at least 12 different G proteins. In a comprehensive assessment of SBI-553's effect on NT-NTSR1 signaling, we observed that SBI-553 blocks NTSR1's activation of some G proteins (e.g., Gq) but not others (e.g., Go), biasing NTSR1 not only toward β -arrestin recruitment, but also toward alternative G protein coupling. Here, we undertake structure-activity-relationship studies to determine whether SBI-553's G protein selectivity profile can be changed by modifying its structure.

Methods: We hypothesized that SBI-553 analogs with different points of substitution would displace select G proteins depending upon the position, size, and direction of substitution. Based on a NTSR1:SBI-553:Go structure, we created a panel of 29 analogs. We assessed the ability of these compounds to antagonize NT-induced NTSR1 Gq, Gi, Go, and G12 activation and to activate β -arrestin independently using bioluminescence resonance energy transfer (BRET)-based assays in HEK293T cells transiently expressing NTSR1. Cells were treated with NT (10 pM-10 μ M) and SBI-553 or its analogs (300 nM - 30 μ M) alone and in combination. Compounds with G protein activation profiles distinct from SBI-553 were evaluated in orthogonal assays. Compounds differing in their ability to antagonize NTSR1 Gq activation in cells were compared in vivo. Following systemic administration and bilateral microinjection into the nucleus accumbens core (NAcC) of C57BL/6J mice, SBI-553 and analog SBI-593 were assessed for their ability to attenuate the Gq-mediated hypothermia induced by the balanced orthosteric NTSR1 peptide agonist PD149163. For NTSR1 signaling assays, 8-point concentration-response curves were generated using a 4-parameter sigmoidal model in GraphPad Prism version 10.1. Curve fits were compared using the extra sum-of-squares F test. For hypothermia inhibition studies, changes between treatment groups over time were identified by two-way repeated measures ANOVA. The proportion of mice exhibiting complete inhibition of hypothermia was compared between treatment groups using a Chi-square test. N = 6-8 mice/treatment. Male and female mice were used. A p-value of < 0.05 was accepted as statistically significant.

Results: Modifications to the SBI-553 scaffold produced NTSR1-targeting compounds with distinct G protein selectivity profiles. Compound SBI-342, an analog of SBI-553 with a dimethoxy-substituted quinazoline, differed from SBI-553 in its ability to antagonize NT-induced Go activation. While SBI-553 did not block NT-induced Go activation, SBI-342 reduced Go activation by 60% ($F(1, 90) = 93.9$, $p < 0.0001$). The action of SBI-342 was specific to NTSR1, as it did not alter Go activation in cells without NTSR1 and did not impair the ability of other GPCRs to activate Go. Compound, SBI-593, an analog of SBI-553 with a methoxy- and trifluoromethyl-substituted quinazoline, differed from SBI-553 in its inability to fully antagonize NT-induced Gq activation. While SBI-553 blocked all NT-induced Gq activation, SBI-593 only

reduced Gq activation by 30% ($F(1, 132) = 17.8, p < 0.0001$). SBI-593 did not alter Gq activation in cells without NTSR1. The difference in SBI-593 Gq antagonism was probe-independent, conserved across receptor species (e.g., human and mouse), and translated to differences in efficacy in a mouse model of NTSR1 agonist-induced, Gq-mediated hypothermia. SBI-553 (12 mg/kg, i.p.) attenuated PD149163 (0.15 mg/kg, i.p.)-induced hypothermia (FTreatment (1, 14) = 4.671, $p < 0.05$) but not SBI-593 (12 mg/kg, i.p.) did not (FTreatment, (1, 14) = 1.817, $p = 0.1990$). SBI-553 and SBI-593 (100 µg, intra-NAcC) both attenuated PD149163 (9.4 ng, intra-NAcC)-induced hypothermia. The proportion of mice exhibiting a complete block of PD14963-induced hypothermia, however, was greater in the SBI-553 (67%) than in the SBI-593 (0%) treatment group ($X^2 (1, N = 12) = 6.0, p < 0.05$). These data suggest that SBI-593 is less effective than SBI-553 at blocking NTSR1 Gq-mediated hypothermia and demonstrate that allosteric modulators with distinct G protein selectivity profiles can exhibit different in vivo activities.

Conclusions: Minor modifications of a single chemical scaffold targeting the NTSR1-transducer interface produced small molecules with distinct G protein activation profiles and in vivo activities. With greater signaling pathway selectivity, biased ligands may serve as a basis for safer, more efficacious therapeutics.

Keywords: G protein-coupled receptors, neurotensin, substance use disorders, allosteric modulator, structure-activity relationships

Disclosure: Nothing to disclose.

P143. Cellular Organization and Signaling of a Striatal Orphan Receptor, GPR88

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Background: Neuronal G protein-coupled receptors (GPCRs) are transmembrane proteins found in cellular membranes (plasma membrane, Golgi, primary cilia) that transduce intracellular signaling to modulate neuronal activity and behavior (neuromodulation). The Gi/o-coupled GPCR, inhibits the elevation of a chemical involved in dopamine neuromodulation, cyclic adenosine monophosphate (cAMP). The primary cilium, a diffusion barrier segregated membrane domain on the neuronal soma, may provide a compartment for insulated signaling. However the functional relevance of localizing to the cilium remains poorly understood for any Gi/o-GPCR. We hypothesize that Gi/o-GPCR signaling from the cilium is distinguishable from plasma membrane signaling and plays a key role in dopamine-related behavior. To examine the consequences of Gi/o-GPCR signaling from the cilium we focused on the striatal receptor, GPR88, because it co-expresses in D1-type medium spiny neurons

Methods: Culture cell experiments using mouse kidney cells or embryonic rat striatal neurons were used for fluorescence spinning-disk confocal imaging studies for receptor ciliary localization. To determine strategies to manipulate receptor localization to primary cilia, a candidate based approach was employed to perturb GPCR transport to cilia. siRNA or Cas9 mediated gene editing was used to screen ciliary transport machinery. Ciliary localization sequences were determined using chimeric and alanine-scanning. Animal studies examining cilia density and length in brain sections, $n = 7-8$ GPR88-KO and WT mice both genders were comprehensively analyzed separately using a machine learning trained AI. Knock-in studies, using $n = 4$ male and female GPR88-Venus mice, stained brain sections were imaged on a spinning-disk confocal. Signaling studies: at low-level

heterologously expressed receptors in cultured cells and at endogenously expressed receptors in cultured rat striatal neurons, we examine the hypothesis that the cilium acts as a location for cAMP signal insulation using cAMP and protein kinase A biosensors on a spinning disk confocal microscope. All data shown are SEM and p value < 0.05 is considered significant.

Results: We screened all reported Gi/o-GPCRs involved in neuromodulation for localization to primary cilia and found 12 Gi/o-GPCRs are significantly enriched in the ciliary compartment in a cultured ciliated cell model. We also determined that Gi proteins localize to primary cilia. We found that all 12 Gi/o-GPCRs required the ciliary adaptor, TULP3. Whereas, some Gs-GPCRs, such as dopamine D1, utilize more than one ciliary transport mechanism. We find that they engage transport protein interactors at distinct receptor domains, GPR88 involving the intracellular loop 3 and D1 involving the cytoplasmic tail. Additionally, in vivo, we find no change in cilia density or length in GPR88-knockouts as compared to wild-type mice. However, employing adult GPR88-Venus knockin mice, we observe neuronal subtype specificity for ciliary transport of GPR88 in GPR88 + /AC3 + /DARPP32 + GABA-ergic MSNs but not GPR88 + /AC3 + /SATB2+ excitatory cortical neurons.

Conclusions: These results suggest GPCRs utilize unique sequence identifiers and transport machinery to gain access into primary cilia, primary cilia house the required components for Gi/o-GPCR signaling and further suggest a functional implication for GPR88 ciliary signaling in striatal but not cortical signaling.

Keywords: cilia, GPCR, Striatal Dopamine signaling

Disclosure: Nothing to disclose.

P144. Egr1 is a Sex-Specific Regulator of Neuronal Chromatin, Synaptic Plasticity, and Behavior

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Background: Women have two-fold increased risk for anxiety and depression disorders compared to men. Clinical data indicate ovarian hormone fluctuations are a critical female-specific risk factor, although the underlying molecular mechanisms are unknown. To address this question, we previously demonstrated that anxiety-related behavior in female mice varies across the estrous cycle. We also showed concurrent hormone-driven changes in neuronal chromatin organization, gene expression, and synaptic plasticity in the ventral hippocampus (vHIP), an area critical for emotion regulation in rodents. Egr1, a transcription factor and estrogen-responsive immediate early gene, was identified as a candidate regulator of these effects. Here, we sought to provide a functional evidence that vHIP Egr1 drives estrous cycle-dependent, sex-specific gene regulation and behavioral and synaptic plasticity.

Methods: We first performed the analysis of the elevated plus maze behavior ($N = 8-12$ /group) and the expression of Egr1 and Ppp1r1b genes in the vHIP ($N = 5-6$ /group) across the physiological estrous cycle in female mice (one-way ANOVA; post-hoc Tukey test). The estrous cycle was tracked using vaginal smear cytology for three consecutive cycles and mice were classified into four estrous cycle phases: proestrus, estrus, metestrus, and diestrus. To address the causal role of Egr1 in gene regulation and behavior, we used AAV-mediated, neuron-specific Egr1 overexpression in the vHIP of intact male and ovariectomized (OVX) female mice. Three weeks following the vHIP injections of either Egr1 or eGFP control viral vectors, we performed analysis of behavior, including the open field (OF), elevated plus maze (EPM), and forced swim test (FST), in males and OVX females ($N = 10-12$ /

group/sex; Welch two-sample t-test). To further explore the role of *Egr1* in gene regulation in the vHIP, we profiled gene expression using RNA-seq (N = 6 animals/group/sex) and chromatin accessibility using ATAC-seq (N = 4 animals/group/sex) in vHIP neurons of male and OVX female mice overexpressing *Egr1* and in their respective controls. Differential gene expression was analyzed using DESeq2 at significance level $\text{padj} < 0.05$. Enrichment analyses were performed using FUMA at significance level $\text{padj} < 0.05$ and using Gene Set Enrichment Analysis (GSEA) with ranked gene lists. The ATAC-seq peak-calling was performed using MACS2. Differential chromatin accessibility analysis was performed by selecting high confidence peaks by overlapping the replicates in each group (n = 4/group/sex) and continuing the analysis with peaks that were called in at least 3/4 replicates. Motif analysis on chromatin accessibility (ATAC-seq) data was performed using HOMER and enrichment analysis on lists of annotated genes was performed with EnrichR.

Results: Using the physiological mouse model, we first show that anxiety-related behavior across the estrous cycle is primarily estrogen-driven and is associated with cyclical changes in *Egr1* mRNA. We observe concomitant increase in *Egr1* mRNA levels in the vHIP and decrease in anxiety indices with a surge of estradiol during the proestrus phase of the cycle. We further show that *Egr1* overexpression in vHIP neurons is sufficient to induce female-specific changes in anxiety- and depression-related behaviors, (OF, $P < 0.05$; EPM, $P < 0.01$; FST, $P < 0.05$), mimicking the high-estrogenic phase of the estrous cycle, with no significant effect in male animals. We further reveal that *Egr1* overexpression induces changes in gene expression ($> 1,000$ DEGs in males and females; $\text{Padj} < 0.05$) and chromatin organization in vHIP neurons underlying this behavioral phenotype, including sex-specific regulation of genes relevant to behavioral fear response and synaptic plasticity. Importantly, we show that *Egr1* preferentially opens *Egr1* motif-enriched chromatin, in a sex specific manner, revealing the first sex-specific immediate early gene and chromatin regulator in the brain. Finally, we show that *Egr1* overexpression increases dendritic spine density in the vHIP of OVX females ($P < 0.001$) and in males ($P < 0.01$), but the effect size was considerably larger in females ($d = 1.532$) than in males ($d = 0.4468$).

Conclusions: Here we show that overexpressing *Egr1*, a candidate estrous cycle-dependent transcription factor, induces sex-specific changes in vHIP neuronal chromatin, gene expression, and synaptic plasticity, along with hippocampus-dependent anxiety- and depression-related behaviors. Taken together, our results causally link *Egr1* to estrous cycle-dependent gene regulation and behavioral plasticity and establish a proof of concept for discovering new targets for sex-specific treatments for anxiety and depression disorders.

Keywords: sex difference, epigenetic regulation, synaptic plasticity, anxiety, depression

Disclosure: Nothing to disclose.

P145. L-Type Calcium Channel Blockade With Verapamil Prevents Noise-Induced Neuronal Dys-Synchrony

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Background: Previous studies have established the protective effects of calcium channel blockade on the peripheral auditory system in response to noise exposure. While these studies implicate L-type calcium channels (LTCCs) in noise generated dysfunction in the auditory periphery, contributions of LTCCs to noise-induced central dysfunction remain unclear. To begin to elucidate the roles of LTCCs in hearing, peripheral and central

auditory function were assessed longitudinally after administration of the widely used antihypertensive medication, verapamil – an LTCC blocker prior to a loud noise exposure. Neuronal synchrony and activity were assessed by analyzing the relationship between farfield potentials (auditory brainstem responses, ABRs) and gap inhibition of the acoustic startle reflex (giASR).

Methods: Twenty-five male Sprague-Dawley rats were divided into four groups and given either verapamil (30 mg/kg) or saline intraperitoneally. The treatment groups were no noise exposure plus saline (n = 6) or verapamil (n = 5) and noise exposure plus saline (n = 7) or verapamil (n = 7). The noise groups were unilaterally exposed to a 16 kHz, 106 dB SPL tone for one hour. ABR and giASR were performed immediately after treatment (day 0) and one (day 1) and five (day 5) day[s] after treatment at 12 and 20 kHz. ABR amplitudes (wave I and V) and thresholds were evaluated. During the giASR testing each group was subjected to a 20 ms startle noise during silence, in the presence of a background tone (at 45- and 60-dB SPL), and in a background tone with a gap prior to startle.

Results: Verapamil did not affect hearing thresholds in the no noise group but did reduce the time to recover from the noise induced temporary threshold shift (TTS). On day 0 of noise exposure there was a significant wave I amplitude reduction in the noise saline group compared to the no noise saline and the no noise verapamil groups at both 12 and 20 kHz ($p < 0.05$). Administration of verapamil prevented the noise induced decrease; therefore, the noise verapamil group was not significantly different when compared to the no noise saline and the no noise verapamil groups on day 0. Similarly, noise exposure resulted in a reduction of Wave V amplitude ($p < 0.05$) and verapamil administration prevented this reduction at both 12 and 20 kHz. However, the no noise verapamil group had a total loss of wave V amplitude on day 0 at both 12 and 20 kHz ($p < 0.05$). Before the treatment, all rats could inhibit their startle. After noise exposure, a trend towards enhanced ability to inhibit startle response was observed. However, five days after treatment the noise verapamil group had a reduction in the ability to inhibit startle compared to the no noise saline and noise saline groups.

Conclusions: The present study demonstrates that voltage-gated calcium channels differentially contribute to peripheral and central auditory function. Blockade of LTCCs does not appear to disrupt neuronal activity (hearing thresholds and giASRs are unchanged). However, this blockade results in a loss of neuronal synchrony centrally. Noise decreases neuronal synchrony both peripherally and centrally and verapamil prevents this outcome. Overall, these findings suggest calcium channel dysfunction may be an underlying mechanism for disorders involving hypo- or hyper-synchrony, but not neuronal hyperactivity. Calcium mobilization, whether by acoustic overexposure or calcium channel blockade may lead to an imbalance of extracellular and intracellular calcium ions. Therefore, exploring relationships among neuronal synchrony, neuronal hyperactivity, and calcium mobilization may enhance our future understanding of mechanisms and aid in identification of therapeutic approaches related to noise induced auditory dysfunction.

Keywords: L-type Voltage-Gated Calcium Channel, neuroprotection, Auditory system, Gap inhibition of Acoustic Startle Reflex, Auditory Brainstem Response

Disclosure: Nothing to disclose.

P146. Uncovering Molecular Rhythmicity Within the Subgenual Anterior Cingulate Cortex at the Resolution of Individual Cortical Layers

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Background: The human brain exhibits diurnal patterns of gene expression in both cortical and subcortical regions. Within psychiatric disorders, alterations to these molecular rhythms have been uncovered. Further, postmortem studies of psychiatric disease have found transcriptional changes that are layer specific. Although prior studies have investigated changes to molecular rhythmicity and laminar signatures of gene expression separately, no study to date has focused on the intersection of the two. Thus, we first sought to characterize these aspects in neurotypical subjects within the subgenual anterior cingulate cortex (sgACC). Given that the sgACC lies at the interface between prefrontal cortical regions responsible for cognition and subcortical regions which process information related to emotion, it is a critical site of inquiry into psychiatric conditions.

Methods: We performed RNA-sequencing on cortical layers 3 (L3) and 5 (L5), isolated using laser capture microdissection, from human postmortem sgACC tissue of neurotypical subjects ($n = 40$; 30 Male, 10 Female). Joint rhythmicity analyses were used to determine and compare 24-hour rhythms in gene expression between L3 and L5 as well as between sexes within layers.

Results: We identified significant transcript expression rhythms ($p < 0.01$) in both L3 and L5 of the sgACC. Strikingly, there were nearly three times as many rhythmic transcripts within L5 (364 transcripts) compared to L3 (119 transcripts). Further, L3 and L5 had minimal overlap in rhythmic transcripts (27 transcripts), suggesting divergent biologic functions between layers. Additionally, significant sex differences emerged in transcriptional rhythms. Within males, substantially more genes were rhythmic in both layers compared to females (163 vs. 79 transcripts in L3 and 269 vs. 102 transcripts in L5), and there was very little overlap in transcripts identified as rhythmic in both males and female subjects within a layer (L3 = 0; L5 = 6, $p < 0.01$). These findings indicate that the underlying biology of gene expression rhythmicity is both layer- and sex-specific.

Conclusions: Together, these findings reveal distinct patterns of transcriptomic rhythms within the sgACC, including normative differences across layer and sex. As previous analyses have shown significant differences in gene expression rhythms in schizophrenia, major depressive disorder, and across subjects with psychosis using bulk RNA-sequencing, we are moving forward with a large, cross-diagnosis cohort ($n = 120$, including subjects with schizophrenia, bipolar disorder, and major depressive disorder) to determine how the interaction of layer- and sex-specific differences in gene expression rhythms are altered in psychiatric illnesses. Our results will be critical in future studies that seek to interpret how disruptions to these rhythms may contribute to psychiatric pathology in this brain region.

Keywords: Anterior Cingulate Cortex (ACC), Circadian Rhythms, Laser Capture Microdissection, Postmortem Human Brain Tissue, RNA-sequencing

Disclosure: Nothing to disclose.

P147. Extracellular Vesicle miRNAs at the Maternal-Fetal Interface may Instruct the Pathogenesis of Neurodevelopmental Disorders via Impacts on Fetal Brain Microglia

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Background: Diseases of pregnancy and of the placenta are linked to offspring neurodevelopmental disorders (NDD) and lifelong psychiatric risk. Extracellular vesicles (EVs) are one placenta-brain mechanism which may impact neurodevelopment.

EVs are nanoparticles ($< 200\text{nm}$) containing various proteins and nucleic acids including miRNAs which are abundantly produced by the placenta. EV miRNA content is changed by placental diseases such as preeclampsia, which is also an NDD risk factor. Here, we examined maternal EV miRNAs in a cohort of children with and without NDD to reveal post-transcriptional prenatal programming mechanisms. We also conducted exploratory preclinical studies to determine EV uptake in the fetal brain in vivo.

Methods: For clinical work, EVs were purified from pregnant participant plasma samples from the Iowa Perinatal Family Tissue Bank (PFTB; IRB: 200910784) if their pregnancy resulted in a child (either sex) with NDD (ASD, ADHD, and/or anxiety disorder; $n = 9$) or no psychiatric diagnosis ($n = 11$) by age 10. Placentas ($n = 3$) were used for placental expression studies. Samples were matched on clinical and demographic features. Exosomes were isolated by ultracentrifugation and RNA isolated for profiling via A-Set TaqMan low density array or qPCR. Differentially expressed transcripts were compared with SFARI Genes (1128 genes, 5/23/23) using enrichment testing via 1-tailed Fisher's exact test. miRNA target scores were generated utilizing a miRNA target prediction based on support vector machines and high-throughput training (miRDB), including targets with prediction scores > 60 and miRNAs with < 800 targets. Functional annotation was performed using miRSystem, gene prediction programs (DIANA, miRanda, miR-Bridge, PicTar, PITA, rna22, TargetScan), and pathway programs (KEGG, Biocarta, PID, Reactome).

For in vitro work, organotypic slices ($N = 3$) from E14 mice were sectioned (100um thick) and incubated for 24 hours in 108 GFP + EV/mL of HEK293-isolated GFP+ EVs and imaged by confocal microscopy. For in vivo work, we crossed a female B6.FVB-Tg(Ada*-Cre)5Xiy/J ("AdaP-Cre"; transgenic expressing cre under Ada promoter and chimeric Tpbpa/Ada placental trophoblast enhancer) with a male B6;129S1-Gt(Rosa)26Sortm1(CAG-CD9/GFP)/Dmfl/J ("TIGER"; Cre-inducible His-tagged CD9/TurboGFP reporter targeted to EVs) to derive a pregnancy ($N = 1$) with GFP+ trophoblast-derived Vesicles. We then ran spectral FLOW cytometry (Aurora5) for CD11b (microglia) and GFP+ EVs in maternal and fetal tissues isolated at E18 from this Tiger x AdaP-Cre pregnancy.

Results: Clinical cohort studies revealed multiple miRNAs as significantly increased (miR-499a-5p, 133b, 525-3p, 493) or decreased (miR-518e, 517c, 455-3p, 518-f) in IDD samples versus controls ($p < 0.05$). All were also expressed in placental EVs. The primary gene targets (enrichment score > 1.5) of differentially-expressed miRNAs are related to neuronal systems, synaptic transmission, axonal guidance, and developmental biology. Of 797 genes with high computationally predicted target scores (> 60 , miRDB), 125 are known ASD risk genes (SFARI) ($P < 0.0001$ overrepresentation). For example, ASD risk genes including XPO1, KMT2C, and DMXL2 are targeted by miR-133b and miR-455-3p. Plac1 was expressed in pregnant plasma EVs, demonstrating trophoblast origins.

Organotypic slice culture experiments in murine brain revealed that HEK293-derived, GFP+ EVs (dosed at physiological concentration: 108 EV/mL) are taken up by E14 brain microglia within 4 hours. Confocal live imaging in cross-section revealed that EVs are internalized by microglia, rather than aggregating on their surface. We also validated these results in vivo. We found that placental EVs localize to the murine E18 fetal brain in the Tiger x AdaP-Cre model. While 19.71% of microglia contained Vesicles, only $< 1\%$ of non-microglia brain cells did. In maternal circulation, 15% of EVs were GFP+ indicating placental origins. $< 1\%$ of maternal spleen and maternal circulating macrophages were GFP+, showing preferential EV uptake by fetal brain microglia versus other macrophage populations.

Conclusions: EV-bound miRNAs are altered in pregnancies resulting in a child with NDD. These miRNAs are also expressed in

placental EVs, and regulate genes implicated in neurodevelopmental processes and autism risk. Our results demonstrate a mechanism by which EVs in pregnancy may alter post-transcriptional, gene regulatory processes in the developing fetal brain, increasing risk for NDDs including ASD. In exploratory animal work, we further found that placental EVs infiltrate late gestation fetal brain in vivo, where they are preferentially taken up by microglia. This does not occur broadly in circulating or maternal macrophages, further evidencing selective EV aggregation in microglia. Collectively, this work demonstrates a plausible mechanism by which placental biology may communicate with developing fetal brain to program neurodevelopmental risk.

Keywords: Neurodevelopmental Disorders, Extracellular vesicles, placenta, pregnancy, translational science

Disclosure: Nothing to disclose.

P148. Elucidating the Molecular Basis of Accelerated Aging in Substance Use Disorder: Integrative Transcriptomic and Methylomic Analysis

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Background: Substance use disorders (SUD) significantly accelerate the onset of age-related diseases and mortality. Accelerated Aging (AA) in individuals with SUD is a crucial factor in these outcomes, as indicated by DNA-methylation changes measurable by Epigenetic Clocks (EC). Chronic substance use exacerbates physical health deterioration and accelerates neurological decline, leading to cognitive impairments and other mental health issues. Despite the importance of AA in individuals with SUD, existing assessments primarily utilize ECs designed for non-brain tissues, potentially overlooking brain-specific aging processes, and the molecular mechanisms underlying AA in brain are not well understood. This study aimed to elucidate the transcriptomic and methylomic mechanisms of AA in postmortem brain from individuals with SUD, utilizing epigenetic clocks specifically designed for brain tissues.

Methods: We employed brain-specific ECs (DNAmClock Cortical, PCBrainAge, and CerebralCortexCommon) to evaluate biological aging in 75 postmortem brain samples (BA9) from the UTHealth Brain Collection ($n=17$ Controls and $n=51$ SUD). K-mean cluster analysis categorized participants into those with accelerated aging (AA+) and those without (AA-). We identified differentially expressed genes (DEGs) between aged and non-aged groups using RNA seq, and further evaluated differentially methylated probes (DMPs) of the DEGs using EPIC methylation arrays, followed by functional enrichment analysis for biological interpretation.

Results: Within SUD, we identified 24 subjects with AA (SUD + AA) and 27 without (SUD-AA). Differential expression analyses between these groups identified 32 DEGs (fold-change $> .58$ or $< -.58$, $p\text{-val.} < 0.2$), including genes implicated in the immune system and inflammation (e.g., CASP10, GBP2, HLA-DRA, TNFRSF4), metabolism and enzymatic activity (e.g., ADH1B, FTCD, KLKB1, STEAP1B), neural and sensory functions (e.g., MTNR1A, ECEL1), and cell signaling and regulatory proteins (e.g., CHN2, HSPB1, GSDMB). DMP analysis of the 32 target genes revealed 9 CpGs with altered methylation levels using the same fold-change and p-value thresholds: MEPE, ECEL1, KLKB1, CHN2, GBP2, HMCN1, GBP2, HLA-DRA, and MTNR1A. While there were no significant differences in aging acceleration between primary substance use diagnoses (opioid, stimulant and alcohol use disorder), we identified a significant overlap in enriched pathways

across the different SUD, including immune response, metabolism, and cell adhesion signaling pathways.

Conclusions: Our findings reveal pathways associated with immune response and inflammation to be significantly enriched in individuals with SUD exhibiting AA. This suggests an inflammatory basis for AA in this population, with prominent involvement of the interferon-gamma (IFN- γ) pathway. These observations align with previous studies that have demonstrated a strong association between inflammation and biological aging. For instance, prior research has identified IFN- γ as a critical factor contributing to accelerated aging. Additionally, tuberculosis-induced DNA hypermethylation and oxidative stress have been linked to premature cellular aging and inflammation. The convergence of our results with these studies underscores the importance of inflammation in the context of AA in SUD. Further investigation into these pathways is essential to elucidate their precise roles and potential as targets for interventions aimed at mitigating accelerated aging in SUD. This study presents a comprehensive multi-omics approach to SUD-related AA. By analyzing postmortem brain samples, we identified critical genomic and epigenetic markers that could inform targeted interventions to mitigate the risk of accelerated aging in individuals with SUD.

Keywords: Epigenetic Aging, substance use disorders, Postmortem Brain Tissue, Transcriptome, inflammation

Disclosure: Nothing to disclose.

P149. Enduring Modulation of Dorsal Raphe Nuclei Regulates (R,S)-Ketamine-Mediated Resilient Stress-Coping Behavior

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Background: Ketamine may be a novel pharmacologic approach to enhance resilience and protect against stress-related disorders, but the molecular targets underlying this response remain to be fully characterized. The multifunctional protein p11 is crucial in the pathophysiology of depression and antidepressant responses. However, it is still unclear whether p11 plays a role in the pro-resilience effects induced by ketamine.

Methods: Wild-type (WT), constitutive global p11 knockout (p11KO), or p11 heterozygous (p11HET), p11 floxed WT (p11flx/flx), conditional knockout of p11 in Ser-expressing neurons (Sert-p11cKO, p11flx/flx Sert-Cre +/−), mice were generated on a C57BL/6J background of both sexes. The group sizes were 6-12 mice/group.

(R,S)-ketamine was purchased from Sigma-Aldrich (St. Louis, MO), dissolved in sterile saline (0.9% NaCl), and administered via intraperitoneal (i.p.) route at a dose of 15 mg/kg.

Animal were pretreated with saline or ketamine and underwent daily immobilization stress for two weeks and where thereafter tested in behavioral paradigms of emotionality.

Postmortem, brain tissue was examined with fluorescent in situ hybridization (RNA Scope), in situ hybridization, immunofluorescence, and matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI).

All statistical analyses were done using GraphPad Prism (GraphPad, San Diego, CA, USA). The D'Agostino-Pearson test was used to assess data normality. The differences among experimental groups were determined by two-tailed unpaired Student's t-test or two-way analysis of variance (ANOVA) followed by Tukey's post hoc test, when appropriate. Differential abundance analysis of neurotransmitters and metabolites (shown in volcano plots) were performed using independent t-tests and the Benjamini-Hochberg multiple-testing correction to control the false discovery rate (FDR < 0.05). The details of statistical tests and

their outcomes are presented in the Supplementary Information. Data are presented as mean $\pm$ standard error of the mean (SEM). A value of $P < 0.05$ was considered significant.

Results: We demonstrated that prophylactic administration of ketamine buffers passive stress-induced maladaptive phenotypes induced by chronic stress exposure. Spatial neurotransmitter and metabolite analysis revealed that prophylactic ketamine was also effective in blunting stress-induced disturbances of tryptophan metabolism in dorsal raphe nuclei (DRN). Additionally, we demonstrated that ketamine prevented chronic restraint stress-induced p11 reduction in DRN, a highly p11-enriched region. Furthermore, we provide novel evidence indicating that p11 deficiency regulates susceptibility to stress-induced depression-related phenotypes, and these behavioral maladaptations are dependent, at least in part, on p11 function in serotonergic neurons. Spatial neurotransmitter and metabolite analysis also showed a reduction of tryptophan and dopamine metabolism in DRN of serotonergic p11-deficient mice. Viral-mediated downregulation of p11 within DRN induced a stress-susceptible phenotype. Finally, our results also unveiled that the ability of ketamine to elicit a pro-resilience response against stress-induced maladaptive phenotypes was occluded when p11 was selectively deleted in serotonergic neurons.

Conclusions: We showed a previously unexplored role of the DRN circuit in regulating stress susceptibility and resilience-enhancing actions of ketamine.

Keywords: (R,S)-ketamine, Emotional stress, s100a10

Disclosure: Nothing to disclose.

P150. TRV045, a Novel and Selective S1PR1 Receptor Agonist, Does Not Desensitize or Downregulate the S1PR1 Receptor in Mouse Brain and Spinal Cord

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Background: TRV045 is a novel S1PR1 selective agonist that has demonstrated analgesic-like properties in animal models of neuropathic pain after both single and repeat dose treatment, without showing sustained lymphopenia known to occur with other S1P modulators such as fingolimod, which also display anti-nociceptive effects in animal models. Both agonist signaling and functional antagonism have been proposed as the underlying mechanism of anti-nociception with S1P modulators like fingolimod (Sim-Selley, L, et al. 2018; Chen, Z, et al. 2019). However, the molecular mechanism of TRV045 has not been studied. We sought to determine whether TRV045 acts through agonist or functional antagonist action by studying whether TRV045 causes: (1) desensitization of S1PR1-mediated G-protein activation (via [35S] GTP γ S binding assay), or (2) downregulation of S1PR1 protein expression (via immunoblotting).

Methods: The neuropathic pain model was established in C57BL/6J young adult male and female mice (7-8 animals per group) by injection of paclitaxel (8 mg/kg i.p.) or its vehicle at days 1, 3, 5, 7. Mice then received single or repeated daily oral doses of either TRV045 (10 mg/kg), fingolimod (1 mg/kg), or vehicle for 14 days. Subsequently, tissue from CNS regions involved in pain processing (including lumbar spinal cord, periaqueductal gray (PAG), amygdala, and cingulate cortex) were isolated 24 hours post last dose. Regions were then tested in the [35S]GTP γ S binding assays in vitro using both S1P and the S1PR1-selective SEW2871 as agonists. GFP-S1PR1 knock-in mice received the same treatments and similar CNS tissue regions were isolated 24 hours post last dose for immunoblot using an anti-GFP antibody. The

anti-GFP antibody method was used due to the absence of an acceptable, highly specific anti-S1PR1 antibody.

Results: TRV045 and the other tested S1PR1 agonists have differing potencies but similar efficacies for G-protein activation in the pain processing CNS tissues isolated from untreated mice. Paclitaxel had no effect on S1PR1-mediated G protein signaling. In both paclitaxel and vehicle groups, acute single dose treatment with fingolimod, but not TRV045, decreased S1P- and SEW2871-stimulated [35S]GTP γ S binding in the lumbar spinal cord and PAG. Acute single dose treatment of neither fingolimod nor TRV045 changed the level of GFP-S1P protein expression in all tissue regions examined with the exception of a slight decrease in cingulate cortex. Repeated treatment with fingolimod, but not TRV045, decreased S1P- and SEW2871-stimulated [35S]GTP γ S binding by approximately 70% in the lumbar spinal cord and PAG. Fingolimod showed significant decreases in both S1P and SEW2871 Emax values and slight increases in EC50 values. Repeated treatment with fingolimod decreased the level of GFP-S1PR1 protein expression by approximately 30% in the lumbar spinal cord and PAG of the paclitaxel group, in the amygdala of both groups and in the cingulate cortex of the vehicle group, whereas TRV045 had no effect on the levels of S1PR1 in all tissues of either group.

Conclusions: We have previously shown that TRV045, like fingolimod, shows clear and sustained analgesic-like properties in animal models of neuropathic pain, without producing sustained lymphopenia. The data presented here provides molecular insights into these actions and suggest that TRV045 behaves differently from S1P modulators like fingolimod at the S1PR1 receptor in CNS pain processing regions. Specifically, unlike fingolimod, which causes S1PR1 desensitization after acute treatment and causes S1PR1 desensitization and downregulation after repeated treatment, TRV045 produces neither downregulation nor desensitization of this receptor after either acute or repeat dose treatment. These data suggest that TRV045 exerts its analgesic-like effects through agonism rather than functional antagonism. TRV045 is an experimental drug not approved by the FDA.

Keywords: sphingosine 1-phosphate receptor, antinociception, Animal Models, neuropathic pain

Disclosure: Trevena, Inc: Employee (Self)

P151. Generation of a Spatio-Molecular Atlas of the Human Nucleus Accumbens

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Background: Located in the ventral striatum, the nucleus accumbens (NAc) receives dopaminergic projections from the ventral tegmental area, and is a key regulator of reward processing and reward-related learning. Molecularly-defined cell populations that are localized to distinct anatomical subregions of the NAc differentially contribute to circuit function and reward-related behaviors in rodent models. However, the extent of molecular conservation in human NAc of these spatially-defined cell types is not well understood. While single-nucleus RNA-sequencing (snRNA-seq) studies in human NAc have identified distinct populations of medium spiny neurons (MSNs) and inhibitory neurons, these studies lack spatial resolution, which is critical given their diverse cellular functions across NAc subregions.

Methods: We used the 10X Genomics Visium and Chromium platforms to generate paired spatial transcriptomic and snRNA-seq data in the postmortem human NAC ($n = 10$ adult, neurotypical brain donors, 6 male/4 female). Tissue blocks containing the NAC were dissected from fresh-frozen coronal hemislabs utilizing established neuroanatomical landmarks. Inclusion of the NAC was confirmed by visualization of histological stains and multiplex single molecule fluorescent in situ hybridization (smFISH) using established marker genes. To encompass the entirety of the structure, NAC tissue blocks were scored and 10 μm cryosections were mounted onto Visium slides (2-5 capture areas per donor). Following tissue collection for Visium, adjacent cryosections from each tissue block were collected for snRNA-seq. Nuclei were isolated and fluorescence activated nuclear sorting (FANS) was performed before Chromium capture. After standard preprocessing and quality control to remove empty droplets, doublets, and poor-quality nuclei (for Chromium) or low-quality spots (for Visium), 103,785 high-quality nuclei and 176,013 Visium spots were retained, respectively. To define cell type-specific clusters in snRNA-seq data, batch correction and graph-based clustering were performed. To define spatial domains in Visium data, we used a data-driven, multi-sample workflow consisting of unsupervised spatial domain detection with PRECAST using the top 2,000 spatially variable genes. We considered a range of spatial domain resolutions (k) and selected $k = 10$. The 10 data-driven spatial domains were biologically annotated based on canonical marker genes and anatomical positioning.

Results: Analysis of snRNA-seq data identified 25 transcriptionally distinct cell types, including 16 neuronal and 9 non-neuronal populations. We identified four dopamine receptor D1 (DRD1)-expressing MSN subpopulations and two DRD2-expressing MSN subpopulations. Two DRD1-expressing MSN subpopulations corresponded to D1+ islands based on marker gene expression of DRD1, OPRM1, and RXFP1. We also identified nine subpopulations of inhibitory neurons, amongst which there were somatostatin (SST)-expressing neurons, and a small cholinergic interneuron population, marked by expression of CHAT. Analysis of Visium data revealed four domains enriched in MSNs, including a domain highly expressing DRD1, OPRM1, and RXFP1, residing within the ventromedial quadrant of the striatum, corresponding to the location of previously characterized D1 islands. We identified inhibitory neurons expressing SST and CORT scattered throughout the NAC, while a different population of inhibitory neurons expressing KIT and ELAVL2 appeared only within most medial sections of the NAC. Additional spatial domains corresponding to white matter, distinct pockets of astrocytes, and other non-neuronal cells were identified.

Conclusions: We generated the first data-driven, transcriptome-wide spatial molecular map of the human nucleus accumbens at cellular resolution, which identified novel spatial domains, including molecularly defined D1 islands. By characterizing spatial gene expression profiles of functionally-relevant cell populations within the cytoarchitecture of the human NAC, our dataset provides a valuable resource for the scientific community.

Keywords: nucleus accumbens, D1+ islands, spatial transcriptomics, single-nucleus RNA-sequencing, medium spiny neurons

Disclosure: Nothing to disclose.

P152. The Alcohol Metabolite Acetate Affects Chromatin and Gene Expression in a Sex- and Brain Region-Specific Manner

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Background: We have recently shown that alcohol-derived acetate is directly incorporated into brain histone acetylation,

and that inhibiting this incorporation blocks ethanol-induced conditioned place preference. The specific post-translational histone modifications fueled by acetate, and the underlying gene expression programs that facilitate the encoding of alcohol-associated memories, however, remain poorly understood.

Methods: We used liquid chromatography coupled with tandem mass spectrometry to identify the effect of acetate on histone post-translational modifications in various brain regions following i.p. exposure to acetate. RNA sequencing was used to assess effects on gene expression. Both sexes were studied ($n = 4$ per sex per group).

Results: We show that acetate is deposited onto histones in a time- and dose-dependent manner, and increases the acetylation of both canonical histones and histone variants. Intriguingly, this effect is specific to the dorsal hippocampus, and only present in female but not in male mice. Moreover, acetate leads to the upregulation of activity-dependent genes and genes previously linked to spatial learning in the dorsal hippocampus of female mice.

Conclusions: Overall, our findings outline in detail the epigenetic and transcriptional mechanisms influenced by the alcohol metabolite acetate, which play a critical role in the formation of alcohol-associated memories. These results will thus guide the development of future therapeutic interventions for alcohol use disorder.

Keywords: Histone acetylation, Dorsal Hippocampus, gene expression, acetate

Disclosure: Nothing to disclose.

P153. Sustained Suppression of Dopaminergic Tone Augments Homeostatic Synaptic Plasticity

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Background: Dopamine is a monoamine neurotransmitter which plays a fundamental role in reward processing, motivation, and movement. Dopamine is primarily synthesized from midbrain structures, the ventral tegmental area (VTA) and substantia nigra. Abnormalities in dopaminergic activity are implicated in variety of neurological and psychiatric disorders, including Parkinson's disease, substance use disorders and schizophrenia. Dopaminergic projections from the VTA to prefrontal cortex play a critical role in motivation, attention and decision-making. A decrease of dopaminergic activity in this pathway is associated with apathy, social withdrawal, and isolation, some of the negative symptoms of schizophrenia. Dopaminergic transmission in the prefrontal cortex can modulate neuronal excitability and neurotransmitter release. However, despite extensive efforts to delineate how dopamine modulates glutamate release, its mechanism remains elusive due to its numerous effectors and the significant heterogeneity of dopaminergic receptors across various brain regions.

Methods: We used primary rat ventral tegmental area-cortex co-cultures to suppress dopaminergic tone acutely and chronically by pharmacological or viral gene delivery methods. The effects of these manipulations on synaptic glutamate release were assessed by whole-cell patch clamp electrophysiology.

Results: We observed that an acute decrease in dopaminergic tone leads to an increase in spontaneous glutamate release frequency through a presynaptic calcium-dependent mechanism involving G-protein coupled receptor activity. However, a long-term reduction in dopaminergic tone results in synaptic upscaling of glutamatergic synapses due to hypoactivity of both D1 and D2 receptors. In addition to chronic antagonism of D1 and D2 receptors

using selective antagonists, common antipsychotic medications such as haloperidol and chlorpromazine also triggered homeostatic synaptic upscaling. These data highlight a key role for dopaminergic tone on postsynaptic glutamatergic terminals. These effects extend beyond first-generation antipsychotics, where long-term treatment with olanzapine, clozapine and aripiprazole also produced similar outcomes resulting in robust homeostatic synaptic upscaling.

Conclusions: Our findings highlight a complex differential interplay of dopaminergic tone on glutamatergic synapses in the prefrontal cortex. We find that the acute suppression of dopaminergic tone affects presynaptic glutamatergic terminals and increases spontaneous release frequency, whereas chronic suppression affects postsynaptic terminal inducing homeostatic synaptic upscaling.

In clinical practice, the antipsychotic action results in cumulative improvement in psychotic symptoms over the course of 3–4 weeks. The effect of long-term D2 antagonism on homeostatic synaptic plasticity might explain some of the long-term therapeutic effects of antipsychotic treatments. Future studies should explore whether targeting homeostatic plasticity mechanisms could reveal novel adjunct therapeutics which could aid to optimize treatment strategies for patients who require long-term antipsychotic treatment.

Keywords: Synaptic Plasticity, Antipsychotic, D2 dopamine antagonists, Dopamine, glutamate

Disclosure: Nothing to disclose.

P154. Neocortical and Hippocampal Organoids Model Tau Accumulation in Alzheimer's Disease

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Background: Alzheimer's Disease (AD) is a chronic neurodegenerative disease that initially manifests with amnesic cognitive decline but ultimately progresses to a global impairment that impacts one's most basic functioning. For unknown reasons, beta-amyloid (BA) plaques begin accumulating in the neocortex and work their way through the limbic system to the hippocampus over a 15-year timespan. In strict contrast, tau's propagation pattern follows the opposite pattern. Tau accumulates first in the entorhinal cortex and the hippocampus before moving into the limbic system, and eventually, the neocortex. While both BA plaques and NFTs have been implicated in the disease pathogenesis, only NFT accumulation and propagation have been correlated with cognitive decline. Unfortunately, the factors that drive tau accumulation in different anatomical regions and cell types remain poorly understood. In this study, we generate an isogenic suite of ApoE3/3 and ApoE4/4 lines from Alzheimer's cases and controls, with male and female backgrounds and establish neocortical and hippocampal organoids as tools for studying Alzheimer's Disease.

Methods: CRISPR/Cas9 Gene Editing: We acquired human induced pluripotent stem cells (hiPSCs) from male and female ApoE4/4 donors with previously diagnosed Alzheimer's disease. We also acquired male and female ApoE3/3 healthy control donors. Using CRISPR/Cas9 gene-editing, we designed guide RNAs to target the ApoE locus and as well as a template for homology directed repair. Ribonuclear protein complexes were delivered to hiPSCs using nucleofection and plated at single cell. Clones were picked and Sanger sequenced to confirm the edit before being further confirmed with next-generation sequencing on a PCR amplicon containing the edit region.

Organoid Differentiation: Cortical organoids were differentiated using previously established protocols [Sloan et. al., Nature Methods, 2017, Sakaguchi et. al, Nature Communications, 2015]. Briefly, iPSCs were dissociated at 80% confluency and passaged 1:3 into Glasgow's modified Eagle's medium (GMEM)-based neural induction medium includes 20% Knockout Serum Replacer (KSR), 1× non-essential amino acids, 0.11 mg/ml sodium pyruvate, 1× penicillin-streptomycin, 0.1 mM β-mercaptoethanol, 5 μM SB431542 and 3 μM IWR1-endo on 6-well ultra-low adhesion plates. Medium was supplemented with CEPT cocktail for the first 6 days. From day 9 to day 25, 10 ng/mL FGF2 and 10 ng/mL EGF were supplemented into the media. After 18 days, organoids were transferred moved to an orbital shaker rotating at 90 rpm, and changed to Dulbecco's modified Eagle's medium (DMEM)/F12-based medium containing 1× GlutaMAX, 1× N2, 1× B27 without vitamin A and 1× antibiotic-antimycotic (anti-anti). For the hippocampal condition only, 3 days of 3 μM CHIR-99021 and 0.5 nM BMP-4 were added to the culture media. At 35 days, organoids were moved into DMEM/F12-based medium containing 1× N2, 1× B27 with vitamin A and 1× anti-anti. From day 55 to day 62, 10 ng/mL LIF were added to base media. From day 63 to d70, 10 ng/mL NT-3 and 10 ng/L BDNF. Organoids were fed every three days.

Results: First, we used healthy donor lines to validate neocortical and hippocampal marker expression in our neocortical and hippocampal differentiations. We used Tbr1 (layer 6 marker), CTIP2 (layer 5 marker), and Cux1 (layer 2/3) marker expression to evaluate neocortical expression and found no statistically significant differences between conditions (p-values > 0.05, n = 3). We then used Prox1 (dentate gyrus marker), KA1 (CA3 marker), and Zbtb20 (pan-hippocampal marker) to evaluate hippocampal marker expression. While there were no significant differences in Prox1 (p-value = 0.52) and Zbtb20 (p-value = 0.55), the expression of KA1 was significantly higher in hippocampal cells (55.97%) compared to neocortical ones (16.23%) (p-value = 0.01, n = 3).

We measured the size of our organoids from all four conditions weekly along the longest diameter. We found that ApoE3/3 neocortical organoids, ApoE4/4 neocortical organoids, ApoE3/3 hippocampal organoids, and ApoE4/4 hippocampal organoids had mean diameters of 999.52 μm, 1262.02 μm, 1105.51 μm, and 940.52 μm respectively at day 90 (Week 13). We performed a 2 way ANOVA with Tukey's Post Hoc test and found no statistically significant differences in size between any pair of conditions (p-values all > 0.85, n = 10 with organoids pooled 10:1).

Lastly, using 1 male and 1 female ApoE4/4 donor with diagnosed Alzheimer's Disease as well as 1 healthy ApoE3/3 donor, we completed an isogenic suite of sex balanced, case-control balanced, and ApoE3/3 and ApoE4/4 balanced lines. PCR amplicons were sequenced with next-generation technology to ensure homozygous editing.

Conclusions: The hippocampal organoid was originally developed in 2015 but has been poorly studied. Although we were initially surprised to find that neocortical marker expression could be found in the hippocampus, large sequencing datasets confirm Cux1, CTIP2, and Tbr1 expression in the hippocampus. While previous literature reports hippocampal organoids contain dentate gyrus and CA3, they also mention missing the CA1 and CA2 regions. Single cell RNA-seq of the hippocampal organoid may offer better insight into the diverse cell types of the developing hippocampus. We plan to evaluate phospho-tau and beta-amyloid levels in these conditions over time to determine their relative susceptibilities to accumulation of these key proteins in Alzheimer's pathophysiology.

Keywords: Alzheimer disease, Aging; Cognition; Stress; Acetylcholinesterase; Splice Variants, Induced pluripotent stem cells (iPSCs), Organoids

Disclosure: Nothing to disclose.

P155. Endogenous Opioid Receptor-Mediated Regulation of Prefrontal Cortex Microcircuitry and Valence Processing

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Background: The medial prefrontal cortex (PFC) is essential for coordinating goal-directed behavior and executive function, processes often disrupted in neuropsychiatric conditions that involve learning context-dependent rules and incorporating past experiences and outcome valence into schema representations used to guide future behavior. Opioid transmission and dysregulated PFC activity have both been implicated in the inhibitory-control deficits associated with addiction and binge-type eating disorders. What remains unknown, while endogenous opioids and their receptors are highly expressed in the PFC, is whether endogenous opioid transmission within the PFC modulates inhibitory control.

Methods: We employed a combination of electrophysiology, optogenetics, calcium imaging, and pharmacological manipulations to investigate Enk signaling in PFC microcircuits across species. Viral vectors were used to express opsins and calcium indicators in specific neuron subpopulations (VIP/SST interneurons and pyramidal neurons) in mice. Optogenetic stimulation and in vivo calcium imaging were performed to assess neuronal activity and Enk release dynamics during associative learning tasks. Whole-cell patch-clamp recordings in acute brain slices from mice and non-human primates were conducted to measure the effects of Enk, MOR, and DOR activation on interneuron excitability. The pharmacological blockade of opioid receptors in the PFC was used to determine the impact of disrupted Enk signaling on reward-related behavior.

Results: We discovered that enkephalin (Enk), along with mu-opioid receptors (MOR) and delta-opioid receptors (DOR), are expressed in distinct neuronal subpopulations within the PFC. Enk was present in both excitatory and inhibitory neurons, with particularly high expression in VIP and SST interneuron subpopulations. MOR was predominantly expressed in SST-positive interneurons, while DOR was found in both SST- and PV-positive interneurons. Electrophysiological recordings from GABAergic interneurons supported these findings, revealing that Enk signaling through MOR and/or DOR inhibits interneurons based on their electrophysiological profiles. Further analysis of interneuron excitability, using principal components analysis and hierarchical clustering, identified distinct clusters of fast-spiking and non-fast-spiking interneurons, each selectively responsive to DOR and/or MOR agonists. Optogenetic stimulation of various PFC interneuron subpopulations showed that MOR and DOR activation inhibit interneurons in a subclass-specific manner. Specifically, SST inhibitory currents were reduced by both MOR and DOR activation, while PV output currents were selectively reduced by DOR activation alone. Enk was also found to suppress inhibitory synaptic transmission onto layer V pyramidal neurons through a presynaptic mechanism in both the non-human primate (NHP) PFC and the human cortex. The functional release of Enk by VIP/SST interneurons or pyramidal neurons appears to inhibit interneurons that express MOR and/or DOR. In vivo calcium imaging of Enk-expressing neurons in the PFC during a head-fixed associative learning task revealed their engagement in response to both rewarding and aversive stimuli. Moreover, pharmacological blockade of opioidergic transmission in SST inhibitory interneurons within the PFC led to an increased lick frequency in response to rewards, suggesting that PFC Enk signaling plays a modulatory role in shaping reward consumption.

Conclusions: These results suggest that Enk signaling may contribute to a disinhibitory microcircuit. Specifically, the release of Enk by VIP/SST interneurons or pyramidal neurons appears to suppress certain interneuron subpopulations through the activation of MOR and/or DOR. This implies that Enk signaling could play a role in a disinhibitory microcircuit across various species, including mice, NHP, and humans. Monitoring the activity of Enk-expressing neurons and the dynamics of Enk release has shown that these cells are likely engaged and release peptides in response to task-related rewards and punishments. Furthermore, blocking opioid signaling in the PFC has been found to affect how mice perceive both rewards and aversive outcomes. These findings provide valuable insight into the microcircuit mechanisms by which opioids regulate PFC circuitry, ultimately influencing behavior by inhibiting specific interneuron subpopulations.

Keywords: Mu opioid receptors, delta opioid receptors, enkephalin

Disclosure: Nothing to disclose.

P156. TBX1, a Driver Gene of 22q11.2 Copy Number Variation, Contributes to Structural, Cellular, and Social and Cognitive Deficits via Neonatal and Embryonic Neurogenesis

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Background: Copy number variants (CNVs) at human chromosome 22q11.2 are associated with social and cognitive deficits and psychiatric disorders. However, how individual 22q11.2-encoded genes alter the developmental trajectories of social and cognitive functions of psychiatric disorders is still poorly understood. Our group has identified TBX1 as a major 22q11.2 driver gene for distinct social and cognitive deficits. TBX1 protein is expressed at high levels in the embryonic brain; while its expression precipitously declines thereafter, it remains enriched in neonatal and adult stem cells in the mouse brain. We have characterized the role of Tbx1 in embryonic neurogenesis and post-embryonic neurogenesis in social and cognitive phenotypes.

Methods: To conditionally initiate Tbx1 heterozygosity in stem cells, we developed nestinCreERT;Tbx1^{flox/+} mice and treated them with Tamoxifen at postnatal days 1 to 5 (P1-5) or postnatal days 21 to 25 (P21-25). They were tested for affiliative social interaction and cognitive flexibility 1 month later. Stem cells of neonatal P0 hippocampus of C57BL/6J pups were cultured to evaluate the role of Tbx1 in the proliferation and cell cycle of neonatal stem cells. The target genes of TBX1 in proliferating neonatal stem cells were identified by ChIP-seq, validated by ChIP-PCR, and Tbx1 knockdown and deletion and mutation of the promoter region of a target gene. To characterize the role of embryonic neurogenesis in brain structures and behavior, we used volumetric magnetic resonance imaging (MRI) analysis to comprehensively evaluate brain region volumes and behavioral alterations relevant to affected structures in congenic constitutive Tbx1 heterozygous mice. We additionally developed Tbx^{flox}-STOP-flox to restore embryonic Tbx1 heterozygosity by crossing this mouse with various Cre lines.

Results: When initiated at P1-5, but not P21-25, Tbx1 heterozygosity in stem cells reduced social interaction and delayed the speed of cognitive flexibility. Tbx1 knockdown reduced the proliferation rate and transition of neonatal hippocampal stem cells from G0/G1 to S cell cycle phase in vitro. ChIP-seq identified

Tbx1 binding peaks in more than a thousand sites. Among them, the knockdown of Tbx1 by siRNA reduced the transcription and expression of Pten in proliferating stem cells in vitro. Segmental deletions and point mutations of the Pten promoter identified a 35bp region as a critical sequence. Our MRI data showed that the volumes of the anterior and posterior portions of the amygdaloid complex and its surrounding cortical regions were most robustly reduced in Tbx1 heterozygous mice. In an amygdala-dependent task, Tbx1 heterozygous mice were impaired in their ability to learn the incentive value of a social partner.

Conclusions: Our data suggest that Tbx1 deficiency impairs social and cognitive functions, at least partly, through a) neonatal stem cells and their progenies and b) embryonic neurogenesis that affects the volumes of the amygdala complex.

Keywords: Amygdala, Social incentive learning, cognitive flexibility, Social Behavior, MRI

Disclosure: Nothing to disclose.

P157. Magnesium-Ibogaine Therapy in Veterans With Alcohol Use Disorder Durably Reduces Alcohol Consumption

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Background: Psychedelics have a track record in anecdotal reports, preclinical and clinical investigations to curb habitual use of drugs of abuse. In particular, the oneirogenic, root derivatives of the tabernanthe iboga shrub have been investigated for the treatment of alcohol and opioid use disorder. Still there are very few prospective clinical trials exploring the neurobiological effects of ibogaine as a treatment for substance dependence. Recently, our group embarked on one of the few prospective studies (Magnesium-Ibogaine: the Stanford Traumatic Injury to the CNS protocol: MISTIC) looking at the use of ibogaine for the treatment of neuropsychiatric disorders in a population of TBI afflicted special operations forces veterans (SOVs). To determine the role of the psychedelic ibogaine on curbing substance dependence, we performed a post-hoc, secondary analysis of our previous open label, prospective study on reductions in alcohol use among a subpopulation of special forces veterans satisfying DSM criteria for alcohol use disorder (AUD). We hypothesized that post Ibogaine treatment, individuals would reduce total amount of alcohol use as measured by alcohol use specific items of the Addiction Severity Index which would correlate with resting state functional connectivity changes pre and post Ibogaine therapy.

Methods: 30 male SOV's independently sought ibogaine therapy at Ambio Life Sciences located outside Tijuana, Mexico, coordinated through a grant by the non-profit Veterans Exploring Treatment Solutions (VETS). 2-3 days before scheduled ibogaine treatment, small groups of SOVs traveled to Stanford for baseline measures and neuropsychological assessments before traveling to Mexico for the MISTIC protocol. Patients returned to Stanford at 4-5 days and 1 month post treatment, with phone call follow ups in a subset of veterans at 3-, 6-, 9- and 12-month post treatment. For the purposes of this study the Addiction Severity Index Lite, and the substance abuse portions of the Mini International Neuropsychiatric Interview and the Structured Clinical Interview for DSM-V was used to assess alcohol consumption and number of drinking days. Paired T-tests were used for changes in number of drinking days at baseline and 1 month post Ibogaine therapy. For the subset of subjects with data and 3-,6-,9- and 12-month post Ibogaine treatment, ANOVA with Bonferroni corrections for repeated measures were used. To assess changes in functional brain connectivity to correlate with the effects on alcohol

consumption, baseline, and 1 month follow up resting state fMRI was performed. Functional Connectivity (FC) was calculated as the Pearson correlation coefficient of the brain activity pairwise time series. We looked at the relationship between FC changes and amount of alcohol drinking days. In this exploratory hypothesis testing, significant interactions in FC pairs were conducted with ANOVA followed by Bonferroni correction between baseline and one month post visits.

Results: 14 SOVs satisfied diagnostic criteria for alcohol use disorder (AUD) as measured by the MINI Neuropsychiatric Interview Substances Scale. All SOVs with AUD had pre and one month post measures of total drinking days in the past month. Of these 14 SOVs, 11 had follow up at 12 months and 6 SOVs had follow up data points at 6- and 9-month post ibogaine treatment. All SOVs reported a significant reduction in alcohol use days 1 month following ibogaine treatment ($n = 30$, $p = 0.0004$). The subset of SOVs satisfying a diagnostic criterion for AUD also demonstrated a statistically significant reduction in total alcohol use days 1 month following ibogaine administration ($n = 14$, $p = 0.002$). In SOVs with AUD that had alcohol use data at 1- and 12-month post ibogaine treatment there was durability in a significant reduction in drinking days per month ($n = 11$, bonferroni corrected $p = 0.001$). Further, in the subset of SOVs for which there was data at 1,6,9 and 12 months, this durability effect persisted throughout the time course ($n = 6$, bonferroni corrected $p = 0.001$). For resting state measurements at pre and 1-month post, we found 2 pairs with increases in FC correlated with decreases in AUD. Specifically the left limbic temporal area—right medial posterior prefrontal cortex showed positive correlation ($n = 9$, $r = 0.83$, $p = 0.01$), and the right sensorimotor striatum—right basolateral amygdala showed a positive correlation ($n = 9$, $r = 0.70$, $p = 0.04$).

Conclusions: These results demonstrate that treatment with Ibogaine can have long lasting and durable reductions in alcohol dependence in this population of veterans. This builds upon our previous study reporting dramatic reductions in symptoms of depression, general levels of disability and neuropsychological functioning. Further, changes in brain connectivity can be detected that correlate with this reduction in alcohol use in brain networks at the interface of emotional processing and action planning. Future work is needed including expanding our sample size and moving from open label to double blinded measurements.

Keywords: ibogaine, Alcohol and substance use disorders, Psychedelic medicine

Disclosure: Headlamp Health: Consultant (Self)

P158. Tolcapone for Negative Symptoms of Schizophrenia Spectrum Disorders, A Pilot Study

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Background: The catechol-O-methyltransferase gene (COMT) codes for the enzyme that methylates and deactivates dopamine (DA) in the prefrontal cortex (PFC). The COMT Val/Met polymorphism has been suggested to affect dopaminergic neurotransmission. Val/Val homozygotes have higher PFC enzyme activity than Met/Met homozygotes, leading to lower PFC DA levels. Tolcapone is a dopaminergic agent and an inhibitor of the COMT enzyme, used as adjuvant therapy in Parkinson's disease. The motivation factor of negative symptoms relies heavily on an intact PFC for optimal function. While COMT has been linked to schizophrenia, research findings on which symptom domain it affects have been inconsistent.

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The main goal of the present study was to investigate whether tolcapone improves the negative symptoms of SSDs when added to antipsychotic treatment as usual.

Methods: Fifty-seven (N = 57, 56.1% male) stable out-patients from AX Mental Health Clinic diagnosed with SSDs according to DSM-5 with a mean age of 44.04 (SD 12.608) years (range 23-65) and a mean duration of illness of 13.97 ($\pm$ 12.219) years entered a 12-week randomized double-blind cross-over pilot phase III interventional clinical trial (two groups randomly allocated to 6 weeks active add-on drug followed by 6 weeks add-on placebo and vice versa) testing the effectiveness of add-on tolcapone 100mg bd to antipsychotic medication as usual on negative symptoms vs. the COMT Val/Met genotype (EudraCT: 2013-002713-35, sponsored by the Greek Ministry of Education, Protocol Approval No. 9717/22-8-13). Data analysis was undertaken with IBM SPSS v.29.0. Multivariate analysis of covariance was employed to test the hypothesis of an interaction effect between COMT genotype and the predictor variable add-on tolcapone on negative symptoms (BPRS, HAMD, SANS, BNSS, PANSS-Negative, CAINS and GAF scores).

Results: Two male patients were excluded from the study as baseline liver function tests (LFTs) were above the upper limit of normal. At entry point, for N = 55 patients [mean BMI = 30.43 ($\pm$ 5.463) kg/m²], mean dose of antipsychotic medication was 37.36 ($\pm$ 30.101) mg in olanzapine equivalents, the mean antidepressant dose (N = 32) was 38.20 ($\pm$ 29.453) mg in fluoxetine equivalents, and the mean benzodiazepine dose (N = 11) 9.75 ($\pm$ 9.402) mg in diazepam equivalents. Baseline (N = 55) CGI-S was 4.62 ($\pm$ 1.027, range 3-7), PANSS total score was 84.02 ($\pm$ 13.166), PANSS-Negative score 20.00 ($\pm$ 3.702), HAMD 9.25 ($\pm$ 3.936), SANS total score 34.04 ($\pm$ 13.314), BNSS total score 26.02 ($\pm$ 9.810), CAINS total score 24.95 ($\pm$ 7.054), and GAF 55.27 ($\pm$ 13.451, range 30-80). Our sample was in Hardy-Weinberg Equilibrium (χ^2 = 0.4521), with N = 12 (21.1%) Met/Met, N = 31 (54.4%) Val/Met, and N = 14 (24.6%) Val/Val individuals. A series of paired t-tests revealed no significant differences between LFTs (SGOT, SGPT, Alkaline Phosphatase, and γ GT) levels between baseline and 2-weekly measurements for 14 weeks total. A series of ANCOVAs were run to determine the effect of COMT genotype on negative symptoms scale scores after controlling for add-on tolcapone. After adjustment for tolcapone add-on, we observed a trend to significance for the difference in SANS total score between the COMT genotype groups [F(2,49) = 2.690, p = 0.078, η^2 = 0.099] and a statistically significant difference in GAF [F(2,49) = 3.726, p = 0.031, η^2 = 0.132]. Post hoc analyses were performed with Bonferroni adjustment. All other negative symptom measures did not differ significantly between genotypes.

Conclusions: This preliminary phase III clinical study showed that improvement in negative symptoms does not seem to be associated with add-on tolcapone depending on COMT genotype. GAF did improve with add-on tolcapone but there was no differentiation between COMT genotype groups. This latter finding may well reflect a possible effect of tolcapone on the cognitive symptoms associated with psychosis. However, our sample was small, and inter-rater variability calls for more carefully designed studies, employing better trained researchers, in the future.

Keywords: Tolcapone, Schizophrenia Spectrum Disorders, Negative Symptoms, COMT Val158Met

Disclosure: Nothing to disclose.

P159. A First-In-Human, Randomised, Double-Blind, Placebo-Controlled Study to Investigate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Oral Ketamine in Healthy Volunteers

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Background: Ketamine, a rapid-acting N-methyl-D-aspartate receptor antagonist effective in treatment-resistant depression (TRD), is usually administered via intravenous or intranasal routes; studies investigating oral dosing are limited. This first-in-human, randomized, double-blind, placebo-controlled, phase 1 study investigated safety and tolerability (primary endpoint), pharmacokinetics (PK), and pharmacodynamics (PD) of a novel immediate-release oral ketamine

Methods: Healthy volunteers (18–55 years) were randomized to each receive two single doses of oral ketamine (40–240 mg) and one oral placebo dose. Treatment-emergent adverse events (TEAEs) and standard PK and PD assessments (e.g., Bond and Lader visual analog scale) were assessed up to 24 hours after dosing. Descriptive statistics were used.

Results: 19 participants were randomized (mean age: 31 years; male, 68%), and 18 completed the study. 8 mild or moderate TEAEs were reported following oral ketamine (40–240 mg) and five following placebo. There were no TEAE-related discontinuations. Most TEAEs (86%) were considered probably related to the study drug. The most common TEAEs with oral ketamine were dissociation (26 events), dizziness (nine events), and headache (nine events). A positive relationship between increasing ketamine doses and dissociation events was observed. PK parameters (C_{max}, AUC_{inf}) of oral ketamine and its primary metabolites (2S,6S;2R,6R-hydroxynorketamine, R/S-norketamine) were dose proportional. Transient changes in mood and dissociation were detected 1 hour postdose with return to predose values after ~4 hours.

Conclusions: There were no unexpected safety signals with oral ketamine. PK properties were consistent with those reported for other rapid-acting formulations. These findings warrant further investigation of oral ketamine in TRD.

Keywords: Ketamine, Pharmacodynamics, Pharmacokinetics, (S)-Norketamine, (2R,6R)-hydroxynorketamine

Disclosure: Janssen: Honoraria (Self). Neurocentrx: Consultant (Self)

P160. SPL026 (DMT Fumarate) in Combination With Selective Serotonin Reuptake Inhibitors (SSRIs) for Patients With Major Depressive Disorder

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Background: Selective serotonin reuptake inhibitors (SSRIs) are the dominant treatment for major depressive disorder (MDD). Recent studies with psychedelics have most often been with participants not currently taking SSRIs. The reason for excluding SSRIs in psychedelic studies is due to both SSRIs and serotonergic (or classical) psychedelics exerting their primary effects through the serotonin system. Theoretically, there are multiple ways in which these compounds could interact acutely. However, SSRI withdrawal can be a disruptive experience. We previously completed a study which reported positive results with SPL026 (N,N-Dimethyltryptamine (DMT) fumarate) in participants with MDD (without SSRIs), with a mean difference between active and placebo at 2 weeks of -7.35 (Montgomery-Åsberg Depression Rating scale (MADRS)) (p = 0.023). Studying the combined use of SSRIs and psychedelics would help advance understanding of their concomitant use to treat patients with MDD.

Methods: This open-label study investigated the safety, tolerability, pharmacokinetics, pharmacodynamics and exploratory

efficacy of a single SPL026 intravenous dose, alone or in combination with SSRIs. The test cohort (N = 12) ("SSRI-Cohort") consisted of participants currently on a stable, but not fully effective, treatment of SSRIs. The control (N = 5) ("Non-SSRI-Cohort") consisted of participants not currently using any pharmacological antidepressant treatment. Participants were recruited with a Hamilton Depression Rating Scale (HAM-D17) score of ≥ 14 .

Results: There were no drug-related serious adverse events (SAEs); 8 drug-related adverse events (AEs) in SSRI-Cohort and 3 in Non-SSRI-Cohort, all AEs were mild-moderate, and the majority resolved during the dosing visit. The most commonly reported treatment-related AEs were within the gastrointestinal disorders class and included nausea (3 [16.7%] participants) and vomiting (2 [11.1%] participants). All other treatment-related AEs were reported by a single participant. There were no clinically significant treatment-related trends in vital sign values (blood pressure, heart rate or temperature), physical findings or ECG parameters. Pharmacokinetics (PK): Overall systemic exposures of SPL026 were similar between the SSRI- and Non-SSRI Cohorts. Geometric mean AUC_{0-inf} was 21.7 h*ng/mL in the SSRI-Cohort (8 participants), and 19.6 h*ng/mL in the Non-SSRI-Cohort (5 participants). Pharmacodynamics (PD): The SSRI-Cohort scored moderately higher on the Mystical Experience Questionnaire and Emotional Breakthrough Inventory, and higher on the Ego Dissolution Inventory suggesting that the study drug evoked a more complete mystical experience, more emotional breakthrough and greater ego dissolution in the SSRI-Cohort compared to the Non-SSRI-Cohort. Exploratory efficacy at 4 weeks: Mean MADRS change in the SSRI-Cohort was -25.8 and -19.4 in the Non-SSRI-Cohort; % response was 100% in SSRI-Cohort and 80% in Non-SSRI-Cohort; % remission was 92% in SSRI-Cohort and 20% in Non-SSRI-Cohort. Decreases from baseline scores on the State-Trait Anxiety Inventory – Trait subscale (STAI-T) were observed at Day 8, Day 15 and Day 29 in both the SSRI-Cohort and Non-SSRI-Control Cohort, suggesting an improvement in trait anxiety symptoms following SPL026 administration.

Conclusions: Although there does not appear to be an effect on the PK of DMT when administered in the presence of SSRIs, there appears to be a possible effect on PD measures. Also, while the efficacy observed in the Non-SSRI-Cohort was comparable to that observed in the SPL026 earlier trial, the antidepressant effects in the SSRI-Cohort were of a greater magnitude. These results imply that participants with MDD do not need to stop ongoing antidepressant treatment to undergo psychedelic treatment – which is of benefit to the participants and reduces the operational complexity of such trials. The results also suggest a potentially enhanced efficacy effect when SPL026 is administered with SSRIs. These findings will be taken forward in our next study in participants with Generalized Anxiety Disorder administered CYB004 (a deuterated DMT analogue), where participants will not need to discontinue ongoing antidepressant/anxiolytic treatment during the trial.

Keywords: psychedelic drugs, Major Depressive Disorder (MDD), Clinical trial, DMT

Disclosure: Cybin Inc: Employee (Self)

P161. An Exploratory Study Evaluating the Possibility of Emergence of Tachyphylaxis, Tolerance, or Withdrawal Upon Multiple Dosing With Sublingual Dexmedetomidine

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Background: Sublingual film formulation of dexmedetomidine, a selective alpha-2 adrenergic receptor agonist, has been approved

for treatment of episodes of agitation in patients with schizophrenia or bipolar disease. It is currently being studied as a treatment for acute agitation associated with Alzheimer's dementia. Dexmedetomidine is also approved in the United States as the intravenous injectable formulation. While not generally observed in larger studies, evidence for withdrawal and tolerance was reported with extended intravenous dosing of dexmedetomidine in some case studies. Because most studies with sublingual dexmedetomidine film were conducted with single doses, there is little to no data on whether repeated frequent dosing of dexmedetomidine administered in this manner or at these dosing levels is associated with tolerance or withdrawal. To assess these phenomena, sublingual dexmedetomidine was administered to a patient population selected for exhibiting frequent, near daily, episodes of agitation.

Methods: The single-arm, open-label study (NCT06041646) was designed to evaluate any emergence of withdrawal, tachyphylaxis, or tolerance after dosing with sublingual dexmedetomidine 180 mcg as needed (PRN) over a period of 7 days in subjects with bipolar disorder or schizophrenia who are agitated frequently (at least 3 days a week) in an inpatient setting. This 7-day treatment period was followed by a 3-day follow-up period during which time no dexmedetomidine was administered with the objective to characterize any potential withdrawal symptoms.

Based on the exploratory nature of this study, a sample size of at least 20 subjects on active drug was considered sufficient to provide adequate clinical information to meet the objectives of the study. Although this study was not statistically powered to evaluate repeat-dose efficacy, changes in agitation were assessed by the Positive and Negative Syndrome Scale – Excitatory Component (PEC) and the Clinical Global Impression-Improvement (CGI-I) scales from pre-dose to 2 hours post-dose for all doses administered. Evaluation of withdrawal phenomenon was based on adverse events (AEs) and hemodynamic effects during the 3-day follow-up.

Results: A total of 29 patients were enrolled, of which 28 patients received study medication, completed the study, and were evaluated for safety. The mean age was 45.4 years, the underlying diagnosis was bipolar disease in 59% of the patients and schizophrenia in 41%, and 65.5% of the patients were male. Over 7 days, patients received between 1 and 7 doses of the study medication, with most (53.5%) receiving 2 or 3 doses.

Mean PEC score reduction was observed following all doses of sublingual dexmedetomidine film, administered PRN over the treatment period. No meaningful difference in effect was observed in patients who received multiple PRN doses, and there was no evidence of tolerance or tachyphylaxis. Similarly, all patients showed improvement in agitation symptoms as assessed by the CGI-I Scale for all doses administered as needed over the treatment period.

In this study, sublingual dexmedetomidine was generally well tolerated – there were no discontinuations due to adverse events (AEs) and there were no serious or severe AEs. No subject met pre-specified objective criteria for withdrawal phenomenon (tachycardia, systolic hypertension, nausea, or vomiting and the emergence of any new AEs on ≥ 2 consecutive days of the 3-day off treatment period). In addition, no evidence of worsening agitation (rebound) was observed, as there was no increase in severity of agitation symptoms upon cessation of dexmedetomidine treatment.

Conclusions: In this exploratory study, as-needed doses of sublingual dexmedetomidine 180 mcg were safe, well tolerated, and reduced the severity of agitation in subjects with schizophrenia or bipolar disorder over a 7-day treatment period. No evidence of tachyphylaxis or tolerance to efficacy was observed in subjects who received multiple doses over the duration of the study. Upon discontinuation of treatment, no withdrawal or rebound phenomena were observed.

Keywords: dexmedetomidine, tachyphylaxis, tolerance, withdrawal, agitation

Disclosure: BioXcel Therapeutics, Inc.: Employee (Self)

P162. A Preliminary Study of the Neurocognitive Effects of the mGlu2/3 Receptor Antagonist TS-161 in Treatment-Resistant Depression

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Background: Individuals with treatment-resistant depression (TRD) frequently present with neurocognitive deficits in several domains including processing speed, attention, verbal fluency, and memory. These cognitive deficits can persist during and between depressive episodes and are associated with a lower quality of life and greater functional impairment. There is a lack of effective pharmacological interventions to treat cognitive dysfunction in patients with depression. Glutamate modulators, including the low-affinity NMDA receptor antagonist memantine, have been used in clinical practice to slow the progression of cognitive impairment in patients with major neurocognitive disorders. Ketamine, a high-affinity antagonist of the NMDA receptor, has been studied for its rapid-acting antidepressant effects and shown to improve cognition in patients with TRD. Preclinical studies have indicated that antagonism of the metabotropic glutamate receptor mGlu2/3 produces antidepressant effects comparable to ketamine and improves cognitive dysfunction. mGlu2/3 receptors are expressed in hippocampal pyramidal cells and excitatory synapses in the prefrontal cortex. As part of a clinical trial of antidepressant efficacy, we aimed to investigate if TS-161, a novel mGlu2/3 receptor antagonist, leads to neurocognitive effects in adult patients with TRD. We specifically examined cognitive domains in which patients with TRD present with deficits, including verbal fluency, processing speed, and verbal memory.

Methods: We analyzed the neurocognitive battery administered during a phase 2 single-site proof-of-concept, placebo-controlled, double-blinded, randomized crossover study conducted at the National Institutes of Health Clinical Center in Bethesda, MD. Inclusion criteria comprised failing at least one pharmacologic or neuromodulation treatment for depression, a current depressive episode of > 4 weeks, an initial Montgomery-Asberg Depression Rating Scale (MADRS) score ≥ 20 , and no mania or psychosis. Patients were not allowed to take additional psychiatric medications during the study. mGlu2/3 receptor antagonist TP0473292 (TS-161) was provided by Taisho Pharmaceutical Co., Ltd. Subjects received TS-161 or placebo (PBO) for 21 days, followed by a 14 day washout, then crossover to the other condition for an additional 21 days. Eight patients with TRD (6 male and 2 female, Age 33.63 ± 10.97 , and years of education 14.38 ± 1.99) completed the Controlled Oral Word Association (COWA), the Hopkins Verbal Learning Test-Revised (HVLTr), and the Digit Symbol Substitution Test (DSST). Cognitive tests were repeated 6 times: one day prior, 2 days after, and the last day of each condition (day 20) (TS-161/Placebo). Alternate forms of COWA and HVLTr were used to attenuate practice effects. Linear mixed models included fixed effects of drug, time, and their interaction. Covariates included baseline cognition, period, and form of test (if applicable). This study was approved by the NIH IRB. ClinicalTrials.gov ID NCT04821271.

Results: For all measures, the drug*time interaction was not significant (all p values > 0.1). Significant drug main effects indicated that the placebo condition was associated with higher COWA total raw scores [$F = 8.66$, $p < 0.01$, (TS-161: Day 2 = 40.25 ± 11.49 , Day 20 = 42.88 ± 14.95), (PBO: Day 2 = 46.62 ± 14.5 , Day

20 = 44.38 ± 13.18)], COWA T-scores [$F = 8.66$, $p < 0.01$, (TS-161: Day 2 = 41.75 ± 11.41 , Day 20 = 44.38 ± 15.08), (PBO: Day 2 = 48.12 ± 14.95 , Day 20 = 45.88 ± 13.26)], DSST raw scores [$F = 7.95$, $p = 0.01$, (TS-161: Day 2 = 75.75 ± 9.02 , Day 20 = 76.88 ± 9.28), (PBO: Day 2 = 79.12 ± 8.2 , Day 20 = 77.14 ± 7.58)], and DSST scaled scores [$F = 12.55$, $p < 0.01$, (TS-161: Day 2 = 11.0 ± 1.69 , Day 20 = 11.12 ± 2.3), (PBO: Day 2 = 11.62 ± 1.85 , Day 20 = 11.43 ± 1.9)]. There were no significant treatment differences in HVLTr's immediate or delayed recall, retention, or recognition discrimination index (all p values > 0.16).

Conclusions: The PBO condition was associated with better verbal fluency and processing speed relative to TS-161, although no differences were detected for any of the HVLTr measures. This finding suggests that TS-161 might diminish practice effects for non-memory-related cognitive tasks. Previous studies demonstrate that in certain individuals, glutamatergic modulators, including ketamine, were shown to lead to impairment in cognition. Although mGlu2/3 antagonism has not demonstrated similar cognitive effects in preclinical models as ketamine, this higher cortical function in human patients may reflect a more nuanced modulation of the glutamatergic pathway. Further studies in different populations, including individuals with major neurocognitive disorders, are needed to test the cognitive effects of TS-161.

Keywords: Major Depressive Disorder, Treatment-resistant depression, Cognition, Processing speed, Attention

Disclosure: Nothing to disclose.

P163. Phase I Results for DLX-001, a Novel Neuroplastogen Under Development for the Treatment of Major Depressive Disorder

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Background: Psychedelic compounds such as psilocybin have demonstrated promise for treating multiple neuropsychiatric disorders, including major depressive disorder. The clinical effects of these compounds are hypothesized to be due to their ability to promote rapid and enduring effects on structural neuroplasticity, and as such, they are increasingly classified as "psychoplastogens." Unfortunately, the dissociative, hallucinatory, and cardiotoxic properties of traditional psychoplastogens will likely limit their widespread clinical use, necessitating the development of non-hallucinogenic analogues, known as neuroplastogens. Here, we describe single and multi-dose safety, tolerability, pharmacokinetic, and pharmacodynamic data from a phase I healthy volunteer study of DLX-001, a novel isoptryptamine neuroplastogen designed to have similar efficacy as psychoplastogens while eliminating clinically undesirable effects.

Methods: This 3-part, randomized, double-blind, placebo-controlled inpatient study was conducted in 106 healthy cytochrome P450 (CYP450) 2D6 genotyped volunteers (HVs) aged 18 to 55 years. Part A (single ascending dose - SAD) included 8 cohorts of 8 subjects each allocated 6:2 to DLX-001 or placebo, in escalating doses from 2 to 360 mg. Part B (N = 10) was an open-label two-way crossover study in which subjects received DLX-001 60 mg under both fed and fasted conditions. Part C (multiple ascending dose - MAD) included 4 cohorts of 8 subjects each allocated 6:2 to DLX-001 or placebo, in escalating doses from 50 to 350 mg daily for 7 days. Safety monitoring included clinical and laboratory assessments, serial 12-lead ECGs, vital signs, and emergence of suicidality (Columbia Suicide Severity Rating Scale - C-SSRS) or psychotic symptoms (Brief Psychiatric Rating Scale -

BPRS). Pharmacodynamic assessments included clinical measures of psychedelic and psychotomimetic effects (Mystical Experience Questionnaire 30 – MEQ-30, Clinician-Administered Dissociative States Scale – CADSS), neuroendocrine effects, and serial quantitative resting state EEG. Non-compartmental PK analysis of plasma DLX-001 was performed up to 30h post-dose in the SAD and on Days 1 and 7 in the MAD. PK analysis of CSF concentrations of DLX-001 was performed in selected cohorts.

Results: DLX-001 was safe and generally well-tolerated at all doses tested. No serious adverse events (SAEs) were observed. No clinically significant changes in vital signs, laboratory measures, ECG, or any behavioral instruments were observed. The most frequent adverse events (AEs) were nausea, headache, and dizziness, which were dose-related, time-limited, and resolved without intervention. While plasma PK demonstrated dose-proportionality in SAD Cohorts 1 through 5 and MAD Cohorts 1 and 2, a strong CYP2D6 phenotype-dependent effect on plasma concentrations and higher-than-expected intersubject PK variability were observed. To address this, subjects with similar CYP2D6-based gene activity scores (AS) were included in subsequent cohorts: in SAD Cohort 6 (160 mg) and MAD Cohort 3 (120 mg) subjects with CYP2D6 AS of 1.0-1.5 (intermediate/normal CYP2D6 metabolizers), and in SAD Cohorts 7 (220 mg) and 8 (360 mg), and MAD Cohort 4 (350 mg) subjects with CYP2D6 AS of 2.0 (normal CYP2D6 metabolizers) were included, respectively, after which intersubject PK variability decreased adequately. No undesirable functional CNS, psychotomimetic, hallucinatory, or dissociative effects were observed at any dose level. Time- and dose-dependent PD effects were observed in serial qEEGs which also coincided with neuroendocrine effects, particularly in the low frequency theta and delta bands, though other qEEG changes were observed as well. DLX-001 was measurable in the CSF of all SAD and MAD subjects who underwent lumbar puncture and received active drug.

Conclusions: This first-in-human study demonstrated a clear lack of psychotomimetic, hallucinatory, or dissociative effects with DLX-001 at concentrations above those expected to be required for efficacy in MDD. Moreover, measurable CSF drug concentrations and observed increases in low frequency EEG bands coinciding with neuroendocrine effects support the conclusion that DLX-001 reaches the CNS and demonstrates central target engagement with no dissociative, hallucinatory or psychotomimetic effects. These data support the continued clinical development of DLX-001 as a first-in-class neuroplastogen with the potential to address significant unmet needs within MDD as a novel, fast-acting, outpatient oral pharmacotherapy.

Keywords: Major Depressive Disorder (MDD), neuroplastogen, Clinical trial

Disclosure: Cerevel Therapeutics: Employee (Spouse/Partner). Delix Therapeutics: Employee (Self)

P164. Safety and Tolerability of an Oral Prodrug of Allopregnanolone (SPT-300) in Healthy Volunteers: A Single-Ascending Dose, Multiple-Ascending Dose, and Food-Effect Study

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Background: Allopregnanolone is an endogenous, neuroactive steroid GABAA receptor positive allosteric modulator in development for the treatment of patients with major depressive disorder (MDD). In preclinical and clinical studies, allopregnanolone modulates the stress response, and clinically, exogenously administered allopregnanolone has demonstrated activity in

mood disorders including post-partum depression. Due to low oral bioavailability, allopregnanolone is only available as an intravenous infusion. SPT-300 is an oral prodrug of allopregnanolone designed with the Glyph™ lymphatic system-directed prodrug platform to reduce first pass liver metabolism and allow for allopregnanolone to be absorbed via the mesenteric lymphatic system and circulate at therapeutic levels.

Methods: 99 healthy volunteers were enrolled in a Phase 1, double-blind, study with single-ascending dose (SAD), up to seven-day multiple-ascending dose (MAD), and open-label food effect (FE) parts (NCT05129865). SPT-300 was dosed orally, once-daily, either during the day or in the evening before bedtime. Safety, pharmacokinetic, and pharmacodynamic data were collected across all study cohorts.

Results: No treatment-related severe or serious adverse events (AEs) were observed in the SAD, MAD, or FE study components. There were no observed clinically significant changes in laboratory parameters or vital signs and the safety profile demonstrated that SPT-300 was well-tolerated across all groups and therapeutic dose levels. The most common AE across the multi-part study was somnolence, which was reported as mild in all cases. Frequency of somnolence increased with dose and typically occurred in the first several hours following dosing, in line with plasma concentrations and the established pharmacokinetic profile of SPT-300.

Conclusions: SPT-300 was generally well-tolerated across SAD, MAD and food effect study cohorts. Reported AEs were on-target, dose-dependent, and relevant to the known pharmacological modulation of GABA receptors. Multiple well-tolerated dose levels were identified with pharmacodynamic activity and provide the basis for dose selection in subsequent clinical investigations, including a planned Phase 2 study in MDD.

Keywords: allopregnanolone, Phase 1, Major Depressive Disorder (MDD)

Disclosure: Seaport Therapeutics: Employee (Self)

P165. Glutamate Levels in Dorsolateral Prefrontal Cortex Associates With Positive Symptoms in Antipsychotic-Naive First-Episode Psychosis Patients: Preliminary 7 Tesla Magnetic Resonance Spectroscopy Data From the Amend Study

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Background: Preclinical and post-mortem data indicate that glutamatergic dysregulation and subsequent excitotoxicity may be a key element in the pathophysiology of emerging psychosis and schizophrenia. Advances in Magnetic Resonance Spectroscopy (MRS) sequences have enabled clinical assessments of regional glutamate levels in vivo. Evidence from previous studies were acquired using MRS at lower field strength and assessing few brain regions simultaneously. Post-mortem findings have shown lower concentrations of glutamate in the dorsolateral prefrontal cortex (DLPFC). In vivo acquisitions are difficult because of partial volume effects. A Meta-analysis has shown higher glutamate levels in the DLPFC among antipsychotic-naïve patients. In vivo clinical studies have also pointed towards aberrant glutamate levels in the anterior cingulate cortex (ACC) and thalamus (THAL), although these studies primarily included chronic and medicated patients. Glutamate levels have been stipulated to be indicative of treatment response.

Based on the revised literature we investigated group differences in the ACC, DLPFC, and THAL. Additionally, we tested

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glutamate levels in the hippocampus (HIPP) and the basal ganglia (BG) due to their established role in the pathophysiology of schizophrenia. Within patients we correlated psychopathology scores from the Positive And Negative Symptom Scale (PANSS) and the Brief Negative Symptom Scale (BNSS) with regional glutamate levels.

Methods: These preliminary analyses present data from the AMEND-study (Add-on MEmantine to Dopamine modulation to improve negative symptoms at first psychosis; protocol paper PMID: 35669271, Clinical Trials number: NCT04789915).

AMEND is an ongoing RCT is powered according to its primary clinical endpoint: to show a two-point difference on the PANSS-negative scale testing the effect of adding memantine/placebo to antipsychotic treatment of antipsychotic-free FEP patients (antipsychotic-naïve or minimally medicated). Recruitment will continue until 18 patients have completed 12 weeks of treatment in each arm.

The patients included in these preliminary analyses were antipsychotic-naïve and underwent thorough examinations including psychopathology (PANSS and BNSS), and levels of functioning (PSP). Glutamate levels were measured at 7T MRS in five regions: DLPFC, ACC, THAL, HIPP, and BG. HC (recruited in ratio 1:2 per patient) underwent similar assessments, excluding psychopathology assessments. No participant had lifetime substance dependence and benzodiazepines treatment was minimized. Anatomical images were acquired for optimal voxel placement. All voxel dimensions were 20 mm x 20 mm x 20 mm. Spectra were quantified using the LC Model analyses software. Group differences were explored using ANOVA corrected for age, BMI, and sex; not corrected for multiple comparisons. Within regions, we correlated glutamate levels with psychopathology subscores. The current analysis includes participants recruited until July 1st, 2024.

Results: Our preliminary data include 41 patients (mean age 22.5 yrs. (± 3.53); 22 female) and 16 healthy controls: (mean age 23.7 yrs. (± 2.82); 9 female). Patients were moderately ill with a mean PANSS total score of 75 (± 12) and PSP 46 (± 14).

Patients and controls did not differ significantly in glutamate levels across all regions (p values > 0.4). However, higher glutamate levels in the DLPFC correlated significantly with more positive symptoms (p value = 0.019). A sub-analysis indicated that this correlation was driven by hallucinatory behavior (P3). No other significant correlations between glutamate levels and psychopathology were found.

Conclusions: Within antipsychotic-naïve patients we find that higher glutamate levels in the DLPFC are associated with positive symptom severity. In these preliminary analyses, we do not observe group differences in glutamate levels between patients and HC. Further analyses of these glutamate data are ongoing, and levels of GABA will be presented at the poster session.

The AMEND-study is ongoing, and the primary endpoint is the treatment effect of adding memantine to antipsychotic treatment, we expect to complete recruitment in Spring 2025.

Keywords: glutamate, 7-Tesla, magnetic resonance spectroscopy (MRS), Antipsychotic-naïve first-episode psychosis

Disclosure: Nothing to disclose.

P166. Effects of Mom Power Parenting Intervention on Mothers With Opioid Use Disorder: Preliminary Results of a Clinical Trial With Multimodal Neuroimaging

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Background: Opioid use disorder (OUD) and related issues of early life adversity, stress and depression disrupt evolutionarily conserved maternal brain neurocircuits (MBN) that govern sensitive parental behavior that are critical to healthy family outcomes. The prevalence of opioid use disorder (OUD) among mothers has risen sharply on the background of an OUD epidemic. Despite gold standard buprenorphine treatment for withdrawal, related public health issues of postpartum depression, polysubstance use, relapse, and developmental risks to children continue to plague affected mothers and their families - underlining the urgent need for compelling models to establish additional and confirm evidence-based therapies. For non-OUD mothers, psychotherapies such as Mom Power (MP) promote mental health across generations by improving caregiving, parental stress and depression via changes in MBN, including the amygdala and hypothalamus. We will present results from a current NIDA-supported clinical trial exploring the effects of MP on symptoms and function of MBN for mothers with OUD.

Methods: Mothers with OUD ($n = 11$) received MP—a 13-session evidence-based parenting group psychotherapy. For preliminary analyses, we used paired t -tests to compare multimodal post- vs. pre-treatment neuroimaging and surveys including Opiate Craving Scale, Edinburgh Postpartum Depression Scale, Post-Traumatic Stress Disorder Checklist and Parenting Stress Index. Neuroimaging included event-related potential (ERP) responses, including N170 and the late positive potential (LPP) to standardized photos of unknown children with Crying, Laughing and Neutral faces; and functional magnetic resonance imaging (fMRI) of empathic attunement comparing “joining” vs. “observing” emotional photos of own vs. unknown child. We conducted a series of paired-sample t tests on the Post-MP vs. Pre-MP responses in the repeated surveys and multimodal brain imaging tasks. Pearson correlation was conducted to evaluate correlations between changes in opioid craving and ERP measures.

Results: Post- vs. Pre-MP analyses for mothers with OUD showed: reduced depression ($t = -2.749$, $p = 0.021$), post-traumatic stress disorder symptoms ($t = -2.330$, $p = 0.042$) and parental stress ($t = -3.723$, $p = 0.005$). In addition, treatment related ERP N170 responses, potentially representing amygdala circuits, were reduced for Crying vs. Neutral faces ($t = 2.892$, $p = 0.018$). Also, fMRI responses that relate to empathic attunement were enhanced for Join vs. Observe own vs. other child's joyful vs. distressed face in MBN = hypothalamus (z -equivalent = 3.67, $p < 0.001$) and bilateral amygdala (right, z -equivalent = 2.56, $p = 0.005$; left, z -equivalent = 2.46, $p = 0.007$). Furthermore, responses in these three regions were $>$ those in an independent sample of OUD mothers who did not receive MP. Finally, Post- vs. Pre-MP reduction in opioid craving correlated with concomitant reductions in depression ($r = 0.748$, $p = 0.008$) and increased LPP, potentially representing fusiform gyrus circuits, to Laughing vs. Neutral ($r = -0.720$, $p = 0.019$) and Laughing vs. Crying faces ($r = -0.808$, $p = 0.005$).

Conclusions: For mothers with OUD in this pilot study, MP psychotherapy improved indices of mental health while concurrently enhancing brain responses to child stimuli in the MBN. Treatment related reduction in ERP N170 to emotional compared to neutral facial expressions is consistent with emotional faces involving lower processing demands compared to neutral faces; and treatment related enhancement of LPP responses to images of a mother's own child compared to other children is consistent with the treatment boosting mother-child attachment to capture more attention and may demonstrate how the intervention increases the motivational salience of own child faces in concert with reduced drug cravings and depression. Increases in fMRI responses in amygdala circuits is consistent with increased empathic mirroring with MP. Despite needed confirmation in a

well powered randomized trial, results provide a compelling model to study the effects of adversity, such as OUD and other substance use disorders, plus potential benefits of interventions, such as MP, to reduce drug cravings and optimize maternal mood, stress and parenting quality.

Keywords: maternal mental health, opioid, social neuroscience, EEG-fMRI, psychosocial intervention

Disclosure: Nothing to disclose.

P167. Olanzapine Added to Risperidone, in Patients With Schizophrenia and Negative Symptoms, a Safety Open-Label Trial

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Background: Despite repeated attempts, there are no U.S. FDA-approved drugs for the treatment of primary negative symptoms in schizophrenia. Trials using an add-on design by which the experimental compound or placebo are added to an antipsychotic drug have consistently failed. There are several explanations for this failure. Most antipsychotic drugs block Dopamine (DA) receptors particularly DA-2 subtype. Since DA neurotransmission is involved in the brain reward system it is possible that antipsychotic drugs enhance avolition which is cornerstone component of primary negative symptoms. Furthermore, blocking negative symptoms in the striatum can generate secondary negative symptoms such as limb rigidity, flat affect and expressionless face.

Risperidone is a compound with high affinities for 5-HT_{2A}, sigma₂, α_{1A}- and α_{1B}-adrenergic receptors but no affinity for DA, cholinergic, and histaminergic receptors. Two previous RCTs demonstrated that risperidone administered as monotherapy was statistically superior to placebo in improving negative symptoms. Based on the studies carried out as of today and the proposed drug labelling, risperidone would be indicated as a monotherapy for symptomatically stable patients suffering from schizophrenia and moderate to severe negative symptoms. However, it is possible that occasionally in clinical practice, administration of risperidone might overlap with administration of an antipsychotic drug.

Methods: This was a safety trial in which risperidone 64mg/day was administered for 7 days (Period 1) followed by adding olanzapine 10 mg/day for 10 additional days (Period 2). Olanzapine 10 mg was selected for this study since it is a very commonly used drug and commonly used dose. All patients were symptomatically stable for > 6 months before entering the trial at which time all psychotropics were discontinued. The study was conducted in inpatient clinical sites to ensure compliance with drug(s) administration.

Results: 17 patients received ≥ 1 dose of study drug and 13 patients completed the study. Of the 4 who dropped out, 2 withdrew consent before the end of the study, 1 due to a major protocol deviation and 1 due to treatment-unrelated serious adverse event. Five patients (29.4%) had treatment-emergent adverse events (TEAEs) related to the study drug, (dizziness, headache, somnolence, heart rate increased, and agitation). The majority of the adverse effects were mild. There were no notable differences in the incidences of related TEAEs between the 2 treatment periods administration of risperidone alone or in combination with olanzapine. All related TEAEs were resolved without sequelae during the study. There were no emergent clinically significant electrocardiographic or laboratory abnormalities. There was no symptomatic worsening during the administration of risperidone alone or in combination with olanzapine. No clinically important pharmacokinetic interactions between the 2 drugs were observed.

Conclusions: Results of this study suggest that the administration of olanzapine, a commonly used antipsychotic, in conjunction with risperidone does not affect its safety or pharmacokinetics.

Keywords: risperidone, negative symptoms, schizophrenia

Disclosure: Minerva Neurosciences: Employee (Self)

P168. Oxytocin-Facilitated Social Cognitive Skills Training for Patients With Schizophrenia

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Background: Impaired social cognition is common in schizophrenia, and this aspect of the illness has debilitating consequences for patients. Psychosocial interventions, such as social cognitive skills training, have shown some promise, though these interventions have limited effects on inferential processes like mentalizing. Oxytocin is a peptide hormone that increases the salience of certain types of social information and may help facilitate social cognitive training. We hypothesized that the administration of intranasal oxytocin prior to social cognitive training would enhance the effectiveness of the treatment.

Methods: In this double-blind, placebo-controlled trial, outpatients with schizophrenia (N = 49) were randomly assigned to one of four conditions. They were assigned to attend 12 weeks of social cognitive skills training (SCST; 24 sessions), focusing on emotional processing, social perception, attributional bias, and mentalizing, or a control condition focusing on health management (HM), and they were assigned to receive intranasal oxytocin (OT) or placebo nasal spray (PL) 30 minutes before each session. They completed a battery of social cognitive tasks at baseline, halfway into training (6 weeks), at the completion of training (12 weeks), and 4 weeks after the completion of training (16 weeks). Outcome measures were two composite scores in the domains of social cue identification and mentalizing.

Results: There were no significant main effects of treatment (OT vs. PL) or training (SCST vs. HM) on either composite domain nor any significant interactions. When examining subdomains, social cognitive skills training tended to improve one component of mentalizing, empathic accuracy ($p = 0.08$). Oxytocin improved performance on another component of mentalizing, The Awareness of Social Inference Test (TASIT), regardless of training group ($p = 0.04$). There were no significant interactions between treatment and training on any of the subdomains.

Conclusions: Overall, the evidence that intranasal oxytocin enhances social cognitive training in schizophrenia is limited, though this was a small-scale study, underpowered to detect treatment effects across the 4 groups. Future studies may identify other novel pharmacologic targets for enhancing social salience in psychosocial interventions.

Keywords: oxytocin, Schizophrenia (SCZ), social cognitive skills training

Disclosure: Nothing to disclose.

P169. Esmethadone (REL-1017) Improves Cognitive Function in Patients With Severe Major Depressive Disorder and Inadequate Response to Standard Antidepressants: A Post Hoc Analysis From a Phase 3 Randomized Controlled Trial

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Background: Background: Subjective cognitive impairment is a key symptom of major depressive disorder (MDD). REL-1017, a novel N-methyl-D-aspartate receptor (NMDAR) uncompetitive antagonist, has shown promise in improving subjective cognitive impairment when administered as adjunctive treatment to patients with MDD in a Phase 2 trial. This study aimed at investigating the effect of adjunctive treatment with esmethadone HCl (REL-1017) on subjective cognitive measures in a subgroup of severely depressed patients unresponsive to standard antidepressants enrolled in a Phase 3 study.

Methods: We conducted a post hoc analysis of subjective cognitive measures from the Montgomery-Asberg Depression Rating Scale (MADRS) and the Symptoms of Depression Questionnaire (SDQ) from the results of a randomized, double-blind, placebo-controlled, Phase 3 trial. This was designed to assess the efficacy and safety of 25mg of REL-1017 as an adjunctive treatment in patients with MDD unresponsive to standard antidepressants, and included 227 patients (Fava et al., 2024). We analyzed results from subjective cognitive measures derived from the MADRS and SDQ scales administered at baseline and day 28 in the subgroup of severely depressed patients (baseline MADRS ≥ 35). The analysis of 8 SDQ items related to cognition followed a previously described methodology (Guidetti et al., 2023). We also analyzed single items related to cognition derived from the MADRS.

Results: Among the 227 randomized patients, 112 were found to have severe depression (baseline MADRS ≥ 35). The analysis of 8 SDQ items related to cognition showed clinically meaningful and statistically significant improvements ($p = 0.01$). Similarly, MADRS item 6 ("Concentration Difficulties") showed a meaningful and significant improvement ($p = 0.03$). Other MADRS items potentially related to cognition, both directly (lassitude, inability to feel) and indirectly (inner tension, reduced sleep), showed clinically meaningful and statistically significant improvements ($p = 0.005$; $p = 0.005$; $p = 0.009$; $p = 0.01$, respectively).

Conclusions: These post-hoc results support the potential efficacy of REL-1017 in patients with severe depression for the relief of subjective cognitive impairment observed in a prior study. Novel antidepressant treatments shown to restore impaired neural plasticity in experimental models of depressive-like behavior may be especially effective for relieving subjective cognitive impairment in patients with MDD. These post hoc findings support the potential efficacy of REL-1017 for improving subjective cognitive impairment in patients with MDD. Further studies are needed to confirm these promising results.

Keywords: NMDA Antagonists, Subjective Cognitive Impairment, Major Depressive Disorder (MDD)

Disclosure: Nothing to disclose.

P170. Measurement-Based Care vs. Standard Care for Major Depressive Disorder in a Lower-Middle Income Country: A Randomized Clinical Trial

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Background: Clinical trials comparing measurement-based care (MBC) to standard treatment for MDD suggest that MBC is a scalable approach to optimise clinical outcomes for individuals with MDD in low-resource settings.

Methods: A two-arm, multicenter randomized trial of 154 adults with moderate to severe DSM-5 major depressive episode in

Pakistan between September 2022 and January 2024. Participants were randomized (1:1) to either MBC (guideline and rating-scale based decisions, $N = 76$), or standard treatment (clinicians' judgement-based decisions, $N = 78$), and prescribed paroxetine (10–60 mg/day) or mirtazapine (7.5–45 mg/day) for 24 weeks. Depressive symptoms were measured with the 17-item Hamilton Depression Rating Scale (HDRS-17). Time to response (i.e., reduction of $> 50\%$ in HDRS-17score) and remission (i.e., HDRS-17score < 7) were the primary outcomes. Outcomes were evaluated by raters blind to study protocol and treatment. Clinicaltrials.gov identifier NCT05431374, registered on June 24, 2022.

Results: Compared to the standard treatment group, more participants in the MBC group achieved response (97.1% vs. 90.4%) at 24 weeks, but this difference did not reach statistical significance. However, significantly more participants in the MBC group achieved remission (91.3% vs. 71.2%). Similarly, time to response and remission were significantly shorter with MBC (response: 4.5 weeks vs. 8 weeks; remission: 6.7 weeks vs. 11.4 weeks). HDRS-17 scores decreased significantly in both groups, but the reduction was significantly larger for the MBC group (18 points vs. 17 points). The MBC group had significantly higher antidepressant dosages from week 8 to week 24. Rates of adverse effects were lower in the MBC group.

Conclusions: MBC is a low-cost and scalable approach to efficiently optimise outcomes for individuals with MDD in Pakistan and other low-resource settings.

Keywords: psychiatric measurement, Major Depression Disorder, Depression, Clinical trial, lower-middle income countries

Disclosure: Mindset Pharma: Advisory Board (Self). COMPASS Pathfinder Ltd: Contracted Research (Self)

P171. Efficacy and Safety of Esketamine Nasal Spray as Monotherapy in Adults With Treatment-Resistant Depression: A Randomized, Double-Blind, Placebo-Controlled Study

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Background: Esketamine (ESK) nasal spray is approved in > 75 countries as an adjunctive treatment with oral antidepressant (OAD) for treatment-resistant depression (TRD). However, whether ESK is efficacious as a monotherapy for TRD is not known. Considering the suboptimal effect of standard of care OADs for many patients with TRD and the associated side effects, whether ESK is efficacious as monotherapy is an important question that could inform clinical practice. This study assessed efficacy and safety of ESK monotherapy compared to placebo (PBO) in adults with TRD.

Methods: This randomized, double-blind (DB), multicenter, PBO-controlled study (NCT04599855) enrolled adults with recurrent or a single (duration ≥ 2 years) episode of major depressive disorder (DSM-5 criteria) without psychotic features (Mini International Neuropsychiatric Interview) and who scored ≥ 34 on the Inventory of Depressive Symptomatology-Clinician rated, 30-item (IDS-C30) scale. Participants were to have a nonresponse ($\leq 25\%$ improvement) to ≥ 2 OADs during the current depressive episode. Study phases were: screening (up to 7 weeks), DB treatment (4 weeks), open-label (OL) treatment/observation (up to 12 weeks), and follow-up (1 week). All participants had a ≥ 2 week OAD-free period and were then randomized 2:1:1 to receive either PBO or fixed dose ESK (56 or 84 mg) twice-weekly for 4 weeks. The safety analysis set included all randomized participants who received ≥ 1 dose of DB study intervention. The DB full efficacy analysis set included participants meeting the following criteria: Montgomery-Asberg Depression Rating Scale (MADRS) total score ≥ 28 at screening weeks 1, 2 and Day 1 (pre-randomization) and $\leq 25\%$ improvement in MADRS total

score from screening week 1 to Day 1. Participants completing the DB phase could enter the OL phase to receive flexible (56 mg or 84 mg) ESK (week 5–8: twice-weekly, week 9–12: once-weekly, and week 13–16: [weekly or every other week at clinician's discretion]). The OL efficacy analysis set included all participants who received ≥ 1 dose of OL ESK. Primary endpoint: change in MADRS total score from baseline to Day 28. Key secondary endpoint: change in MADRS total score from baseline to Day 2 (~24 hours) post first dose. Other efficacy endpoints: improvements in MADRS total score and Patient Health Questionnaire [PHQ]-9 total score through Day 28. Changes in MADRS score through OL phase were also assessed. Safety was monitored throughout the study. Primary and key secondary endpoints were analyzed using a mixed-effects model with repeated measures and a predefined testing hierarchy to control multiplicity.

Results: DB phase: safety analysis set: 476 adults; full efficacy analysis set: 378 adults (ESK 56 mg: 86; ESK 84 mg: 95; PBO: 197). Participants mean (SD) age: 45.4 (14.06) years (9.8% ≥ 65 years), majority (61.1%) were female. Baseline mean (SD) IDS-C30 score: 45.8 (7.10), mean (SD) MADRS total score: 37.3 (4.88) and mean (SD) PHQ-9 total score: 20.0 (3.87). In the DB phase, a high proportion of participants across all groups completed treatment (94.7% overall). Mean (SD) change from baseline to Day 28 in MADRS total score was -12.7 (11.82) for ESK 56 mg, -13.9 (11.89) for ESK 84 mg, and -7.0 (10.07) for PBO. Differences for both ESK groups vs PBO were statistically significant ($p < 0.001$); least-square (LS) mean difference (SE) between ESK and PBO were -5.1 (1.42) for 56 mg and -6.8 (1.38) for 84 mg. At Day 2, significant improvements in MADRS total score were observed, with LS mean differences (SE) for ESK vs PBO of -3.8 ([1.29]; $p = 0.004$) for ESK 56 mg and -3.4 ([1.24]; $p = 0.006$) for ESK 84 mg. At Day 28, higher response rates (ESK 56 mg: 30.5%; ESK 84 mg: 29.2%; PBO: 15.1%) and higher remission rates ($\text{MADRS} \leq 10$): ESK 56 mg: 14.6%; ESK 84 mg: 21.3%; PBO: 6.5%) were observed in both ESK groups vs PBO. Changes in mean (SD) PHQ-9 score from baseline to Day 28 for both ESK groups vs PBO were ESK 56 mg: -7.1 (6.35); ESK 84 mg: -7.0 (6.54); PBO: -2.8 (5.92), with LS mean treatment differences (SE) of -3.7 ([0.80], 95% confidence interval (CI): -5.28 , -2.15 for 56 mg) and -4.1 ([0.77], CI: -5.65 , -2.61 for 84 mg). At Day 28, in both ESK groups vs PBO, higher response rates based on PHQ-9 score (≥ 6 -point improvement/ $\geq 50\%$ improvement) were observed: ESK 56 mg: 53.0%/37.3%; ESK 84 mg: 51.7%/39.1%; PBO: 25.3%/12.9%. Improvements in MADRS score observed in ESK groups through Day 28 continued during the OL phase. For DB phase, participants on PBO who started ESK (PBO/ESK) in OL phase, MADRS score improved from the first OL assessment and achieved similar improvement as the ESK/ESK groups around Day 70. The most common ($\geq 10\%$) treatment-emergent adverse events during the DB phase for combined ESK groups and OL phase were nausea, dissociation, dizziness and headache.

Conclusions: The results of this study show a statistically significant and clinically meaningful improvement of depressive symptoms after 4-weeks of ESK monotherapy treatment, with onset as early as Day 2 (~24 hours) after the first ESK dose (56 mg and 84 mg doses). Improvements in depressive symptoms continued in the 12-week OL treatment phase. No new safety signals were identified. These results provide important new data to inform treatment options for patients with TRD.

Keywords: TRD, Esketamine nasal spray, Safety, clinical efficacy

Disclosure: Janssen Research and Development, LLC: Employee (Self). Johnson and Johnson: Stock / Equity - Privately Held Company (Self)

P172. N-Acetylcysteine Reduces Frontal Glutamate Levels and Heavy Drinking Days in People With Co-Occurring Bipolar and Alcohol Use Disorders: Results From a Randomized, Double-Blind, Placebo-Controlled, Crossover Study

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Background: Nearly 40% of people with bipolar disorder (BD) develop alcohol use disorder (AUD), with co-occurring AUD worsening the course of BD via more severe manic and depressive symptoms, more frequent mood episodes, treatment resistance, and increased risk for suicide. Clearly effective pharmacotherapies for co-occurring BD and AUD (BD + AUD) are lacking, in part because underlying disease mechanisms remain poorly understood. Convergent lines of evidence support frontal glutamate dysregulation as a candidate target for adjunctive pharmacotherapy for BD + AUD. N-AcetylCysteine (NAC), a safe and well-tolerated medication FDA-approved to treat acetaminophen overdose, has been shown to restore glutamate homeostasis in animal and human studies, with randomized controlled trials supporting the efficacy of NAC for reducing symptoms in people with BD or AUD. Despite these encouraging findings, NAC has never been evaluated in people with BD + AUD. Against this background, we conducted an NIH/NIAAA-funded (R01AA025365) randomized, double-blind, placebo-controlled, crossover study of adjunctive NAC (2400mg/day) and gabapentin (1200mg/day) for BD + AUD.

Methods: Fifty-four individuals diagnosed with BD I or II, prescribed daily use of at least one 1 FDA-approved mood-stabilizing medication, and current, moderate to severe AUD were enrolled across a 5-year period. Exclusions included serious medical illness, history of brain injury, and medication dose changes of $\geq 20\%$, ≤ 2 weeks before testing. Enrolled participants completed 3, 1-week conditions (i.e., gabapentin, NAC, placebo) in a randomized order. Each condition consisted of an in-person study visit for assessment of drinking (via Timeline Followback) and mood symptoms (via Young Mania [YMRS] and Montgomery-Asberg [MADRS] Rating Scales) and dispensing of medication (Day 1), titration to maximum medication dose (Days 1-5), MRI scanning (Day 5), and medication washout (Days 5-7). Each MRI included acquisition of T1-weighted images for proton MR spectroscopy (1H-MRS) voxel placement (i.e., in dorsal anterior cingulate cortex; dACC) and tissue segmentation, followed by echo-time (TE) optimized resting-state acquisitions of GABA+ (via MEGA-PRESS with TE=68ms) and glutamate (i.e., represented by "Glx" or glutamate+glutamine, via PRESS with TE=40ms), respectively. MEGA-PRESS and PRESS data were processed using the Osprey MATLAB toolbox and LCModel 6.3, respectively, with metabolite values normalized to unsuppressed water, corrected for within-voxel tissue fractions, and expressed in mmol/kg. Linear mixed models; each containing the main effect of treatment, period, and sequence to ensure the crossover design and washout were successful; with unstructured covariance matrices and REML estimation were estimated using IBM SPSS software to assess the effect of treatment on dACC GABA+ and Glx levels as well as % heavy drinking days (%HDD) and MADRS scores.

Results: Forty-nine (i.e., 91% of enrolled) participants completed at least one experimental condition, providing a total of 138 completed posttreatment MRI scans (NAC $n = 44$, gabapentin $n = 46$, placebo $n = 47$), with unusable (e.g., poor quality) data representing a small proportion (i.e., ~5%) of this total. NAC and gabapentin were very well tolerated, with relatively few participants (~15%) reporting mild-moderate adverse events (none requiring discontinuation) that were distributed equally across conditions ($p > 0.50$), adherence was excellent, with adherence successfully demonstrated (via urinary riboflavin detection) for ~95% of post-treatment visits, and the study blind was successfully maintained, with correct condition guessing observed at chance levels (n.s. different from 50%). Treatment condition was significantly associated with Glx levels ($F = 4.76$, $p = 0.012$), with post-hoc pairwise comparisons demonstrating that only NAC

($M[SD] = 21.50[2.55]$) was associated with significantly different (i.e., lower) Glx levels relative to placebo ($M[SD] = 22.59[2.56]$; $p = 0.003$; $SMD = 0.43$). In contrast, treatment condition was not significantly associated with GABA+ levels ($F = 0.96$, $p = 0.388$). Although, controlling for baseline scores, treatment condition was significantly associated with Montgomery-Asberg Depression Rating Scale (MADRS) scores ($F = 3.71$, $p = 0.030$), post-hoc pairwise comparisons revealed that MADRS scores did not significantly differ between NAC ($p = 0.128$) or gabapentin ($p = 0.228$) and placebo conditions. Conversely, controlling for baseline heavy drinking, treatment was significantly associated with percent heavy drinking days ($F = 3.90$, $p = 0.025$), with post-hoc pairwise comparisons revealing that only NAC ($M[SD] = 31.86[4.60]$) was associated with significantly different (i.e., lower) %HDD relative to placebo ($M[SD] = 40.94[4.55]$; $p = 0.015$; $SMD = 4.25$). There was no evidence of carryover effects across analyses.

Conclusions: NAC and gabapentin were both safe and well-tolerated in BD + AUD participants, but only NAC significantly decreased dACC glutamate (i.e., Glx) levels and heavy drinking days relative to placebo, whereas gabapentin did not significantly modulate any evaluated biomarkers or clinical variables. These findings may suggest that NAC exerts therapeutic effects on heavy drinking in individuals with BD + AUD via decreasing frontal glutamate levels. Replication and extension of our findings across a longer dosing interval will be necessary, however, to supporting this interpretation more definitively.

Keywords: Bipolar Disorder, Alcohol Use Disorder, Comorbidity, N-acetylcysteine, Glutamate Homeostasis

Disclosure: Nothing to disclose.

P173. Investigation of the Antidepressant Effects of the mGlu2/3 Receptor Antagonist Prodrug, TS-161, in Treatment-Resistant Depression: A Randomized Double Blind Placebo Crossover Study

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Background: Conventional monoaminergic treatments for major depressive disorder (MDD) have proven to be only modestly effective, while glutamatergic modulation has shown rapid antidepressant effects even after failed conventional treatment. Ketamine is highly efficacious but produces psychotomimetic side effects. The preclinical antidepressant profile of mGlu2/3 receptor antagonists and effects at the neuronal level have been comparable to those of ketamine but without evidence for its side effects and misuse potential. mGlu2/3 receptors are highly expressed in hippocampal pyramidal cells and at prefrontal excitatory synapses where they decrease glutamate transmission via several mechanisms. mGlu2/3 receptor antagonism influences serotonergic and dopaminergic signaling, as does ketamine. In assays of antidepressant efficacy, mGlu2/3 antagonists rapidly reversed behaviors associated with depression, including increased immobility, decreased sucrose preference, and delayed novelty-suppressed feeding. We sought to evaluate this novel glutamate-mediated mechanism with a potent selective antagonist prodrug, TS-161, in adult patients with MDD and at least one failed treatment trial. We also examined synaptic plasticity biomarkers involved in the antidepressant response to TS-161, testing the broader hypothesis that glutamatergic modulation is involved in fast antidepressant response.

Methods: At analysis of this phase 2 single-site proof of concept double-blind randomized crossover study, we recruited 11 patients with MDD (7 male, 2 female, Age 35.4 ± 11.3 (range 21–50.8)) who failed ≥ 1 previous medication, ECT, or TMS. Diagnosis was confirmed with Structured Clinical Interview for DSM-5. Inclusion criteria included: age 18–65, current episode > 4 weeks, an initial Montgomery-Asberg Depression Rating Scale (MADRS) score ≥ 20 , and no mania or psychosis. Exclusion criteria included: previous non-response to ketamine, substance use disorder, serious or unstable medical illness, or past major head injury or unresolved seizures. Concomitant antidepressants were not allowed, and patients were tapered followed by 2 weeks drug-free. TP0473292 (TS-161), an orally bioavailable prodrug of TP0178894 (a potent and selective mGlu2/3 receptor antagonist), was provided by Taisho Pharmaceutical Co., Ltd. A flexible dose design was used to better assess safety and efficacy (50–100mg/day); a double-dummy blind dosing design was utilized with any dose decrease due to intolerance done blindly. Drug or placebo (PBO) was given daily for 3 weeks, followed by 2–3 week washout, then crossover to the other condition. The primary outcome was MADRS total score measured at 230 minutes (day 0), and days 1, 2, 3, 7, 14, and 21 per period. We used a linear mixed model to test for drug effects with fixed effects of time, drug, and interaction. Covariates included period and period-specific baseline MADRS values. Remission was defined as MADRS score ≤ 10 ; response was defined as 50% reduction. Peripheral blood was assessed for brain derived neurotrophic factor (BDNF; Biosensis ELISA). Magnetoencephalography (MEG) was conducted at multiple time points using a CTF 275-channel whole-head MEG scanner. MEG data were source-localized in gamma frequency (30–58Hz), a proxy measure for excitation-inhibition balance, and extracted in key brain regions of interest implicated in glutamate-mediated antidepressant response, including insula, dorsolateral PFC, and anterior cingulate cortex (ACC). Non-parametric tests were used due to non-normality of the gamma power data. Statistics were run with SAS software (Cary, NC) and Graphpad Prism 10 (Boston, MA). All procedures were conducted at the National Institutes of Health Clinical Center in Bethesda, MD, and were approved by the NIH IRB. ClinicalTrials.gov ID NCT04821271.

Results: Adverse events were mild and consistent with phase 1 data. Two had blind dose decreases but completed the study. Two participants withdrew (one each on days 0 and 2). Nine patients completed both treatment conditions. There was no significant drug*time interaction ($p = 0.80$) and there were no significant differences in MADRS scores at individual timepoints or collapsed over time (estimated overall means: PBO = 26.5 ± 1.2 , TS-161 = 27.9 ± 1.2 , $p = 0.07$). There were no remitters in either group, and there was one responder to TS-161 (MADRS from 30 to 11 by day 7). Gamma power at resting state was not significantly different in TS-161 vs. PBO but trended higher in ACC (TS-161 = 0.11 ± 0.11 , PBO = 0.07 ± 0.03 , $p = 0.097$), insula (TS-161 = 0.11 ± 0.11 , PBO = 0.07 ± 0.02 , $p = 0.16$), and dlPFC (TS-161 = 0.12 ± 0.10 , PBO = 0.08 ± 0.03 , $p = 0.24$). BDNF levels trended higher during TS-161 vs. PBO (6 timepoints in linear mixed model, TS-161 = 24.1 ± 2.0 ng/mL, PBO = 21.3 ± 2.1 ng/mL, $t = 1.95$, $df = 80.8$, $p = 0.055$).

Conclusions: In this analysis, TS-161 did not meet the predicted endpoints based on MADRS scores, remission, or response. The study medication was generally well tolerated with no major safety events in MDD patients. Limitations include the underpowered recruitment total. A trend that was not statistically significant for increased gamma power and BDNF during TS-161 suggests neurobiological engagement of glutamatergic modulation. Further larger studies or those in patients not necessarily treatment resistant are needed to further test this novel antidepressant mechanism.

Keywords: Clinical trial, Fast-acting Antidepressant, mGlu2/3 antagonist, magnetoencephalography, BDNF

Disclosure: Nothing to disclose.

P174. Pharmacologic Augmentation of Clinical Gains With Computerized Targeted Cognitive Training in Chronic Psychosis: Preliminary Evidence From a Single-Site, Double-Blind Study

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Background: Computerized targeted cognitive training (TCT) improves symptoms, cognition and functioning in schizophrenia (SZ), but these benefits are modest. We previously proposed a strategy of Pharmacologically Augmented Cognitive Therapy ("PACT") for SZ, in which pro-cognitive drugs specifically augment the neurocognitive and clinical benefits of cognitive therapies. Here we tested the ability of 2 drugs to enhance clinical measures in psychosis patients undergoing 30h of TCT. Both the psychostimulant, d-amphetamine (AMPH) and the non-competitive NMDA receptor modulator, memantine (MEM), acutely enhance auditory learning in psychosis patients, using a frequency modulation task that is part of a well-characterized auditory TCT suite. Because the clinical gains from TCT presumably reflect learning that takes place during TCT training, we hypothesized that, administered throughout training, these agents would also enhance the benefits of a full course of TCT.

Methods: Methods: Antipsychotic-medicated individuals with chronic psychosis ($n = 68$; mean age (range) = 47.03 (21-65); M:F = 39:29) were tested twice, one week apart. Baseline clinical measures were obtained after either placebo (PBO: Test 1) or test drug (PBO, AMPH (5 mg po) or MEM (20 mg po): Test 2). Participants then completed up to 30 TCT sessions (2-3 sessions/week; $n = 50$ completed 30 sessions) in 3 groups: "AMPH group" received AMPH (5 mg po) 1-h before each TCT session; "MEM group" titrated to 10 mg MEM bid prior to starting TCT and maintained that dose throughout training; "PBO group" received PBO dosed identically to either AMPH or MEM. Outcome measures were acquired after 10, 20 and 30 TCT sessions, and 12 weeks post-training. Pill identity (active/PBO) was blind to subjects and staff. Primary outcome measures were PANSS (total, positive and negative symptom subscales); secondary outcome measures included YMRS, PHQ-9, PSYRATS Hallucinations and WHODAS. Outcomes were also assessed via attrition rates, rates of positive C-SSRS responses and other adverse events (AEs) and ratings of treatment satisfaction; for AMPH group subjects and corresponding PBO group subjects, potential adverse effects were assessed using the AIMS and AMPH Cessation Symptom Assessment. Outcome scores were treated as continuous variables. To test the hypothesis that active drugs would augment the therapeutic impact of a full 30-session course of TCT, the effect size of the score change was calculated using Cohen's d ; positive " d " values reflect clinical improvement, and negative values reflect clinical worsening. A "threshold" for positive active drug effects was then set at $d = 0.4$ above PBO values. Planned comparisons identified time points when "threshold" improvement was reached.

Results: PBO vs. active drug groups did not differ in attrition or AEs. PANSS total scores declined from baseline to P30 in all drug groups. Effect sizes (Cohen's d) for these declines were 0.355, 0.638 and 0.927 for PBO, MEM and AMPH groups, respectively. MEM subjects missed the "threshold" of a $d = 0.4$ decline (improvement) over PBO (i.e., threshold $d > 0.355 + 0.4 = 0.755$), while AMPH subjects exceeded this threshold. PANSS positive

scores also declined from baseline to P30 in all groups ($d = 0.230$, 0.404 and 0.927 for PBO, MEM and AMPH groups, respectively). Values for MEM subjects did not reach the threshold, while those in AMPH subjects exceeded it. PANSS negative scores declined from baseline to P30; in this case, little change was detected in the PBO group ($d = 0.172$) or AMPH group ($d = 0.255$), while gains in the MEM subjects ($d = 0.696$) exceeded the threshold. Though not a primary outcome measure, PANSS general scores declined in all groups from baseline to P30 ($d = 0.489$, 0.501 and 0.830, for PBO, MEM and AMPH groups, respectively). Across PANSS measures, patients with the highest baseline symptoms showed the most clinical improvement.

Comparisons of PHQ-9 baseline vs. P30 scores revealed minimal declines among PBO group subjects ($d = 0.057$), but small-to-medium effect size declines among AMPH ($d = 0.457$) and MEM group subjects (0.372), with AMPH group subjects equaling the threshold. YMRS scores declined among AMPH group subjects ($d = 0.808$), but not MEM ($d = 0.030$) or PBO ($d = 0.094$) group subjects; AMPH group subjects well exceeded the threshold. PSYRATS hallucinations scores also declined among AMPH group subjects from baseline to P30 ($d = 0.552$) in contrast to MEM or PBO groups ($d = (-0.031)$ and (-0.072) , respectively), with AMPH group exceeding the threshold. WHODAS disability scores declined from baseline to P30 among AMPH group subjects ($d = 0.658$), but not MEM and PBO group subjects ($d = (-0.006)$ and (-0.055) , respectively). BPRS psychosis subscale scores did not detect declines among PBO, MEM or AMPH subgroups ($d = 0.175$, 0.120 and 0.154, respectively).

No measures detected significant symptom worsening in any group; treatment satisfaction exceeded subjects' expectations. Benefits among AMPH group subjects were durable 12 weeks after treatment cessation.

Conclusions: Results suggest that these pharmacologic regimens may augment or accelerate TCT-induced clinical gains in psychosis patients. The ability of AMPH or MEM to augment the clinical impact of TCT in this small, single-site study does not justify the routine use of either medication to enhance TCT effects in psychosis patients. Pharmacologic augmentation of TCT in SZ patients remains a promising therapeutic approach warranting further assessment in larger samples.

Keywords: amphetamine, memantine, targeted cognitive training, schizophrenia

Disclosure: Nothing to disclose.

P175. The Role of Oxytocin in the Relationship Between Social Factors and Chemotherapy-Associated Cognitive Decline

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Background: Chemotherapy can cause debilitating side effects such as cognitive decline. Interestingly, social factors, including having an intimate long-term partner or a robust social network can be protective. While the mechanisms by which this occurs are currently unknown, oxytocin (OXT) is an important hormone for social interaction and can influence cognition. Thus, we hypothesized that social factors reduce chemotherapy-associated cognitive decline, and that this relationship is mediated by circulating OXT and OXT receptor (OXTR) expression.

Methods: Forty-eight partnered, female breast cancer patients from a longitudinal study conducted at the OSU Comprehensive Breast Center were included. Participants completed self-reported and objective cognitive assessments and provided blood samples at 3 timepoints: pre-chemotherapy, during chemotherapy, and approximately three months post-chemotherapy. Participants also

completed retrospective, self-reported couple satisfaction and perceived social support indexes to gain insight on their social factors over chemotherapy treatment. Plasma was used to quantify [OXT] using immunoassays. Peripheral blood mononuclear cells were analyzed for OXTR gene expression using RT-qPCR. Linear mixed effects models were used to assess changes over time, and regression models were used to quantify associations between change scores and social factors.

Results: Subjective cognition and objective memory decreased over chemotherapy treatment ($p = 0.0042$ and $p = 0.009$ respectively), and these decreases were associated with lower couple satisfaction from pre- to post-chemotherapy ($p = 0.0086$, estimate: 0.11 and $p = 0.006$, estimate: 0.03, respectively). While an age- and education-normalized composite measure combining all four objective cognitive assessments did not change over chemotherapy treatment ($p > 0.05$), reductions in the cognitive composite over chemotherapy related to both lower couple satisfaction ($p = 0.032$, estimate: 0.01) and perceived social support ($p = 0.019$, estimate: 0.01). Additionally, plasma [OXT] decreased from pre- to during chemotherapy ($p = 0.002$), whereas PBMC OXTR tended to increase ($p < 0.09$). While the decrease in [OXT] was not associated with couple satisfaction ($p > 0.05$) or perceived social support ($p > 0.05$), it was strongly associated with longer relationship duration ($p = 0.008$, estimate: -0.011). In contrast, the increase in OXTR from pre- to during chemotherapy tended to be associated with lower couple satisfaction ($p = 0.08$, estimate: -0.01). Finally, changes in [OXT] and OXTR gene expression were not associated with the changes in the three cognitive measures over chemotherapy ($p > 0.05$ for all).

Conclusions: These findings indicate that while social factors were protective against chemotherapy-associated cognitive decline, they were not related to OXT/OXTR alterations due to chemotherapy. Understanding how changes in OXT/OXTR over chemotherapy may relate to side effects requires further investigation. Understanding biological mechanisms is necessary to develop novel preventative and interventional therapies to mitigate or reduce the adverse side effects of chemotherapy.

Keywords: Social Factors and Functioning, Cognition, oxytocin

Disclosure: Nothing to disclose.

P176. Building a Foundation for Indigenous Cultural Resilience Neuroscience Research: Using the American Indian Multimedia Stimulus Set to Measure Functional Connectivity of the Default Mode and Salience Networks

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Background: American Indian (AI) peoples have long practiced cultural traditions that support mental health and well-being. However, these populations face significant stress due to historical and ongoing colonization. Understanding how cultural protective factors, such as a connection to traditional tribal culture, confer resilience against stress and mental health issues is crucial. The default mode network (DMN) is a network of brain regions, including the ventromedial prefrontal cortex, posterior cingulate cortex, and precuneus, that is active during rest and self-referential thought processes. The DMN is associated with functions such as introspection, memory retrieval, and social cognition, all of which are critical for understanding one's cultural identity and sense of self. The salience network (SN) includes brain

regions such as the dorsal anterior cingulate cortex and anterior insula and is involved in detecting stimuli in the environment and filtering stimulus inputs for salience. Studies have shown that alterations in DMN and SN connectivity are linked to various mental health disorders, including depression and anxiety. Examining the DMN and SN's functional connectivity (FC) in response to culturally relevant stimuli may provide insights into how cultural connections enhance mental health and resilience in AI populations. Thus, we aimed to (1) examine the within-network FC of the DMN and SN when viewing cultural versus non-cultural (comparator) stimuli in AI adults, and (2) investigate the relationship between FC contrasts (cultural versus comparator) and participants' demographic, cultural, and clinical characteristics.

Methods: In this pilot study, we developed and validated video and audio stimuli reflecting AI cultural content, along with matched comparator stimuli, for use in neuroimaging research. The sample consisted of 45 AI adults (ages 21-64; 84% female, 16% male) with available neuroimaging data. Participants viewed the cultural and comparator stimuli while undergoing functional magnetic resonance imaging (fMRI). We analyzed within-network FC between key nodes of the DMN (ventromedial prefrontal cortex, posterior cingulate cortex, precuneus) and SN (dorsal anterior cingulate cortex, anterior insula), using the Brainnetome atlas for anatomical parcellation. We calculated average correlations across relevant nodes for each network. Participants also provided self-reports of their demographic, cultural, and clinical characteristics.

Results: The DMN and SN showed positive within-network FC on average when viewing both cultural and comparator stimuli. Linear regression analyses revealed no significant differences in average DMN and SN FC values when comparing cultural and comparator stimuli (B 's = -0.23-0.20, p 's = 0.23-0.49). However, bivariate correlation analyses indicated that lower self-reported historical loss symptoms were associated with greater FC within the DMN ($r = -0.53$, $p = 0.004$) and SN ($r = -0.41$, $p = 0.028$) when viewing cultural versus comparator video stimuli. For audio stimuli, lower acculturation correlated with greater FC in the DMN ($r = -0.36$, $p = 0.028$), while higher enculturation correlated with greater FC in the SN ($r = 0.41$, $p = 0.021$) when viewing cultural versus comparator stimuli. Age and sex were not significantly related to FC differences (r 's = -0.05-0.19, p 's = 0.21-0.96), and the cultural findings remained significant when controlling for these variables. Additionally, greater FC in the DMN during cultural audio stimuli was associated with higher anxiety symptoms when controlling for age and sex ($B = 0.31$, $p = 0.047$).

Conclusions: The findings suggest that the novel stimulus set is effective in probing cultural characteristics at the neurobiological level. Specifically, higher FC to cultural versus comparator stimuli within the DMN and SN correlates with cultural characteristics such as lower historical loss symptoms and higher enculturation. These results highlight the potential for using culturally relevant stimuli to understand the neurobiological basis of resilience among AI peoples. Future research should further explore the interactions between cultural and clinical characteristics to develop targeted interventions that enhance mental health equity for AI populations.

Keywords: Culture, Resilience, American Indian, fMRI Functional Connectivity

Disclosure: Nothing to disclose.

P177. Reaction Time as a Transdiagnostic Cognitive Marker of Goal-Directed Attention, Learning and Memory

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Background: Reaction time is an indicator of cognitive performance that reflects different facets of cognition and learning, including attentional, information processing, working memory, motor preparation and movement execution processes. Alterations in these capacities are frequently associated with neuropsychiatric conditions. Yet, psychiatric disorders often present with complex profiles that do not adhere strictly to diagnostic criteria. This creates the need for objective tools that can better identify a constellation of cognitive, behavioral, and neural features. High-dimensional characterization of behavioral metrics with reaction time can enhance our understanding of patients' clinical profiles and lead to more precise interventions. So far, increased reaction time variability is a recognized feature in ADHD, ASD and other psychiatric conditions, however pinpointing the exact cognitive processes responsible remains complex and research aimed at disentangling these processes is still elusive. To further investigate the role of reaction time in cognition and mental illness, we paired established psychiatric questionnaires with a context generalization learning task of attention and memory, then analyzed the association between traits, task behavior and reaction times.

Methods: Subjects participated in a context generalization task, where they responded to visual stimuli defined by color, shape, texture and size and earned points for selecting the rewarded action. They could use several qualitatively unique cognitive strategies to successfully learn the task: focusing on the goal-directed feature (shape), memorizing each stimulus individually, or creating a composite representation of stimuli associated with actions. Each strategy resulted in specific patterns of actions and errors, reflecting the distinct cognitive strategies. The task was paired with questionnaires assessing for traits associated with ASD, attention-deficit/hyperactivity disorder (ADHD), obsessive compulsive disorder (OCD), depression, schizotypy and psychosis. The distinct cognitive strategies, are associated with specific psychiatric traits, such as rigidity, associated with ASD, OCD and schizotypy. Participants were recruited online. 744 participants completed the first online session, which is the focus of this analysis. 52 participants were excluded for taking extended breaks during the task, which had no time limit. For our investigation we focused on specific reaction time variables (RT variables), including the initial mean reaction time (first 8 trials), the final mean reaction time (final 24 trials), the overall mean reaction time (across all trials), and coefficient variation of reaction time (final 24 trials). Investigating reaction time, we found that cognitive strategies differ significantly between their mean RT variables (initial mean, mean across all trials and final 24 trials) and between their RT variability. We hypothesized that RT variables would a) predict distinct psychiatric traits b) integrating RT as an outcome will update the cognitive strategies in a more informative way and will reflect unique psychiatric traits.

Results: We found that higher scores of ADHD, ASD, OCD, schizotypy traits predict slower mean RT variables. Additionally higher scores of ADHD, OCD, schizotypy and schizophrenia traits also predict increased reaction time variability. When we included RT variables in behavioral metrics of the task, cognitive strategies were further subdivided based on reaction times. Interestingly, the cognitive strategies of discriminative goal directed attention and non-goal discriminative attention were each further sub divided into qualitative cognitive strategies of higher or lower reaction time variability and slower/faster mean reaction time. Moreover, we found that these new cognitive strategies differed significantly in ASD (p -value = 0.002) and OCD traits (p -value = 0.036), but not ADHD traits. The cognitive strategy of goal-directed attention and higher variability in reaction time showed elevated ADHD, ASD, and OCD trait scores. In contrast, the strategy with a mix of goal-directed and non-discriminative attention, along with highly variable reaction time, exhibited very low psychiatric trait scores.

Conclusions: We examined the association of psychiatric traits with mean reaction time and reaction time variability in a task that distinguishes unique strategies of goal-directed attention, learning, and memory. We found that slower and more variable reaction times

were associated with higher traits of ADHD, ASD, OCD, and schizophrenia. This supports reaction time as a potential transdiagnostic marker. The cognitive strategy with high discriminative goal-directed attention but variable and slower reaction times showed elevated ADHD, ASD, and OCD traits, unlike the cognitive strategy with mixed goal-directed and non-discriminative attention but similar reaction time variability. Notably, cognitive strategies significantly differed in OCD and ASD traits. These findings underscore the role of reaction time in attentional cognitive processes and its potential utility in disentangling pathologies such as ADHD, ASD, and OCD, leading to more precise interventions. Future research should focus on further decomposing reaction time to provide deeper insights into the distinct cognitive processes it reflects.

Keywords: Attention, ADHD, Cognition

Disclosure: Nothing to disclose.

P178. Emotional GPS: Tracking the Emotional Self Through Functional Segregation of the Anterior Insula

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Background: Emotional Meta-Awareness (EMA)—the capacity to recognize and understand one's own emotions—is essential for effective emotional regulation and overall mental well-being. The right anterior insula plays a pivotal role in interoceptive awareness 1, a key component underpinning EMA, yet the cognitive and neural mechanisms that drive EMA remain insufficiently understood. Traditional self-report measures are prone to bias, as they depend on the very construct they aim to assess. To overcome this limitation, we developed a novel EMA marker by tracking the alignment between subjective (self-report) and objective (pupil size) emotional intensity during movie viewing. We hypothesized that EMA would be (1) impaired during dysphoric provocation compared to the neutral condition, (2) linked to higher self-ratings on interoceptive awareness (Noticing scale) of the Multidimensional Assessment of Interoceptive Awareness (MAIA 2), and (3) associated with greater functional segregation of the ventral anterior insula (vAI) as measured by modularity degree.

Methods: Pupil size was tracked during dysphoric and neutral emotional provocation in the scanner (5 minute movie clip for each condition). Participants ($n = 17$) then rewatched the clips, annotating their initial emotional intensity. EMA was defined as the dynamic correlation (15s window, 1s step) between subjective (annotations) and objective (pupil size) arousal markers, adjusted for time lags using principal component alignment. Modularity degree (MD) was calculated on signed, unthresholded connectivity matrices over 15-second windows using the community Louvain algorithm (214 regions: Schaefer 2003, 8 subcortical regions from Harvard-Oxford parcellation 4, 6 insula sub-parcellations from Deen parcellation 5). Linear mixed effects models predicted EMA based on MD for the 9 insula sub-regions, accounting for emotion state and the MAIA Noticing index.

Results: Dysphoric stimulation significantly impaired EMA across individuals ($\beta = -0.82$, 95% CI [-1.44, -0.19], $p = 0.013$). A significant interaction between Noticing and emotion category showed that higher Noticing was linked to higher EMA in the neutral condition, but this association was lost during dysphoric stimulation (interaction (condition [neutral vs. dysphoric] x Noticing): $\beta = -0.75$, 95% CI [-1.34, -0.15], $p = 0.016$). Additionally, lower Noticing scores were linked to higher vAI modularity, while this association waned with higher Noticing scores

(interaction vAI modularity x Noticing: $\beta = -0.46$, 95% CI [-0.74, -0.18], $pFDR = 0.01$).

Conclusions: These findings suggest that while EMA is generally supported by interoceptive sensitivity, this relationship breaks down under dysphoric provocation. The compensatory role of ventral anterior insula modularity in individuals with lower interoceptive awareness highlights the brain's adaptive mechanisms in maintaining emotional awareness even when typical interoceptive cues may be less accessible to some individuals.

Keywords: Emotional Meta-Awareness, Interoceptive Awareness, Insula, Stress, Pupil Size

Disclosure: Nothing to disclose.

P179. Assessing Dynamic Gabaergic Function in Psychosis Spectrum Patients: Preliminary Results From an Ongoing Study

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Background: There is considerable evidence implicating GABAergic dysfunction in schizophrenia and bipolar disorder. GABAergic agents treat these psychosis spectrum patients, often for anxiety, but the response of catatonic patients to GABA-enhancing benzodiazepines suggests a deeper link between GABA and this psychosis spectrum. Here, using a pharmacologic challenge with lorazepam, a benzodiazepine that allosterically modulates GABA receptors, we measure both blood oxygenation level dependent (BOLD) change and regional cerebral blood flow, to investigate the effects of GABAergic agents on the brain in psychosis.

Methods: Participants were 29 patients (11 men/18 women) with schizophrenia spectrum disorders (schizophrenia, schizoaffective disorder, and psychotic disorder not otherwise specified), 21 bipolar I patients with a history of psychosis (8 men/13 women), and 40 demographically matched healthy control participants (18 men/22 women). Participants took part in two identical imaging sessions using a 3T Philips Achieva scanner, during which functional MRI data was acquired as they passively viewed positive, negative and neutral images from the International Affective Picture System (IAPS). Regional cerebral blood flow data was collected using arterial spin labeled (ASL) MRI. In addition, a high-quality structural image was acquired.

Approximately 2 hours before each session, participants were given either lorazepam or a placebo orally. Each participant took part in both a lorazepam session and a placebo session, the order of which was randomized. During each session, both the participant and data collection staff were blind as to whether the participant had received the drug or placebo. The two sessions were approximately a week apart.

Functional MRI data were preprocessed using the fMRIprep standard preprocessing pipeline, and responses to positive, negative and neutral images were modeled. ASL MRI data was preprocessed and modeled using BASIL, available within FSL. For both fMRI and ASL data, second level analyses were performed in SPM to explore the main effects of session (drug, placebo), diagnosis (schizophrenia spectrum, bipolar I, healthy control), as well as session x diagnosis interaction effects.

Results: Across all participants, the functional MRI data showed a significant reduction in BOLD activity in visual cortex during the lorazepam session ($p < .05$, FWE). Additionally, there was a significant session by diagnosis interaction effect observed in bilateral dorsolateral prefrontal cortex ($p < .001$, unc). There were

no significant main effects of diagnosis. During the lorazepam session, regional cerebral blood flow was also significantly decreased in posterior cingulate cortex and bilateral temporal cortex across all participants ($p < .05$ FWE). We found no significant effects of diagnosis, or session by diagnosis interaction effects in the analysis of regional cerebral blood flow.

Conclusions: These results provide evidence that the administration of a GABAergic agent results in regional changes in both BOLD and regional cerebral blood flow. These results are preliminary, and data collection is ongoing.

Keywords: GABA, ASL, BOLD fMRI signal, psychosis

Disclosure: Nothing to disclose.

P180. Heterogeneity in Cognitive Capacity and Impulsivity Predicts the Subjective Cost of Self-Control

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Background: A growing body of work has demonstrated that the deployment of cognitive control is perceived as subjectively effortful to humans. We recently extended this work to the domain of self-control by measuring the monetary cost choosers were willing to pay in order to use precommitment strategies to avoid tempting rewards that may lead to self-control failures. This work suggests that the extent to which individuals value precommitment can be taken as a proxy for how costly they find exercising self-control to be. Here, we extended this work to examine how heterogeneity in cognitive capacity and impulsivity—two factors widely known to influence goal-directed behavior—shape the perceived cost of self-control.

Methods: 90 healthy dieters completed a decision-making study in which they first completed a cognitive battery of tasks that included a working-memory measure (Automated Operating Span, or OSPAN) and an intertemporal choice task in which choosers made binary choices between smaller, sooner and larger, later monetary rewards. Participants returned a week later and completed our self-control decision task, during which they viewed images of low, medium and highly-tempting snack foods and reported how much they would be willing to pay to avoid each these food rewards across different amounts of time. OSPAN scores and proportion of immediate (vs. delayed) rewards chosen were used to index working-memory capacity and impulsivity, while average WTP at each level of temptation served as a metric of self-control costs.

Results: Linear mixed-effects models revealed that greater working-memory capacity predicted higher WTP to avoid self-control across all temptation levels ($p < .001$), suggesting that higher working-memory may lead to a better capacity to prospectively estimate how costly self-control will be once temptations are encountered. In contrast, higher impulsivity predicted lower WTP to avoid temptation ($p < .001$), suggesting that self-control costs are estimated to be lower in more impulsive individuals.

Conclusions: Our findings provide novel insight into individual variability underlying self-control costs by demonstrating that working-memory and impulsivity have distinct and inverse effects on the prospective estimation of these costs. This finding suggests that cognitive capacity and impulsivity may be predictive of the extent to which individuals perceive precommitment to be a valuable strategy and point to constructs often related to goal-directed control as impacting how subjective self-control costs are constructed. Future work may seek to determine the extent to which working memory and impulsivity not only predict the

estimation of self-control costs but the subsequent success of such strategies.

Keywords: Self-control, Decision Making, Cognitive Functioning
Disclosure: Nothing to disclose.

P181. Memory Biases for Alcohol-Related Events Within and Outside the Lab Associated With Harmful Drinking Behavior

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Background: Memory plays a key role in alcohol use, as what people learn and remember about their experiences with alcohol can drive later drinking. However, we know little about how individuals who engage in riskier patterns of drinking encode and retain such alcohol-related episodes, and whether laboratory observations of these processes extend to memories for real drinking experiences. In a set of experiments, we measured the formation of memories for alcohol-related memoranda in the laboratory (Study 1) and, using smartphones, the formation of memories for real alcohol drinking events (Study 2).

Methods: In this ongoing set of experiments, we have recruited risky social drinkers (engaging in regular binge episodes but not meeting diagnostic criteria for AUD, current $N = 26/35$) and light social drinkers ($N = 22/35$). After an intake appointment to assess drinking profiles, participants completed both in-lab (Study 1) and real world (Study 2) experiments. In Study 1, participants encoded trial-unique object/scene pairs while undergoing fMRI. Of these 80 pairs (or "events"), 50% contained photographs of alcoholic beverages and the 50% contained photographs of neutral objects. The next day, we assessed participants' memory for the objects, object/scene associations, and their affective state during encoding. After completing these memory tests, participants began Study 2. In this study, participants responded to prompts on their smartphones. On a given day, they would receive two notifications to enter the current time and report details of what was happening at that moment (where they were, how they were feeling, etc). They would also be prompted to initiate one of these surveys when they had their first drink of the day. The next morning, they would be asked to retrieve memories for the events reported the previous day. Participants completed these smartphone prompts for 14 days. In both settings, we analyze memory accuracy per feature (i.e., how well they remember different features of an event) as well as a novel measure of memory "co-accuracy", reflecting how tightly memory for different event features are integrated (i.e., how likely a participant is to remember one feature if they have forgotten another).

Results: The smartphone-based approach yielded high completion rates (completion rate for encoding prompts: 91.4%; completion rate for paired retrieval prompts: 82.1%; $N = 113$ drinking encoding/retrieval pairs for risky drinkers thus far). Participants also showed above-chance memory in both laboratory and real-world settings. Critically, risky drinkers formed distinct memories for alcohol-related events in both settings, showing greater integration (lab: varies by memory feature; $p = .03$, real world: $p = .006$). We also observed differences in memory biases between settings. For example, although accuracy in-lab was generally worse for alcohol vs neutral events ($t(1815) = -1.8$, $p = .07$), risky drinkers had significantly more accurate memory for real drinking events compared to other daily experiences ($t(372) = 3.23$, $p = .001$). Preliminary analyses of fMRI data obtained during in-lab encoding suggest that risky drinkers also engage distinct neural mechanisms when forming memories for alcohol-related events. When we examined patterns of responses associated with individual events, we found that risky drinkers showed more integrated (that is, more

similar) hippocampal representations for emotionally salient alcohol events. Ongoing analyses investigate how these memory properties contribute to subsequent alcohol intake reported during smartphone monitoring.

Conclusions: Our smartphone-based memory sampling approach provides a feasible method to study memory for real and clinically meaningful experiences. Comparing these results to in-lab memory assessments highlights important distinctions between memories for experiences in the laboratory and the real world. Together, these findings provide ecologically valid insight to the organization of alcohol-related memories associated with maladaptive drinking.

Keywords: Alcohol, Memory Bias, ecological momentary assessment, Functional MRI (fMRI), episodic memory
Disclosure: Nothing to disclose.

P182. A Candidate Neural Substrate for the "Effort Paradox": Temporal Interference (TI) Stimulation to the Dorsomedial Prefrontal Cortex Modulates Effortful Foraging and its Effect on Mood

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Background: Effort-based decision-making (EBDM) has emerged as a critical paradigm for understanding the pathophysiology of motivational deficits, yet key questions remain. Notably, the impact of effort on mood and affect is ambiguous, as effort may both act as a cost and add value (e.g., Ikea effect). Moreover, while most people discount rewards based on effort, a subset of "effort seekers" seem to prefer rewards that require greater effort. Importantly, these phenomena may depend on function of the dorsomedial prefrontal cortex (dmPFC), which has been implicated in EBDM across species. Here, we first present behavioral results from four independent samples ($n = 399$) during a novel effortful exploration task to examine the effects of effort, reward, and exploration on mood. Next, we present data on two samples using temporal interference stimulation of the dmPFC to identify a causal role for dmPFC involvement in the effects of effort on mood.

Methods: A total of seven samples were collected using the newly developed Virtual Effortful Exploration Task (VEET), which involved effortful foraging within a quasi-naturalistic, three-dimensional environment. Specifically, participants navigated a map containing rooms with varying levels of effort (i.e., friction that decelerates player movement; 3 levels: High, Medium, Low) and reward (i.e., points per coin; 3 levels: High, Medium, Low) with the objective of collecting coins and maximizing points. Samples 1-5 ($n \sim 100$ each) were collected online using the Unity platform. Data were analyzed using linear-mixed effects models testing the effects of trial-wise mood, reward, and effort on trial-wise mood ratings. The first two samples were collected for purpose of initial hypothesis formation, sample 3 was collected as a replication sample for results obtained in samples 1 and 2, and samples 4 and 5 introduced a manipulation of effort vs. non-effortful exploration. Samples 6 and 7 were collected in the lab while participants underwent temporal interference (TI) stimulation at 20hz targeting dmPFC. Data were analyzed using linear-mixed effects models testing the effects of trial-wise mood, reward, and effort on trial-wise mood ratings.

Results: In samples 1-4, we found that effort expenditure, exploration, and reward independently predicted momentary mood (all p 's $< .001$). Critically, we observed that effort appeared to have dual, opposing effects on mood: On one hand, increased

effort expenditure predicted more negative mood, and on the other hand, we found that the removal of effort in samples 4 and 5 resulted in a reduced impact of reward on mood (p 's < .01). As such, reward predicted subsequent mood only when foraging required effort. In samples 6 and 7, we examined the effects of temporal interference (TI) stimulation to dmPFC on effort and its effects on mood. In sample 6, we observed that TI stimulation of dmPFC directly enhanced effortful activity, and also moderated the impact of effort on momentary mood such that greater effort become a positive predictor of mood for most participants. Replication analyses for sample 7 are ongoing.

Conclusions: Taken together, these data help shed light on the interplay between reward, effort and exploration on momentary mood, and help unpack the role of dmPFC in underlying "effort seeking" behavior.

Keywords: Effort, exploration-exploitation tradeoff, Mood, Non-invasive Neuromodulation, dorsomedial prefrontal cortex

Disclosure: Boehringer Ingelheim: Consultant (Self)

P183. Affective Reactivity During Naturalistic Movie Viewing in MEG: Implications for Depression

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Background: Depression is characterized by low mood and decreased response to positive stimuli. A hurdle to identifying objective biomarkers for depression is that the depressed state is not static. Advances in neuroimaging have enabled analyses of brain synchronization using "naturalistic" stimuli to ascertain inter-subject correlations (ISC) across participants. In such paradigms, participants passively view stimuli such as short film clips during scanning to evaluate group-level brain synchrony across a dynamic stimulus. Applications to clinical populations are limited, but essential; these approaches move beyond static images to recreate exposure to emotional stimuli in laboratory settings, providing a window into potential brain processes underlying alterations in affective dynamics in individuals with mood disorders. This study examined affective reactivity in individuals with mood disorders in combination with emerging techniques related to naturalistic viewing during magnetoencephalography (MEG) scanning. Specifically, we evaluated changes in affective response to a mood induction task in a sample of individuals with mood disorders and healthy volunteers.

Methods: Forty-two participants with mood disorder diagnoses (MD, 20F/22M) and 30 healthy volunteers (HVs, 21F/9M) underwent MEG scanning (CTF 275 system) and viewed four film clips in random order. Two of the clips were selected to be linked with changes in sadness or negative affect and two for changes in positive affect. Current mood was assessed using the Positive and Negative Affect Schedule (PANAS) before and after each film clip. MEG data were inspected for bad channels and cleaned using Independent Component Analysis (ICA) before bandpass filtering into frequency bands (Delta, Theta, Alpha, Beta, and Gamma) and Hilbert transforming the timeseries. These data were then source modeled to each participant's unique individual cortical surface using a Boundary Element Model (BEM) forward solution with a dSPM inverse operator in MNE-Python. Pairwise subject correlations were performed in source space using AFNI across all participants; ISC analysis was conducted in R using a Linear Mixed Effects (LME) model (3dISC) to identify group effects.

Results: MDs and HVs demonstrated altered mood reactivity in response to viewing the movie clips. Specifically, HVs reported

increased negative affect and decreased positive affect after the sad movie clips, suggesting that they became "sadder" with the mood induction. This pattern of mood reactivity was not found for the MDs. Source reconstructed brain responses showed widespread increases in ISC in HVs particularly in the alpha and gamma frequencies, mimicking other studies comparing clinical groups to healthy volunteers. In the gamma frequency, we also found significant increases in ISC in the anterior cingulate, a region implicated in the pathophysiology of depression, for all movie clips. More focal increases in synchrony in MDs compared to HVs were found in early visual cortex and the medial temporal lobes for one of the negative and one of the positive movies.

Conclusions: ISC is a powerful tool which permits brain-related metrics that map onto the process of mood induction using naturalistic stimuli such as movies. Findings confirm altered affective reactivity and neural synchrony in individuals with MDs as compared to HVs in response to a mood induction paradigm. Future studies could capitalize on these techniques by acquiring behavioral measures that may map onto ISC, which can be used for future diagnostic and treatment efforts.

Keywords: Depression, MEG, inter-subject correlation

Disclosure: Nothing to disclose.

P184. Alternating Tasks: Exploring the Influence of Emotion and Instructions on Flexibility

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Background: Flexibility is commonly assessed using multiple neuropsychological tests (e.g., Dimensional Change Card Sort (DCCS) and Trailmaking Test (TMT)). While these tests assess flexibility in the cognitive domain, flexibility in the emotional domain may be more relevant for good mental health (e.g., Malooly et al., 2013). Additionally, while these tests use two different methods to test flexibility, both provide switching instructions. The DCCS uses a cued task-switching paradigm; whereas, Trailmaking B requires completion of a single task alternating between sets (e.g., number, letter, number). Yet, individuals must exert flexibility without the aid of external instructions in the real world. Thus, we characterized how individuals flexibly generated thoughts within and across cognitive and emotional domains to understand how self-initiated flexibility uniquely relates to these other 'standardized' neuropsychological measures of cognitive flexibility. Relationships between objective measures of flexibility and self-report of emotion regulation and anxiety-related symptoms were also explored.

Methods: 146 college-aged adults (19.1 years, SD = 1.0, 18-22 years old, 60.9% female, 24.0% Hispanic, 30.1% minority) completed the NIH Toolbox DCCS task, a computerized TMT and a verbal generation flexibility task. Symptom measures (e.g., Emotion Regulation Questionnaire, Obsessive-Compulsive Inventory, and Screen for Adult Anxiety-Related Disorders) were also collected. In the verbal generation task, individuals were presented with two categories (dual task) and instructed to verbally generate as many items as possible in 60 seconds, alternating between the categories. Cognitive condition presented two non-emotional categories (e.g., things that grow). Emotional condition presented two emotional categories (e.g., annoying habits). In addition, the cognitive-emotional condition presented one category of each. Video recordings of the sessions were reviewed for accuracy. Repeated measures ANOVA were used to test significant condition (e.g., cognitive, cognitive-emotional, emotional) effects in the number of items generated. In separate models, DCCS switch cost, reaction time difference between

correct switch and correct repeat trials, and TMT-B completion time were included.

Results: In the dual alternating task, the most responses were observed in the cognitive condition, while the emotional condition elicited the least (i.e., cognitive > cognitive-emotional > emotional, task effect: $F(2,144) = 56.5$, $p < 0.001$, $\eta^2 = 0.44$). DCCS switch cost did not influence this effect ($p > 0.6$). However, TMT-B completion time interacted with the condition, such that individuals with faster reaction times on TMT-B generated more items in the cognitive ($r(146) = -0.246$, $p < 0.001$) and the mixed condition ($r(146) = -0.227$). TMT-B time was not associated with the emotional condition ($p > 0.4$) or DCCS switch cost ($p > 0.8$). Several measures tended to be related to self-reported symptoms. DCCS switch cost, a measure of inflexibility, tended to be positively correlated with emotion suppression ($r(144) = 0.14$, $p > 0.09$) and obsessive compulsive symptoms ($r(144) = 0.14$, $p > 0.08$). Contrary to expectations, the number of items generated in cognitive-emotion condition tended to be correlated with generalized anxiety disorder (GAD) symptoms ($r(144) = 0.15$, $p < 0.08$).

Conclusions: Several conclusions can be drawn from these behavioral results. First, flexibility within the emotional domain may be distinct from flexibility in the cognitive domain. This distinction should be taken into consideration when examining neuropsychological profiles of psychiatric disorders. Second, the instructions and task-structure of existing neuropsychological tests may assess different types of flexibility, which impact the interpretability of the results. Future work is needed in clinical populations to understand the relationships between objective measures of flexibility to symptoms. Additionally, we need to determine the utility of these measures for capturing actual behavior in real-world situations.

Keywords: affective neuroscience, neuropsychology, cognitive flexibility

Disclosure: Nothing to disclose.

P185. Uncertainty and Value Estimation in the Human Prefrontal Cortex Govern Exploratory Behavior

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Background: Successful navigation in complex environments requires individuals to strike a balance between exploration and exploitation, a fundamental aspect of mental flexibility. Decisions to explore are influenced by how we combine uncertainty about our choices with the value of those choices. Deciphering the neural underpinnings of how these measures are computed in the human brain is crucial for understanding healthy, adaptive behaviors and pathological, maladaptive behaviors in mental illness.

Methods: Surgical epilepsy patients with intracranial electrodes performed a three-armed bandit task while we recorded intracranial EEG data from the ventral medial prefrontal cortex (vmPFC), dorsal medial prefrontal cortex (dmPFC), and dorsolateral prefrontal cortex (dlPFC). Local field potentials were analyzed with 2D time-frequency cluster-based permutation statistics and transfer entropy.

Results: In dlPFC, theta band power negatively tracked the relative uncertainty about choice during learning ($Z = -3.3$, $p < 0.001$) and positively tracked the relative value of choice during decision-making ($Z = 3$, $p < 0.001$). In dmPFC, high gamma positively tracked the relative uncertainty about choice during learning ($z = 2.11$, $p < 0.05$) and negatively tracked the relative

uncertainty about choice during decision-making. Dynamic causal modeling revealed a directional connection from dmPFC to dlPFC, consistent with the results from a transfer entropy calculation.

Conclusions: Decisions to explore are guided by the integration of choice value and uncertainty in dlPFC, based on computations in dmPFC. These results suggest that dysfunction in dmPFC-dlPFC interactions may be particularly important in conditions such as anxiety with distortions in how individuals balance uncertainty about the world with the potential benefits of choices.

Keywords: electrophysiology, explore-exploit dilemma, uncertainty, Value-based Decision-Making

Disclosure: Nothing to disclose.

P186. Trait and State-Dependent Multidimensional Phenotyping

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Background: Computational phenotyping characterizes an individual through computational modeling of behavior across several cognitive tasks. Combined computational metrics from learning, decision-making, and metacognition tasks map onto clusters of transdiagnostic symptomatology (Wise et al., 2023). More recently, the field has noted the importance of state-driven effects on these same cognitive computations (Hitchcock et al., 2022, Schurr et al., 2024). To account for these effects, here we evaluate how affective state manipulations can shift a computational phenotype and whether these changes are predictive of mental health symptoms.

Methods: 42 participants (24 women) completed a series of mental health questionnaires (PHQ-9, BDI, BAI) and were randomized to 3 negative valence manipulations (stress, craving, negative emotion). On 2 sessions (experimental vs sham), they completed 3 tasks assessing choice impulsivity, tolerance for risk and ambiguity in gains and losses, and choice confidence (a measure of metacognition.) We used computational modeling to derive a multidimensional subject- and session-specific phenotype. We applied cross-validated LASSO logistic regression and LASSO-GLM to test associations between symptomatology and baseline vs experimental computational phenotypes.

Results: The baseline computational phenotype predicted the presence of any depression symptomatology in the PHQ-9. Notably, choice stochasticity, choice impulsivity, and risk tolerance survived the LASSO regularization procedure. The computational phenotype was symptom-specific: anxiety was associated with greater ambiguity aversion for losses and reduced tolerance for risk in gains, while depression was associated with reduced choice confidence. Interestingly, the affective perturbations induced changes that explained more anxiety variance than the baseline model.

Conclusions: Computational phenotypes are susceptible to state-driven changes. Deviations in computational phenotype in response to emotional state perturbations were predictive of anxiety. We conclude that efforts to characterize psychopathology as a function of computational measures of behavior benefit from accounts of affective state. Longitudinal studies that track states are key to uncovering how behavioral dynamics can improve clinical prediction.

Keywords: Computational Cognitive Neuroscience, Value-based Decision-Making, Mood and cognition, Anxiety and Depression

Disclosure: Nothing to disclose.

P187. Associations Between Working Memory Contributions to Reinforcement Learning and Mental Health Phenotypes in a Representative Online Hypothesis-Generating Sample

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Background: Working memory (WM) contributions to reinforcement learning (RL) have been reported to differ between participants with and without schizophrenia. It remains unexplored whether such processes are related to distinct dimensional phenotypes in the general population. We aimed to validate RLWM computational phenotypes in predicting self-reported phenotypes relevant to mental health (i.e., anxiety, depression, behavioral inhibition, disability, mania, motivation/pleasure, schizotypy) in a large online sample demographically representative of the US general population ($N = 2,300$). In addition to traditional total survey scores, we used dimensionality reduction to probe the latent structure underlying covariation of these phenotypes related to psychiatric symptoms. We compared the validity of parameters in predicting total scores and factor scores.

Methods: Participants completed a task which manipulates WM demands in the service of RL to disentangle various WM and RL contributions to learning and choices (RLWM, $N = 1,665$ after quality control exclusions, ages 18-65 years). The RLWM model was adapted from Collins and Frank (2012) using a recent variant described in Westbrook et al. (2024) and fit to choice data using a two-pass hierarchical maximum likelihood estimation (MLE) procedure with Nelder-Mead optimization. Parameters were the WM reliance, capturing the weight on WM versus RL systems for guiding choice, the WM capacity, reflecting maximum number of unique stimuli held in working memory, the decay rate of items in WM (reflecting interference from intervening trials), the RL system learning rate, the perseveration parameter reflecting the tendency to persist with a response after an error, and undirected noise (a component of decision noise potentially reflecting lapses of attention). Following Fox et al. (2024), unsupervised factor analysis was conducted on all scaled survey items, using an oblique rotation, and the number of factors was determined with an eigenvalues scree plot (motivation and pleasure scores were recoded so that higher scores reflected lower motivation and pleasure). We used sparse partial least squares analysis (SPLS) to predict i) 9 traditional survey total score metrics and ii) 3 survey factor scores from 6 RLWM parameters. All variables were scaled residuals after controlling for demographic factors (age, sex at birth, gender, trans-gender, race, hispanic ethnicity, education, and income). We used cross validation to select the number of components and threshold parameter for each SPLS test and compared the variance explained (R^2) between survey and factor scores. We used linear regression to test whether parameters significantly predicted self-reported phenotypes related to mental health better than a null model (using FDR correction across symptom tests), and compared F-statistics in predicting total vs. factor scores.

Results: RLWM parameters significantly predicted cognitive-perceptual schizotypy (SPQ-CP; $F = 5.8$, $p < 0.001$) and mania total scores ($F = 3.2$, $p < 0.018$). SPLS results captured a pattern in which SPQ-CP was associated with increased perseveration (learning less from negative feedback) and undirected noise ($R^2 = 0.019$). Mania was associated with increased WM decay rate, increased perseveration, and decreased WM reliance ($R^2 = 0.010$). Factor analysis yielded three interpretable factors. Factor 1 had positive

loadings for depression, anxiety, disability, and schizotypy; Factor 2 had positive loadings for motivation/pleasure reductions and mania and negative loadings for interpersonal schizotypy; and Factor 3 has negative loadings for behavioral inhibition and positive loadings for disorganized schizotypy. Higher scores on Factor 1 were significantly associated with decreased learning rate, increased undirected noise, and decreased WM reliance ($R^2 = 0.009$, $F = 2.3$, $p < 0.044$). Higher Factor 2 scores were significantly associated with increased WM decay rate, decreased perseveration, and decreased WM reliance ($R^2 = 0.017$, $F = 5.6$, $p < 0.001$).

Conclusions: Results of this hypothesis-generating study support the utility of the RLWM task as a computational phenotyping tool useful for studying mechanisms of psychiatric symptoms (especially schizotypy, mania) in community samples. Parameters were similarly predictive of both total and factor scores (comparable F-statistic and R^2). We will test the out-of-sample replicability of these findings in an independent sample of 5,000 participants.

Keywords: schizotypy, computational psychiatry, working memory, Reinforcement learning, dimensional psychopathology

Disclosure: Nothing to disclose.

P188. Hair Cortisol Concentration Associates With Cognitive Performance in Healthy Late Peri- and Early Postmenopausal Women

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Background: Perimenopause is associated with decrements in cognition, including attention and verbal learning and verbal memory. The brain regions underlying these functions--the prefrontal cortex and hippocampus--are susceptible to the effects of estrogen and cortisol. Whether long-term markers of hypothalamic-pituitary-adrenal axis activity, such as hair cortisol concentration levels, are associated with these cognitive decrements over perimenopause has not been studied. The present project sought to determine the degree to which hair cortisol concentration is associated with cognitive performance on tests of (1) verbal memory and verbal learning, and (2) attention and working memory among healthy women in late perimenopause or early postmenopause.

Methods: Fifty-nine participants who were generally healthy, not on hormone therapy, and in the late perimenopause or early postmenopause completed online cognitive tests and provided a hair sample under supervision via Zoom. Verbal learning and verbal memory was assessed using the California Verbal Learning Test - Third Edition (CVLT-3; immediate recall, delayed recall, overall recall). Attention and working memory were assessed using the 3-back trial of the letter n-back task (true positives, false positives). Hair samples were assessed for hair cortisol concentration using an enzyme immunoassay. Hair cortisol concentration levels were log transformed due to a right skew. A multiple linear regression with an adjusted and unadjusted model was used to assess the association between hair cortisol and verbal learning and verbal memory, while negative binomial regressions were used for the 3-back tests due to their count nature. All adjusted models included race/ethnicity, education, and age as covariates.

Results: Regarding attention and working memory performance on the 3-back trial of the n-back, hair cortisol had a negative and statistically significant association with true positive responses in both the unadjusted model ($RR = .78$, 95% CI [.65, 0.94], $p = .011$) and the adjusted model ($RR = .77$, 95% CI [.64,

0.94], $p = .01$), such that higher levels of hair cortisol were linked with lower true positive scores. Conversely, hair cortisol was positively and statistically associated with false positive response scores in both the unadjusted model ($RR = 1.97$, 95% CI [1.11, 3.49], $p = .02$) and the adjusted model ($RR = 1.92$, 95% CI [1.15, 3.19], $p = .01$; i.e., higher levels of hair cortisol was associated with higher false positive scores). In all models, no statistically significant associations were found among any of the covariates and true/false positive response scores.

Regarding verbal learning and verbal memory performance on the CVLT-3, hair cortisol showed no significant associations in either the unadjusted or adjusted models with respect to the immediate recall index scores (unadjusted model: $B = -3.60$, 95% CI [-15.65, 8.45], $p = 0.551$; adjusted model: $B = -4.10$, 95% CI [-17.37, 9.17], $p = 0.538$), delayed recall index scores (unadjusted: $B = -4.82$, 95% CI [-16.09, 6.45], $p = .395$; adjusted: $B = -3.73$, 95% CI [-16.24, 8.78], $p = .552$), nor overall recall index scores (unadjusted: $B = -4.80$, 95% CI [-16.78, 7.16], $p = .424$; adjusted: $B = -4.44$, 95% CI [-17.58, 8.70], $p = .500$). No covariates in any of the models showed statistically significant associations.

Conclusions: This work suggests that a marker of hypothalamic-pituitary-adrenal (HPA) axis activation that captures total cortisol secretion over multiple months (i.e., hair cortisol) is associated with cognitive performance on an attention and working memory task. Study designs allowing for causal interpretations are needed to inform whether and how interventions may be helpful to promote HPA axis health and improve cognition during the menopause transition.

Keywords: executive function, chronic stress, natural menopause, language, midlife

Disclosure: Nothing to disclose.

P189. Obsessive-Compulsive Personality Disorder is Associated With Cognitive Inflexibility in Individuals With Coronary Artery Disease

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Background: Cardiovascular conditions, such as coronary artery disease (CAD), are often accompanied with comorbid mental disorders, which may have a significant effect on important clinical outcomes such as general cognitive impairment and executive dysfunction. Obsessive-compulsive personality disorder (OCPD) is a common psychiatric comorbidity in CAD. OCPD has a unique cognitive profile marked by rigid thinking and difficulties in executive functioning, which may subsequently impact adherence to behavioral change, which is often required for individuals with CAD undergoing rehabilitation. Nevertheless, there is a lack of evidence on the influence of OCPD on cognitive performance in CAD.

Our objective was to examine the influence of OCPD on the cognitive performance related to inflexibility and executive planning in individuals with CAD who are undergoing rehabilitation. To do this, we conducted cognitive tests specifically designed to assess aforementioned functions.

Methods: A total of seventy-eight persons (with a median age of 59 years and a range of 53-66 years; 11.5 % female) who had CAD were included in the study. These individuals were examined after three days of being admitted to the hospital for cardiac rehabilitation, which took place within two weeks of having either unstable angina or myocardial infarction. The Compulsive Personality Assessment Scale (CPAS) was used to assess features associated with OCPD. The Cambridge Automated Neuropsychological Test Battery (CANTAB)

was used to conduct neurocognitive testing, which included assessments of set shifting (Intra-Extra Dimensional [IED] Set Shifting) and executive planning (Stockings of Cambridge [SOC]).

Statistical analysis was performed using IBM SPSS Statistics for Windows (version 29.0.0.0; SPSS Inc, Chicago, IL, USA). Descriptive statistics were used to explore the samples' sociodemographic, and clinical characteristics. Categorical analysis within groups was completed using χ^2 or Mann-Whitney U tests. Associations between neuropsychological tests and OCPD traits between participants was completed using Spearman's correlational analysis.

Results: Ten individuals with CAD fulfilled the operational criteria for DSM-5 OCPD. Individuals with comorbid OCPD made more IED intra-dimensional shift reversal errors (2.0 [2.0–4.0] vs. 1.0 [1.0–2.0], $p = .004$), reflecting a difficulty inhibiting previously learnt responses. When all participants were analysed as a group, negative associations were found between individual OCPD traits and other aspects of cognitive performance. Hoarding was associated with increased initial thinking time on the SOC at five moves ($p = .242$, $p = .033$), while the need for control and rigidity were each associated with increased initial thinking time on the SOC at two moves (respectively, $p = .259$, $p = .022$; $p = .239$, $p = .035$), reflecting slower executive planning. A preoccupation with details was associated with fewer errors on a compound discrimination stage of the IED ($p = -.251$, $p = .026$).

Conclusions: Initial findings indicate that individuals with CAD and coexisting OCPD features exhibit higher levels of rigidity and slower cognitive planning compared to those without OCPD. This may have consequences for the effectiveness of rehabilitation.

Keywords: Obsessive-compulsive personality traits, cognitive inflexibility, executive function

Disclosure: Nothing to disclose.

P190. Computational Modeling of Cognitive Flexibility Reveals Novel Dissociations Across Disorders With and Without Repetitive Behaviors

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Background: Impaired cognitive flexibility causes dysfunction in many neuropsychiatric disorders. How the underlying computations differ between disorders remains incompletely understood. We examined latent processes underlying cognitive inflexibility in 251 patients (94 female) and 107 healthy controls (52 female) encompassing schizophrenia, depression (current major depressive disorder or dysthymia) with and without an addictive disorder, and autism spectrum disorder (ASD) with depression. We predicted that accounting for perseverative tendencies, unrelated to expected value, would dissociate disorders.

Methods: Computational models of reinforcement learning were applied to probabilistic reversal learning data in a hierarchical Bayesian approach. We examined value-free "stickiness" parameters, a tendency to prefer a recently chosen stimulus or location (side of computer screen, where applicable) regardless of outcome, as well as value-based learning rates and sensitivity to reinforced values.

Results: Individuals with ASD and depression ($0 \notin 95\%$ posterior highest density interval [HDI]) and those with both an addictive disorder and depression ($0 \notin 75\%$ HDI) showed abnormally high stimulus stickiness. Stimulus stickiness, however, was unaffected in depression without these comorbidities ($0 \in 75\%$ HDI). Individuals with schizophrenia also demonstrated increased stimulus stickiness but accompanied by decreased location stickiness (all

0 $\notin$ 95% HDI). Conversely, our published works on obsessive-compulsive (0 $\notin$ 95% HDI) and gambling (0 $\notin$ 75% HDI) disorder have shown abnormally low stimulus stickiness. Reinforcement sensitivity was diminished in ASD, schizophrenia, and depression with addiction (0 $\notin$ 75% HDI). Learning rates were unaffected across disorders (0 $\in$ 75% HDI).

Conclusions: Accounting for value-free influences underlying cognitive flexibility provided novel dissociations transdiagnostically, with implications for psychiatric classification and treatment.

Keywords: addiction, Cognitive / behavioral flexibility, Schizophrenia (SCZ), autism spectrum disorder, Depression

Disclosure: Nothing to disclose.

P191. Brain Energy States are Diagnostically Distinct and Capture Neural Dynamics, Changes in Cognition and Psychopathology Better Than Regional Activation and Connectivity

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Background: Neuroimaging studies have reported altered regional activations (1st order properties) and functional connectivity using pairwise co-activations (2nd order properties) in schizophrenia (SZ), bipolar disorder (BD) and major depressive disorder (MDD). These models provide limited systems level information because 1st order models consider brain regions in isolation and average BOLD responses over an entire task period or a task block losing temporal evolution of BOLD response. 2nd order models assume independence of pairwise correlations and ignore regional activations. Better models are needed to obtain an integrated picture of these processes represented as different states of activations and non-activations within networks. Pairwise maximum entropy model (MEM) or Ising model can integrate both 1st and 2nd order terms to capture temporal evolution of neural dynamics as a collective process in a single scalar quantity called energy states that are inversely related to the probability of occurrence.

Methods: We present data from 2 independent but mutually complementary studies applying MEM to examine differences in brain energy states.

For study 1, we selected N components, binarized the BOLD responses in these components using a threshold and implemented a pairwise MEM model using gradient descent followed by evaluation of the fit of the model. We derived a vector of energy values along a sequence of time points for each subject. We used Principal Component Analysis (PCA) to describe states in terms of a lower-dimensional manifold and visualize energy landscape in a three-dimensional state space.

For study 2, we used resting fMRI to examine the default mode network (DMN) connectivity consisting of 17 HCP regions per hemisphere. We created 3 subsets composed of size ≤ 9 regions, namely, an 8-node bilateral system and two 9-node unilateral systems. We applied gradient descent to perform MLE to find the coefficients of the MEM based on the log-likelihood function given the set of observed brain states.

Results: Study 1 examined 23 adolescent-onset SZ (AOS), which constitutes about 12% of all SZ and a more severe form of SZ, and 53 healthy controls (HC). Using executive function (EF) task-fMRI data, we computed group energy landscape features. Average energy over the course of the fMRI negatively correlated with EF and was a stronger predictor than 1st or 2nd order statistics. AOS spent more time in higher energy states corresponding to lower probability of occurrence and exhibited impaired EF and more severe psychopathology. Energy landscapes featured attractors

that were larger in HC than in AOS and corresponded to two distinct subnetworks: fronto-temporal (FT) and parieto-motor (PM). FT basin size significantly correlated with EF in controls but not in AOS.

Study 2 examined a transdiagnostic sample of SZ, BD and MDD from UK Biobank ($n = 132$) and a matched control ($n = 132$). Within clinical groups, averaged subject-specific energy level distributions were higher in SZ but lower in BD compared to HC for both bilateral and unilateral default mode network (DMN). MDD energy distributions were higher compared to HC only in the right hemisphere DMN.

Conclusions: Our data from Study 1 suggests that the neural dynamics is less efficient in AOS relative to HC in that HC spent more time in lower energy states occurring with higher probability and hence may achieve task performance and exhibit more stable and efficient brain states. Diagnostically distinct energy states among BPD, MDD and SZ suggest that probability distributions of temporal patterns of synchronously active nodes may underlie each diagnostic entity. Examination of functional dynamics using the MEM, which is well-established in fields such as quantum physics, has been applied to neuroscience only recently. The MEM approach is especially useful to investigate statistics of the time-varying dynamics during cognitive tasks because the model includes BOLD responses at every repetition time (TR) of the fMRI acquisition providing arguably the best temporal resolution for fMRI data analysis compared to traditional first- or second-order comparisons as well as most models of dynamic functional connectivity. Further, investigation of brain networks using a pairwise MEM and associated energy landscape provides a useful representation of network organization that can be compared between different tasks, conditions, or subject groups. Subject-specific MEMs factor in the individual variations missing from group-level inferences offering an improved measure of biologically and clinically meaningful correlates of brain activity. By applying combined mathematical and advanced statistical methods to two sets of unique data, our investigations reveal biologically important and clinically relevant findings linking brain energy states to psychopathology and cognition. These results offer great potential for further follow up for treatment development and for designing potential preventive strategies.

Keywords: Schizophrenia Spectrum Illness, Maximum entropy model, Transdiagnostic biology, Biological definition of psychiatric disorders

Disclosure: Nothing to disclose.

P192. The Effects of Bipolar Disorder Granule Cell Hyperexcitability and Lithium Therapy on Pattern Separation in a Computational Model of the Dentate Gyrus

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Background: Induced pluripotent stem cell (iPSC) derived hippocampal dentate granule cell-like neurons from individuals with bipolar disorder (BD) are hyperexcitable and more spontaneously active relative to healthy control (HC) neurons. These abnormalities are normalized after the application of lithium in neurons derived from lithium responders only. How these abnormalities impact hippocampal microcircuit computation is not understood. We aimed to investigate the impacts of BD-associated abnormal granule cell (GC) activity on pattern separation (PS) using a computational model of the dentate gyrus (DG).

Methods: We used numerical parameter optimization to fit the parameters of biophysically realistic GC models to

electrophysiological data from iPSC GCs from patients with BD. These cellular models were incorporated into DG networks to assess impacts on PS using an adapted spatiotemporal task. Relationships between BD, lithium and spontaneous activity were analyzed using linear mixed effects modelling.

Results: Lithium and BD negatively impacted PS, consistent with clinical reports of cognitive slowing and memory impairment during lithium therapy. Our simulations show that lithium's deleterious effect on PS in nonresponders is likely due to impairment of the DG's "winner-take-all" network dynamics. By normalizing spontaneous activity levels, lithium improved PS performance in LR only ($\beta = 0.39$, CI: 0.22 – 0.56, $p < 0.001$).

Conclusions: Improvements in PS after lithium therapy in lithium responders may be attributable to the normalization of spontaneous activity levels rather than reductions in GC intrinsic excitability as we hypothesized. Our results agree with a hypothesized relationship between behavioural mnemonic discrimination and DG PS, as previous behavioural research has suggested that mnemonic discrimination improves after lithium therapy in lithium responders only. Our work can be expanded on in the future by simulating the effects of lithium-induced neurogenesis on PS.

Keywords: Bipolar Disorder, Induced pluripotent stem cells (iPSCs), Computational Neuroscience, dentate gyrus, pattern separation

Disclosure: Nothing to disclose.

P193. Volatility-Related Belief Updating and Persecutory Delusions: Sensitivity to Change Following a Randomized-Controlled Trial of Psychotherapy

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Background: Cross-sectional studies have shown that paranoia and persecutory delusions are associated with elevated prior expectations of environmental volatility. Expecting more frequent change in one's environment is therefore a putative cognitive mechanism of persecutory thinking. Modulation of volatility-related belief updating following treatment of persecutory delusions in schizophrenia would offer stronger evidence of a causal relationship. We aimed to test that in a randomized-controlled trial.

Methods: 63 individuals with schizophrenia-spectrum disorder or delusional disorder (35% female, 43% Black or African-American) were randomized to receive either an established cognitive-behavioral therapy for psychosis (CBTp) protocol or an active control therapy (befriending) over 8 weeks. At baseline, all participants endorsed a persecutory delusion with > 50% conviction as well as elevated levels of worry. The final sample includes 28 CBTp and 25 befriending participants. A 3-option probabilistic reversal learning (3PRL) task was conducted pre/post treatment and volatility parameters were modeled using a Hierarchical Gaussian Filter (HGF). Delusion severity was assessed with the Psychotic Symptom Rating Scale (PSYRATS) and the Positive and Negative Syndrome Scale (PANSS). Linear mixed models were used to examine change with treatment and associations between computational and clinical variables. The study was pre-registered NCT04748679.

Results: Clinically, both treatments resulted in a significant reduction in persecutory delusion severity, as measured by the PSYRATS total score ($F(1,100) = 50.9$ ($p < .001$), Cohen's $d = 1.2$). Positive symptoms also improved as measured by the PANSS positive symptom scale (Cohen's $d = 0.99$, $p = .001$). PANSS general symptoms reduced with treatment as well (Cohen's $d = 1.1$,

$p = .001$) but no significant change was observed in PANSS negative symptoms (Cohen's $d = .49$, $p = .15$). Computational parameters were investigated for change pre/post treatment. Across both treatment groups we observed a significant reduction in prior expectations of volatility ($F(1,100) = 8.5$ ($p = .004$), Cohen's $d = .63$), sensitivity to volatility ($F(1,100) = 7.7$ ($p = .007$), Cohen's $d = .60$) and an increase in meta-volatility learning rate ($F(1,100) = 8.5$ ($p = .004$), Cohen's $d = .59$). There was no evidence of differential impact of CBTp or befriending on belief updating, as all treatment by time interactions for belief updating metrics were non-significant. Finally, we found that change in PSYRATS was not significantly associated with change in volatility priors ($F(1,102) = 1.3$, $p = .26$); however reduced volatility priors were associated with reduced PANSS positive symptoms ($F(1,111) = 11.1$, $p = .001$), particularly item P1 (delusions) ($F(1,112) = 4.9$, $p = .03$). This same pattern of significance was observed for sensitivity to volatility and meta-volatility learning rate as well. Critically, no significant associations were observed between volatility-related belief updating metrics and severity of hallucinations or PANSS general symptoms, suggesting specificity to delusions.

Conclusions: Volatility-related belief updating, including elevated prior expectations of environmental volatility, reduces in the context of psychotherapeutic treatment and is associated with reduced severity of positive symptoms, particularly delusions. These data provide initial support for the notion that over-estimating the frequency of change in one's environment is a cognitive mechanism underlying delusions.

Keywords: Randomized-Controlled Trial, Delusions, computational modeling, Psychotherapy, Volatility

Disclosure: Nothing to disclose.

P194. Clinical Outcome Prediction in Anorexia Nervosa via Neurocomputational Markers of Gastrointestinal Interoception

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Background: Anorexia nervosa (AN) has one of the highest mortality rates among psychiatric disorders and is associated with nearly 50% relapse rates in the first year after inpatient treatment, underscoring the need to better understand the pathophysiological mechanisms underlying AN and to develop prognostic biomarkers for this disorder. Altered gastrointestinal (GI) interoception has been proposed as a contributing factor to the maintenance of AN pathophysiology. Within a computational framework, this involves biased interoceptive prior beliefs (PB), abnormal learning rates (LRs), and abnormal precision-weighting of GI signals. This study utilized a Bayesian model of GI interoception to examine whether neural and computational measures could predict relapse risk and symptom severity in AN individuals 6 months after hospital discharge. We hypothesized that, relative to healthy comparisons (HCs), those with AN would exhibit biased PBs against GI signals, lower LRs from GI signal perturbation, and reduced GI sensory precision. Additionally, we hypothesized that these computational measures and associated evoked neural responses could predict outcomes and the severity of eating disorder symptoms at 6 months.

Methods: This single-blinded, randomized crossover study collected longitudinal data from 52 female participants: 25 weight-restored inpatients with AN (mean age: 18 ± 3 years, mean body mass index (BMI): 21.4 ± 3.6) and 27 HCs (mean age: 22 ± 5 years, mean BMI: 23.2 ± 3.4). Eating disorder severity was assessed using the Eating Disorder Examination Questionnaire (EDE-Q) at pre-discharge and six-month follow-up. During the pre-discharge

visit, GI interoceptive sensitivity was assessed using an ingestible vibrating capsule (Vibrant Ltd) delivering two different intensities of nonpainful mechanosensory stimulation. Participants were instructed to press a button when they perceived each vibration. Computational measures derived from participant responses included PB, LRs, forgetting rate (FR), and interoceptive precision (IP). Group differences were tested using linear mixed effect models, with group, age, and BMI as fixed variables, and computational measures as dependent variables. 31-channel scalp electroencephalogram (EEG) was continuously recorded during the capsule vibration task. 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 segmented into epochs ranging from -200 to 3000 ms after the capsule vibration onset, which served as the temporal reference. For statistical analyses, we computed the average event-related potential 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 (400–720 ms after the vibration onset) that we previously identified and replicated as an EEG marker of gut mechanosensation. Relapse at 6 months was assessed remotely using a standardized operational definition of illness status based on a combination of self-reported symptoms, behaviors, and BMI. Linear and logistic regression models were used to test whether pre-discharge computational measures predicted outcomes (full relapse vs. no full relapse) and EDE-Q global score at 6 months.

Results: Relative to HCs, the AN group exhibited stronger PB against perceiving GI signals, as evidenced by lower PB values ($p = .002$; $b = -.08$; 95%CI [-.10;-.03], Cohen's $d = -0.54$). Individuals with AN showed lower FR values than HCs ($p < .001$; $b = -.39$; 95% CI [-.59;-.20], $d = -0.89$). The AN group also exhibited lower LR values from Vibration trials ($p = .002$; $b = -.09$; 95%CI [-.15;-.03]), $d = -0.55$), and greater LR values from No-vibration trials ($p < .001$; $b = .10$; 95%CI [.04;.15]), $d = -0.82$) vs. HCs. There were no significant group differences in IP ($p = .77$; $b = -.003$; 95%CI: [-.02;.02]). Across the entire sample, relapse outcome at 6 months was successfully predicted by PB (Odds Ratio (OR) = 4.81; $p = .03$) and LR from No-vibration trials (OR = .09; $p = .03$). Similarly, EDE-Q score at 6 months was successfully predicted by PB (Adjusted R squared (R^2) = .45; $p = .001$), LR from No-vibration trials ($R^2 = .56$; $p < .001$), and FR ($R^2 = .31$; $p = .001$). Across the entire sample, avERP in response to weak vibrations was significantly correlated with LR from No-vibration trials ($r = -.62$; $p < .001$), LR from Vibration trials ($r = .49$; $p < .001$), FR ($r = .59$; $p < .001$), and PB ($r = .31$; $p = .03$). The avERP in response to strong vibrations was significantly correlated, yet in a more moderate way, with LR from No-vibration trials ($r = -.34$; $p = .02$), LR from Vibration trials ($r = .36$; $p = .01$), and FR ($r = .33$; $p = .02$).

Conclusions: These initial findings provide evidence that individuals with AN have altered GI interoception, characterized by biased interoceptive prior beliefs and abnormal learning rates, which may contribute to the maintenance and relapse of AN. Moreover, the identification of specific computational measures as predictors of relapse and symptom severity underscores their potential as prognostic, and possibly, treatment target biomarkers.

Keywords: Eating disorders, Computational Neuroscience, Interoception, Biomarker

Disclosure: Nothing to disclose.

P195. Shared and Unique Neuroanatomical Alterations in Non-Affective and Affective Early Psychosis Patients: Findings From an Optimized Machine-Learning Approach

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Background: Identifying neural signatures that can reliably differentiate clinical from non-clinical populations represents a well-known challenge in psychiatry. This is especially true for early psychosis, which includes affective (AFF) and non-affective (NAFF) patients. Statistical approaches based on machine-learning (ML) algorithms have been heralded as a possible solution to such a challenge. When ML approaches are applied to psychiatric populations, data characteristics (e.g., different sample sizes between clinical and non-clinical groups, datasets from different sites), validation strategy and model selections can significantly affect outcome measures. Moreover, ML models need to achieve an acceptable performance and obtain interpretable findings. Addressing these challenges and achieving these goals are essential for impactful clinical research as well as to enable progression toward effective precision medicine.

Methods: Two independent structural MRI datasets (i.e., HCP-EP project and University of Pittsburgh) were acquired from early course AFF and NAFF psychosis patients and healthy comparison (HC) subjects, including a total of $N = 328$ subjects: [HC: $N = 147$, age (mean $\pm$ SD years) = 24.75 ± 4.65 , sex (male/female) = 91/56], [NAFF: $N = 139$, age = 22.43 ± 3.78 , sex = 96/43], and [AFF: $N = 42$, age = 23.87 ± 4.38 , sex = 17/25]. Data were preprocessed and analyzed with FreeSurfer, which was used to extract volume, area, thickness, and mean curvature. These cross-site measures were harmonized using the ComBat method for each group separately, considering age and sex as covariates. Then we employed an optimized machine learning approach using a three-split method (i.e., training, validation, and unseen testing samples) with Bayesian optimization and nested cross-validation strategy. During two loops, in the 5-fold outer loop, we took out 20% of the data as unseen test split folds and used 80% for training/validation folds. In every fold of the inner loop ($k = 5$), we used the Bayesian optimization approach to select the best-fitted model with optimized hyperparameters to validate the model. Four comparisons were run, including: 1) HC vs. patients (both AFF and NAFF), 2) HC vs. NAFF, 3) HC vs. AFF, and 4) NAFF vs. AFF. Average percentage accuracy (Acc) of the 5-test folds was the main outcome measure for the analyses including all features as well as for the analyses selecting features yielding at least medium effect size (ES) in between-group comparisons.

Results: A total of 278 MRI measures were extracted. Between-group comparisons that included all features yielded the following results: 1) HC vs NAFF + AFF patients: mean Acc = 70.38%, 2) HC vs NAFF: mean Acc = 70.64%, 3) HC vs AFF: mean Acc = 74.67%, and 4) AFF vs NAFF: mean Acc = 73.56%. We then repeated these comparisons by selecting only features that yielded at least a medium effect size (ES) and found: a) two features in HC vs patients (mean Acc = 59.02%): *rh_entorhinal_meancurv* (t -value = -4.89, $p < 0.001$, ES = -0.54, CI = [-0.01, -0.004]) and *lh_inferiortemporal_thickness* (t -value = 5.55, $p < 0.001$, ES = 0.61, CI = [0.05, 0.10]); b) four reproducible features in HC vs NAFF comparison (mean Acc = 59.32%): *lh_entorhinal_meancurv* (t -value = -5.380, $P < 0.001$, ES = -0.66, CI = [-0.01, -0.005]), *rh_entorhinal_meancurv* (t -value = -6.22, $p < 0.001$, ES = -0.73, CI = [-0.01,-0.008]), *lh_fusiform_thickness* (t -value = 4.986, $p < 0.001$, ES = 0.59, CI = [0.04, 0.09]), and *lh_inferiortemporal_thickness* (t -value = 5.129, $p < 0.001$, ES = 0.607, CI = [0.04, 0.10]); c) six reproducible features in the HC vs AFF comparison (mean Acc = 74.38%) ; *lh_medialorbitofrontal_area* (t -value = 4.34, $p < 0.001$, ES = 0.76, CI = [86.72, 230.72]), *lh_rostralanteriorcingulate_area* (t -value = 3.398, $P < 0.001$, ES = 0.59, CI = [43.89, 165.44]), *lh_inferiortemporal_thickness* (t -value = 3.741, $p < 0.001$, ES = 0.655, CI = [0.03, 0.12]), *lh_middletemporal_thickness* (t -value = 3.25, $p = 0.001$, ES = 0.57, CI = [0.03, 0.12]), *lh_entorhinal_volume* (t -value = 4.23, $p < 0.001$, ES = 0.74, CI = [150.42, 412.37]), *lh_rostralanteriorcingulate_volume* (t -value = 3.449, $p < 0.001$, ES = 0.60, CI = [145.73, 535.23]); and d) two features repeated for NAFF vs AFF (mean Acc = 74.79%); *lh_medialorbitofrontal_area* (t -value = 2.33, $p = 0.02$, ES = 0.41, CI = [14.20,171.21]), and *lh_transversetemporal_volume* (t -value = -2.742, $p = 0.007$, ES = -0.483, CI = [-174.52, -28.45]).

Conclusions: By applying an optimized machine learning approach to neuroanatomical features, here we were able to differentiate early psychosis patients from healthy control subjects across two different datasets with good accuracy. We also identified shared and unique neuroanatomical alterations that could reliably discern non-affective and affective psychosis patients from healthy comparison subjects, as well as the two patient groups from one another. Together, these findings show the utility of such an approach in characterizing the pathophysiology of early psychosis, which in turn may contribute to the development of precision medicine interventions in these patients.

Keywords: early psychosis, machine learning, Human Neuroimaging

Disclosure: Nothing to disclose.

P196. Serum Levels of D-Cycloserine Predict Antidepressant Response From Intermittent Theta-Burst Stimulation

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Background: Transcranial magnetic stimulation (TMS) is an important treatment option for treatment resistant Major Depressive Disorder. Pairing stimulation with mechanistically informed adjuncts, such as the NMDA receptor partial agonist D-Cycloserine, has emerged as a strategy to enhance treatment outcomes. However, at high concentrations D-Cycloserine antagonizes the NMDA receptor, and therefore important dosing questions remain due to individual differences in volume of distribution and possible accumulation with repeated dosing.

Methods: We recruited $n = 12$ individuals (males and females) with a moderate-severe depressive episode for a 4 week (20 treatments) open-label trial pairing intermittent theta-burst stimulation (iTBS) with D-Cycloserine (25mg/17.5kg). We serially sampled peripheral blood for pharmacokinetic characterization, first prior to treatment with a 100mg dose, and then in conjunction with iTBS+D-Cycloserine treatment after the first 25mg/17.5kg dose, the 5th dose (accumulation), and the 6th dose (start of the second week - elimination).

Results: iTBS+D-Cycloserine was associated with a potent antidepressant effect, achieving 83.3% response and 75.0% remission at treatment end that was sustained at 4 weeks of follow-up. The percent MADRS score decrease was predicted by serum D-Cycloserine concentration ($r^2 = 0.51$, $p = 0.0086$). Although we found evidence for accumulation after five doses (mean difference 2.16µg/mL, 95%CI 0.66-3.66), the concentration remained within the NMDA receptor agonist range and returned to drug-naïve levels with the 6th dose. Serum concentrations did not significantly differ between 100mg and 25mg/17.5kg doses.

Conclusions: Our data demonstrate the safety of extended D-Cycloserine dosing and confirm the antidepressant effects of iTBS+D-Cycloserine. Weight-based dosing may not be required, however clinical outcomes may depend on achieving adequate D-Cycloserine serum concentrations.

Keywords: theta burst transcranial magnetic stimulation, d-cycloserine, Major Depression Disorder

Disclosure: Nothing to disclose.

P197. CYB003 Produces Robust and Enduring Antidepressant Effects in Patients With Major Depressive Disorder (MDD)

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Background: Available medication treatments for Major Depressive Disorder (MDD) have limited efficacy, require daily dosing, are associated with short and long-term side effects, and do not address the maladaptive patterns of thinking underlying these conditions.

Clinical studies¹ 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. CYB003 is a novel deuterated analog of psilocin that may offer benefits over psilocybin.

The aim of this clinical study was to determine safety, PK and tolerability of ascending doses of CYB003, and to evaluate the short-term and long-term efficacy of CYB003 in treating symptoms of MDD. We previously presented top line short-term (up to Day 42) efficacy data from this study at the ACNP2. This poster will present the 12-month durability of effect data.

Methods: A seamless Phase 1/2 study was designed to evaluate safety, tolerability, and therapeutic efficacy of ascending doses of CYB003. In this study 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. Patients remained 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 3 weeks apart. All participants received psychological support based on Cybin's EMBARKTM framework. MADRS scores were collected at baseline and up to 16 weeks after the first dose to assess acute and medium-term (Day 126) efficacy.

The primary endpoint for the efficacy assessment was the change from baseline at the end of the double-blind phase of the study (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) was performed after unblinding at Days 21, 42, and 126.

To further evaluate the durability of effect of two doses of CYB003 up to 12 months, a follow-up study (CYB003-001b) was initiated for patients who had completed the CYB003-001 study.

Results: CYB003 demonstrated a favorable safety and tolerability profile. Side effects for all doses were mild or moderate and mostly self-limiting, and no severe or serious AEs occurred. 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.

CYB003 showed a rapid improvement with a difference of 14 points on the MADRS total score compared to the placebo-group, leading to a clinically meaningful effect size of 2.15 ($P = 0.0005$) at the end of the double-blind phase (Day 21) for the 12 mg dose. Similar results were obtained in the 16 mg dose group with a difference of 13-point improvement over placebo (Effect size 2.54, $P = 0.008$).

This resulted in a response rate of 53.3% and 44.4% and a remission rate of 20% and 22.2%, compared to 0% response and remission in the placebo group for the 12 mg and 16 mg groups, respectively, at the end of the double-blind phase (Day 21).

A second dose of CYB003 on Day 22 led to further improvement of the response and remission rates for the 12 mg (78.6% and 78.6%) and 16 mg (75% and 50%) groups, respectively, 3 weeks after a 2nd dose (Day 42).

On Day 126, the last assessment in the study CYB003-001, the 12 mg group showed a mean difference of 22.9 points improvement over baseline with corresponding 73% responders and 60% remitters. In the 16 mg group, 75% of patients were responders/remitters.

Long term efficacy was assessed in the follow-up study (CYB003-001b) with clinical and safety assessments at Days 270 and 364. Within the 12 mg group, an improvement of 18 points on

the MADRS total score from baseline was observed at the 1 year follow up, with responder and remitted rates of 60% and 50% respectively. In the 16 mg dose group, a difference of 19 points on the MADRS total score relative to baseline was observed at the Day 270 follow up, which translated into responder and remitted rates of 71% and 57% respectively. Data for Day 364 is outstanding and will be presented at the conference. No relevant safety concerns were noted during the long-term follow-up.

Conclusions: CYB003 is safe and well tolerated and shows a rapid and sustained improvement in depressive symptoms. These improvements were maintained long-term as seen by the decreases in MADRS scores and clinically meaningful improvement and response and remission rates even after 12 months of follow-up.

1Carhart-Harris, R. et al. (2021) *N Engl J Med*, 384, 1402–1411.

2Kremppien, S. et al. M83, ACNP 62nd Annual Meeting: Poster Abstracts P251 – P500. *Neuropsychopharmacol.* 48 (Suppl 1), 211–354 (2023). <https://doi.org/10.1038/s41386-023-01756-4>.

Keywords: Psilocin, Major Depressive Disorder (MDD), Psychedelic therapy, Human Clinical trial, MADRS

Disclosure: Nothing to disclose.

P198. Theta Burst Stimulation to the Dorsomedial Prefrontal Cortex in Young Adults With Depression – Effects on Neural Reward Processing

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Background: Depression is marked by altered positive affect (PA) which has been previously correlated with altered frontostriatal neural responses to reward. Greater dorsomedial prefrontal cortex (dmPFC) activation during reward, especially during the anticipatory phase, has been associated with dysregulated PA through its connectivity with the ventral striatum (VS).

Methods: We conducted a randomized study to evaluate the modulatory power of transcranial magnetic stimulation, and more specifically theta-burst stimulation (TBS), on neural activity of the dmPFC. We recruited 29 adults with depression who received in a randomized, counterbalanced design, three types of TBS followed by fMRI during a reward guessing task. Participants received either intermittent (iTBS; potentiating), continuous (cTBS; depotentiating), or sham TBS (control) in a counterbalanced order. We used VS-dmPFC functional connectivity to localize the dmPFC and targeted this region with neuronavigation. We explored activity in the dmPFC during reward anticipation win (or loss) (compared to neutral) as well as VS-dmPFC connectivity during win (or loss) (compared to neutral). We compared iTBS or cTBS to sham.

Results: We found that cTBS significantly altered dmPFC anticipation loss-neutral activity compared to sham [$t(18) = -2.4$, $p < 0.05$], while iTBS did not [$t(17) = 0.4$, $p = 0.7129$]. When exploring these effects (compared to sham), cTBS reduced activity during both anticipation loss and neutral. Neither cTBS nor iTBS altered dmPFC anticipation win-neutral activity or VS-dmPFC connectivity during either anticipation loss-neutral or win-neutral.

Conclusions: A single session of cTBS is capable of modulating neural activity during reward processing. We previously showed, in this same cohort, that we can significantly alter VS-dmPFC resting state connectivity. This analysis of task-based reward processing shows we can significantly alter dmPFC neural activity during reward processing. This has potential implications for improving positive affect, which may be especially important with respect to treating symptoms of anhedonia.

Keywords: Depression, TMS, iTBS, Reward, Reward Anticipation
Disclosure: Nothing to disclose.

P199. Assessment of Mitochondrial Function and Ketamine's Mechanism of Action in Patients With Treatment Resistant Depression

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Background: Over 300 million people world-wide suffer from major depressive disorder (MDD). Unfortunately, only 30–40% of patients with MDD receive remission after conventional monoamine antidepressant therapy. In recent years, ketamine has transformed the treatment of MDD by delivering fast-acting antidepressant effects within hours, in contrast to conventional antidepressants which require weeks to produce therapeutic effects. Numerous research efforts have sought to identify ketamine's mechanism of action in mood disorders, with several studies suggesting its involvement in regulating neuroinflammation. Circulating cell-free mtDNA (cf-mtDNA) is typically considered a pro-inflammatory antigen capable of triggering inflammatory responses in target cells. Depressed subjects have been shown to have an increase in cf-mtDNA levels. We hypothesize that ketamine exerts its therapeutic mechanism of action through mitochondrial regulation.

Methods: Thirty-nine unmedicated individuals with MDD (23F) and 25 healthy volunteers (HVs, 16F) participated in a randomized, double-blind trial comparing intravenous ketamine (0.5 mg/kg) to placebo. Blood was obtained at baseline and at three post-infusion timepoints (230 minutes, Day 1, and Day 3). Plasma was then aliquoted into cryotubes and stored at -80 degrees Celsius until thawed for assay. In this secondary analysis from a placebo-controlled double-blind inpatient crossover ketamine trial, cf-mtDNA levels were determined by qRT-PCR.

Results: Our analysis standardized the cf-DNA estimation in plasma samples from depressed subjects. The cf-DNA values from MDD and healthy volunteers showed consistent results in cf-mtDNA levels across the different time points. The data analysis on the effects of ketamine on cf-mtDNA levels are currently in progress.

Conclusions: Based on our hypothesis, our prospective findings would show a significant positive effect of ketamine on cf-mtDNA levels. Additional investigation on other markers of mitochondrial function are still warranted.

Keywords: Depression, Mitochondria, Ketamine, Circulating cell-free mitochondrial DNA, Biomarker

Disclosure: Nothing to disclose.

P200. Elevated MAOA and Altered Bioenergetics Underlying Elevated Emotionality in Depression

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Background: Treatment for major depressive disorder (MDD) using conventional antidepressants (ADs) is only effective in approximately 50% of patients often with partial efficacies. Current understanding of the mechanisms of action of conventional monoaminergic ADs does not explain their delayed and

partial effects, representing unmet needs for studying disease pathophysiology and treatment resistance. Notably, elevated expression of monoamine oxidase A (MAOA) in corticolimbic brain areas has been consistently reported in MDD via positron emission tomography (PET) imaging studies. MAOA is an outer mitochondrial membrane protein that catalyzes the oxidative deamination of various monoamines, while producing hydrogen peroxide and electrons to fuel bioenergetic machinery in cells. In human brains, MAOA is widely expressed in both neurons and glia, and primarily responsible for metabolizing monoaminergic neurotransmitters (e.g., serotonin and norepinephrine) produced in the brainstem. Altered MAOA activity or expression levels in the brain are associated with changes in diverse behavioral traits, such as mood, cognition and personality, likely through deregulated monoaminergic neurotransmission. High MAOA level is thought to contribute to low brain serotonin levels and hence depressed mood, or resistance to conventional monoaminergic AD treatment. However, the causal link of elevated MAOA to the pathobiology of MDD and/or treatment resistance has not been tested, and the mechanisms underlying these psychopathological conditions remain to be studied.

Methods: To test this causality, we have developed a humanized model of elevated MAOA in mice, in which human MAOA gene is selectively expressed in relevant neuronal or glial cell types within corticolimbic areas using adeno-associated virus transduction system, and studied the impacts on emotional behavior, neurochemistry (microdialysis) and cellular metabolic functions, with particular emphasis on mitochondrial bioenergetic pathways.

Results: Increasing MAOA (by ~60% compared to control, a level comparable to that seen in MDD in PET studies) in pyramidal neurons in the medial prefrontal cortex (mPFC) resulted in anhedonia-like behaviors in mice ($N = 16$), while elevated MAOA in astrocytes in PFC resulted in anxiety behaviors ($N = 16$), with concurrent decrease (~40%) in serotonin levels. We also investigated the effects of elevated MAOA in primary neurons/astrocytes on mitochondrial bioenergetics (e.g., reactive oxygen species (ROS), membrane potential, oxygen consumption rate [OCR], ATP production rate) and maladaptive proteomic changes by mass-spectrometry. Elevated MAOA lead to significant increase in ROS production, and reduction in mitochondrial membrane potential, OCR, and ATP production differentially in neurons vs. astrocytes. These bioenergetic measures were at least partially rescued by antioxidant treatment but not by serotonin supplementation.

Conclusions: Elevated MAOA in distinct cell types differentially caused impaired neurochemical profiles, emotionality behaviors, and mitochondrial bioenergetic functions, suggesting a novel mechanism for neuron/glia interplay that regulates emotional behavior. We will further assess the impact of elevated MAOA in mediating resistance to conventional and new AD treatment regimen. The mitochondrial metabolic assay system can be used as a platform to test the efficacy of novel treatment regimen at mechanistic levels.

Keywords: Mitochondria, Monoamine Oxidase-A, Treatment-resistance

Disclosure: Nothing to disclose.

P201. Differences in Bipolar Disorder and Major Depressive Disorder Genetics and Genetic Expression

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Background: Bipolar Disorder (BD) and Major Depressive Disorder (MDD) are among the leading causes of disability worldwide and

confer a significant mortality risk. Both disorders overlap in their depressive symptom clinical presentation, but they are differentiated by the presence of mania and hypomania in BD. There are significant heritable components to these chronic disorders, and recent genome-wide association studies have identified hundreds of genetic loci associated with increased risk to both. However, the genetic mechanisms by which genetic variants increase risk and how they differ between BD and MDD are unknown. Here we conducted a differential expression analysis of RNA sequencing data from post-mortem human brains to illuminate the genetic mechanisms underlying these disorders

Methods: Donor brains were obtained by the Lieber Institute for Brain Development and identified as having lived with a diagnosis of MDD or BD. We analyzed MDD samples and BD samples from the amygdala (total $n = 353$, MDD $n = 231$, BD $n = 122$) and subgenual Anterior Cingulate Cortex (sACC) (total $n = 351$, MDD $n = 228$, BD $n = 123$). RNA-seq was utilized to quantify expression of genes, transcripts, exons, and junctions (i.e. the feature types). Linear models tested each feature for differences in expression between BP vs MDD, controlling for potential confounders. False-discovery rate (FDR) adjusted $p < 0.05$ within each feature type was used to identify significant features. Then, gene enrichment analysis of FDR significant features was carried out to identify genetic pathways that share a common biological process, and these pathways were tested for FDR significance.

Results: Roughly similar numbers of significant differentially expressed features were identified in both the amygdala ($n = 129$ genes, $n = 2703$ transcripts, $n = 2897$ exons, and $n = 992$ junctions) and sACC ($n = 129$ genes, $n = 518$ transcripts, $n = 1673$ exons, $n = 1024$ junctions). Of these significant genes, 33 were shared between both brain regions (amygdala and sACC). Genes down-regulated in BD were enriched for biological pathways related to nucleotide synthesis, mRNA processing, and cytoplasmic translation (adjusted $p < 0.05$) in both brain regions.

Conclusions: Our findings suggest transcriptional differences between MD and BP are shared across two regions of the human brain selected for their involvement in the neural circuits subserving mood regulation. Since we tested the differential expression of two mood disorders with overlapping but distinct clinical profiles, these significant differences might identify specific genetic differences and – by extension – their downstream cellular pathways implicated in each disease. Accordingly, these expression targets may represent particularly robust disease markers and pharmaceutical targets. Future work will investigate functional significance of these gene features and investigate their potential for therapeutic intervention.

Keywords: Major Depression Disorder, bipolar disorder (BD), genetics, transcriptomics

Disclosure: Nothing to disclose.

P202. Prediction of Optimal Treatment Selection Between ECT and Ketamine to Treat Suicidal Ideation

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Background: Despite an increased focus on prevention, suicide remains a major public health issue. Untreated and partially responsive treatment of depression is a significant risk factor for suicidal behavior. Electroconvulsive therapy, ECT, and ketamine are two treatments commonly used for treatment resistant depression. Both are thought to have anti-suicide properties but the low frequency of completed suicides and the heterogeneity of

para-suicidal behavior makes it difficult to design studies that compare the two treatments. This is further complicated by the conflicting results reported by recent studies that have compared the relative antidepressant efficacy of the two treatments.

Previously, we have applied the Personalized Advantage Index (PAI) machine learning algorithm to a clinical population from a single academic medical center to identify characteristics that predict optimal treatment selection between ketamine and ECT to treat depressive symptoms⁷. Subsequently, we applied this approach to predict optimal treatment selection to treat suicidal ideation in the same cohort. To test the generalizability of the PAI approach, we hypothesized that it could be applied to predict optimal treatment selection for individual patients across multiple treatment centers using the ideation severity subscale of the Columbia Suicide Severity Rating Scale (C-SSRS).

Methods: This study leveraged electronic health record (EHR)-derived, de-identified data from NeuroBlu Data, a longitudinal behavioral health real-world database comprising structured and unstructured patient-level clinical data⁷. 3730 patients in this database underwent treatment with either ECT ($n=123$) or ketamine ($n=3607$) for an acute episode of depression. The C-SSRS scale was collected within 30 days prior to the start of the treatment course and again within 45 days of completion of the treatment course. To limit the impact of potential confounding by indication in this naturalistic treatment sample, propensity score matching on age, sex, visit type, and the baseline Ideation Severity subscale score of the C-SSRS (IS-CSSRS) was used to create equal sized cohorts of 122 ketamine and ECT patients ($n=244$ total). Individual PAI scores were computed using leave-one-out cross validation using a series of random forest regression (RFR) models with 1000 regression trees to predict end IS-CSSRS using N-1 patients. Predictor variables across patients included medication history, and treatment type (ECT or ketamine). Two end-IS-CSSRS predictions were made for each held-out patient in the cohort: one using the patient's true treatment label and a counterfactual prediction in which the treatment label was switched to the treatment the patient did not receive. The prediction resulting in the lowest end- IS-CSSRS score was considered the patient's predicted optimal treatment. We compared end- IS-CSSRS score distributions between patients who received a predicted optimal versus non-optimal treatment using a two-sample, one-sided t-test.

Results: Patients who received the treatment predicted to be optimal had a significantly lower post-treatment IS-CSSRS than those who received non-optimal treatment (mean difference = 1.41, $t=6.13$, $df=133.3$, $p<0.001$, Cohen's $d=0.9$). The magnitude of this difference increased considerably among the 31% of patients with the largest predicted advantages for one treatment versus another (mean difference = 3.24, $t=10.7$, $df=41$, $p<0.001$, Cohen's $d=1.73$). Factors that predicted response included age, baseline IS-CSSRS, prior outpatient treatment, number of medications tried prior to treatment, and the number of total clinical encounters prior to beginning treatment. Notably, ECT was predicted to be an optimal treatment for only 35% of patients who received it, while ketamine was predicted to be an optimal treatment for 86% of patients who received it (chi square = 63.9, $df=1$, $p<0.001$).

Conclusions: Patients who received the treatment predicted to be optimal by our model had significantly lower post-treatment IS-CSSRS compared to those who received a predicted non-optimal treatment. The data suggest that ketamine had a more robust effect on the severity of suicidal ideation in this cohort of depressed individuals. These results in a multi-center curated database mirror our results in a single center and suggest that the method is sufficiently robust to detect treatment differences. The strengths of this study include prospective collection of the data at the time of treatment, and the clinical population that comprises the dataset. The limitations of the data include the

relatively small sample size for machine learning approaches, the limited number of variables available for propensity score matching which may raise concern about confounding by indication, and the heterogeneity of the settings in which the treatment was delivered, and in which the data were collected. Additional studies with larger, more heterogeneous datasets that include potential biomarkers are indicated. Our future work will seek to directly test the external validity of the model using leave-one-site-out cross validation.

Keywords: electroconvulsive therapy, (R,S)-ketamine, machine learning

Disclosure: Nothing to disclose.

P203. The Effect of TNF-Alpha Antagonist on Functional Connectivity Within Corticostriatal Reward Circuitry in Depressed Patients With High Inflammation

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Background: Increasing evidence suggests that alterations in inflammation may contribute to the pathophysiology of major depressive disorder (MDD). Specifically, stimulation of cytokines leads to reduced connectivity within the reward circuitry, a circuitry heavily implicated in MDD. In particular, increased peripheral inflammation has been shown to be associated with decreased functional connectivity (FC) between ventromedial prefrontal cortex (vmPFC) and ventral striatum (VS), as well as vmPFC connectivity to dorsal striatum regions. To date, only a few studies have explored the impact of pharmaceuticals that target inflammation on reward circuitry in depressed patients. Here, we present novel results from a randomized-controlled clinical trial where a single dose of TNF-alpha antagonist, infliximab, was administered to a sample of patients with MDD and high inflammation.

Methods: 51 patients (both sexes included) with CRP > 3 mg/L were randomized to receive a single infusion of either the TNF-alpha antagonist infliximab (5mg/kg) or saline solution as part of a double-blind, placebo-controlled, randomized clinical trial. Resting state fMRI was collected prior to infusion at baseline and 14 days post infusion.

Results: At baseline, consistent with the previous literature, we observed a negative association between a composite score of inflammatory markers and the vmPFC-right VS functional connectivity. Following randomization, as was expected, a single dose of infliximab was associated with a group (placebo vs. infliximab) by time (baseline vs. 14 days) interaction ($F(1,48)=11.02$, $p=0.002$, eta squared = 0.187) such that patients receiving infliximab exhibited a significant reduction in the inflammatory composite score ($t(26)=2.699$, $p=0.012$, $d=0.51$), while those receiving placebo did not ($p>0.2$). We did not observe a group (placebo vs. infliximab) by time (baseline vs. 14 days) interaction for the vmPFC to VS connectivity. Instead, considering other striatal regions, we found that a single dose of infliximab was associated with a group (placebo vs. infliximab) by time (baseline vs. 14 days) interaction ($F(1,45)=5.02$, $p=0.03$, eta squared = 0.1) of vmPFC-right posterior caudate connectivity, controlling for age, sex and study sample. While patients who received infliximab showed an increase in their connectivity ($t(26)=-2.1$, $p=0.046$, $d=-0.4$), those who received placebo did not change ($p>0.3$). We did not observe this interaction for the vmPFC-other striatal regions connectivity.

Moreover, we observed a negative association between the change in the inflammatory composite score and the change in

vmPFC-right posterior caudate connectivity for all patients ($r = -0.301$, $p = 0.040$); suggesting that a reduction of larger magnitudes of inflammatory markers was associated with greater post-infusion increase in connectivity. This association became marginal after controlling for age, sex and study sample ($r = -0.272$, $p = 0.075$). Using multivariate analysis, at baseline the rsFC between VS and voxels of vmPFC predicted the levels of TNF alpha ($p < 0.02$). Furthermore, Changes in rsFC between right VS and voxels of vmPFC predicted the changes in the levels of TNF alpha ($p = 0.01$) 14 days post infusion.

We are also planning to examine the relationship between changes in the rsFC within the reward circuitry and changes in fatigue and psychomotor slowing symptoms, as well as the distribution of TNF gene expression.

Conclusions: These results corroborate the effect of peripheral inflammation on rsFC in reward circuitry, and the potential for vmPFC-VS connectivity as a target for anti-inflammatory depression treatment strategies.

Keywords: Major Depressive Disorder, Inflammation, reward circuitry, TNF-alpha antagonist, resting state functional connectivity

Disclosure: Nothing to disclose.

P204. Human Hippocampus Expression Disruption in Major Depression and Connection to Age-Related Changes

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Background: Hippocampal neuroplasticity regulates emotional responses, which are altered in major depressive disorder (MDD). Human brain postmortem studies found smaller hippocampus dentate gyrus (DG) and fewer granule neurons (GNs) in MDD, which may reflect impaired neuroplasticity resulting in MDD symptoms. Differential age-related changes in gene expression (GEX) are possible confounders for MDD related changes, and the current analytic practice to adjust for age as a shared effect should be further investigated.

Methods: Brain tissue was obtained from the Brain Collection of the New York State Psychiatric Institute, which includes brain samples from the Republic of Macedonia, and the Human Brain Collection Core of the National Institute of Mental Health. Diagnoses were established according to the Diagnostic Statistical Manual of Mental Disorders through structured proxy-based interviews. The sample included were 12 sudden death neurotypical controls with no psychiatric diagnoses (CTRL), and 15 subjects with Major Depressive Disorder (MDDs) and a major depressive episode within 4 months of death. Number of samples per donor, cells profiled per donor, RNA integrity number (RIN), post-mortem interval (PMI), and age did not differ between the two groups. As part of a larger study, we simultaneously profiled single nuclei (sn) gene expression and open chromatin (RNA and ATAC, 10X Genomics) of over 350,000 nuclei from hippocampus proper tissue. Multiome data was batch corrected and integrated using Harmony followed by Weighted Nearest Neighbor (WNN) analysis to performed unsupervised cell clustering. Then supervised machine learning (ML) was used for identifying rare cell types.

For the current analysis, we focused on GNs, and differentially expressed genes (DEG) related to MDD were identified using limma, with age, RIN, PMI and batch as covariates. Subsequently, CTRL and MDDs were separately split by age, using 50 years as cutoff, and age-related changes were tested in CTRL and MDDs separately. The proportions of overlap of age-related and MDD-related DEGs were tested using two-sided exact binomial tests against that expected by chance alone.

Results: MDD-related DEGs in GN were 101, including 7 previously linked to psychiatric disorders like depression, schizophrenia, and mood disorders according to the PsyGnet database. Age-related changes in GEX included 3394 DEGs in CTRL and 3550 in MDDs. Of the 101 MDD-related DEGs, the overlap with age-related DEGs in controls included 16 genes, which was significantly lower than what is expected by chance alone (p -value = 0.029), likely because of age adjustment performed during the test for MDD-related DEGs. When testing aging-related DEGs in the MDD group, the overlap with diagnosis-related DEGs was 27, close to what it would be expected by chance alone if the diagnosis analysis did not adjust for age (p -value = 0.732).

Conclusions: These findings suggest that there could be differences in the age-related dynamics of gene expression in MDD versus neurotypical subjects, which may confound lists of significant DEGs obtained using standard covariate adjustment methods, even when the samples are balanced on age. Future analyses should include detailed examination of age-related gene expression profiles in both neurotypical and MDD subjects, and a robust testing of MDD-related DEGs adjusted for differential age effects.

Keywords: snRNA-seq, gene expression, MDD, Hippocampus, Biology of aging

Disclosure: Illumina, Inc.: Stock / Equity - Publicly Traded Company (Self)

P205. Customized Psilocybin Treatment in Major Depressive Disorder: A Comprehensive Systematic Review of Markers of Response

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Background: Major depressive disorder (MDD) is a global leading cause of disability; however, the most common treatments for MDD have shortcomings including a delay in onset of action, and limited efficacy rates and accessibility. Psilocybin, a classic psychedelic, is a serotonin 2A receptors (5-HT_{2A}) agonist. Psilocybin has shown efficacy in treating MDD, including treatment-resistant cases. Despite its efficacy, there is substantial variability in antidepressant response to psilocybin and its use might be associated with side effects. There are no well-established markers of antidepressant response to psilocybin and identifying markers of response to psilocybin in MDD will lead to increased success rates. There has been a growing body of literature investigating the antidepressant effects of psilocybin, but most studies have relatively small sample sizes making it difficult to accurately determine the replicability and clinical usefulness of markers of response. Therefore, we conducted a systematic review summarizing the literature investigating clinical, neurocognitive and biological markers of response to psilocybin in MDD.

Methods: This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. Three electronic databases were searched: MEDLINE (PubMed), Embase, and PsycINFO. Searches were conducted on July 1, 2024. Electronic searches were complemented by manual searches. Briefly, this systematic review included studies that: 1) examined adult participants (i.e., aged 18 or older); 2) assessed individuals with a major depressive episode in MDD; 3) were published in English or Spanish; 4) were randomized controlled trials (RCTs) or open label trials that administered at least one dose of psilocybin; 5) had at least a total sample of ten participants; 6) prospectively measured depressive symptoms with a self-reported or clinician-administered standardized scale; 7)

examined the association between pre-treatment and/or post-treatment clinical, neurocognitive or biological markers, and changes in depressive symptoms. We excluded manuscripts that studied participants with depressive symptoms due to other psychiatric disorders such as bipolar disorder, medically- or substance-induced depressive disorder, persistent depressive disorder; included individuals with serious/unstable medical and/or neurological diseases (e.g., end of life cancer or diseases requiring palliative care); combined psilocybin with concurrent neuromodulation (concurrent psychotherapy and/or oral antidepressants were allowed); included manuscripts typically considered to have lower levels of evidence per the Evidence-Based Medicine Pyramid such as case reports or series, commentaries, editorials, and conference abstracts; and review articles.

Results: After the removal of duplicates, we identified 337 titles and abstracts. Thirty-nine manuscripts were reviewed in full, and twenty-three articles were ultimately included in this systematic review. Fifteen examined clinical markers, four investigated brain-based biological markers, and three assessed neurocognitive markers. The most investigated marker of response was quality of psychedelic experience, a post-treatment marker that measures positive psychedelic-induced experience (i.e., better insight, elevated conscientiousness, and/or subjective sense of connectedness/unity). Four studies, using two independent samples, investigated the relationship between improvement of depression and quality of psychedelic experience (different scales were used in all four distinct studies). Three studies (using two independent samples) were positive, identifying statistically significant associations between greater improvement of depression and better quality of psychedelic experience either with total scores or sub scores in the chosen scales. Other markers of antidepressant response to psilocybin that were observed in two studies (using two independent samples) were the association between greater improvement of depressive symptoms and 1) quality of therapeutic alliance with the treatment facilitator/guide, and 2) early improvement of depressive symptoms. In terms of brain-based biological measures, greater improvement of depression was correlated with post-treatment increases in global brain network integration/neuroplasticity. These changes were observed in three independent samples, two used functional magnetic resonance imaging and one used EEG. There was not clear convergence of the results of the neurocognitive studies.

Conclusions: There is variability in antidepressant response to psilocybin and a more customized approach is needed by identifying markers and predictors of response. Clinical variables that showed convergence with greater improvement of depressive symptoms in two independent samples included: 1) quality of psychedelic experience, 2) quality of therapeutic alliance with the treatment facilitator/guide, 3) early improvement of depressive symptoms, and 4) post-treatment increases in global brain network integration/neuroplasticity. Many studies were based on the same samples. Additional studies, particularly with larger and more representative samples, are needed.

Keywords: Psilocybin, Depression, Precision psychiatry, Personalized treatment, Markers of response

Disclosure: Nothing to disclose.

P206. Meaningful Patient-Reported Outcome Improvements at Weeks 1, 2, and 6 With AXS-05 for Major Depressive Disorder: Responder Analysis of the GEMINI Trial

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Background: Major depressive disorder (MDD) presents with emotional, physical, and cognitive symptoms, leading to reduced social and occupational functioning and diminished quality of life (QoL). As some symptoms are not easily observable by clinicians, their evaluations can differ from those of patients. Thus, understanding the patient perspective is crucial for achieving effective treatment outcomes. Patient-reported outcomes (PROs) are essential for comprehensively assessing the impact of treatments on MDD and determining whether the treatment is meaningful. PROs evaluating MDD symptom severity (Quick Inventory of Depressive Symptomatology [QIDS]), functioning (Sheehan Disability Scale [SDS]), and QoL (Quality-of-Life Enjoyment and Satisfaction Questionnaire – Short Form [Q-LES-Q-SF]) were utilized to assess AXS-05 (dextromethorphan 45 mg-bupropion 105 mg), a novel oral N-methyl-D-aspartate (NMDA) receptor antagonist and sigma-1 receptor agonist approved for treating MDD in adults.

Methods: In the GEMINI trial—a Phase 3, double-blind study randomizing adult MDD patients (N = 327 [female, n = 212]) to receive AXS-05 or placebo once daily for 3 days, then twice daily for 6 weeks—significant clinical improvement with AXS-05 was observed as early as Week 1, continuing through Week 6. Post-hoc analyses evaluated whether PRO improvements in symptom severity, functioning, and QoL at Weeks 1, 2, and 6 were considered meaningful to both patients and clinicians. Anchor-based methods estimated PRO responder thresholds using pooled data from both study arms. Week 6 results for Patient Global Impression of Improvement (PGI-I) and Clinician Global Impression of Improvement (CGI-I) were used to identify meaningful responder thresholds. Estimated thresholds for meaningful change in QIDS, SDS, and Q-LES-Q-SF were computed as the mean change from baseline among patients achieving “Much Improvement” (PGI-I)/“Moderate Improvement” (CGI-I) or “Very Much Improvement” (PGI-I)/“Marked Improvement” (CGI-I). These thresholds were confirmed by Week 6 cumulative distribution functions. Responder proportions for each PRO at Weeks 1, 2, and 6 were calculated, and differences across AXS-05 and placebo arms were tested using Chi-square or Fisher’s exact tests.

Results: Among patients and clinicians reporting “Much/Moderate Improvement” or “Very Much/Marked Improvement,” associated PRO changes from baseline were 10 and 14 points on QIDS, 12 and 17 points on SDS, and 24 and 44 points on Q-LES-Q-SF, respectively. For the “Much/Moderate Improvement” threshold, more patients receiving AXS-05 than placebo were QIDS responders at Weeks 1 (12.7% vs. 5.4%), 2 (24.7% vs. 4.7%), and 6 (38.7% vs. 17.4%); SDS responders at Weeks 1 (19.1% vs. 6.8%), 2 (27.0% vs. 13.7%), and 6 (41.1% vs. 24.0%); and Q-LES-Q-SF responders at Weeks 1 (12.3% vs. 4.9%), 2 (18.7% vs. 13.0%), and 6 (33.5% vs. 21.6%) (all statistically significant [P < 0.05], except Q-LES-Q-SF at Week 2). Similarly, for the “Very Much/Marked Improvement” threshold, more AXS-05 patients were QIDS responders at Weeks 1 (5.8% vs. 1.7%), 2 (12.4% vs. 2.6%), and 6 (22.3% vs. 6.9%); SDS responders at Weeks 1 (11.6% vs. 2.6%), 2 (17.4% vs. 4.3%), and 6 (24.8% vs. 11.3%); and Q-LES-Q-SF responders at Weeks 1 (5.4% vs. 0.6%), 2 (8.7% vs. 1.3%), and 6 (15.4% vs. 5.8%) (all statistically significant [P < 0.05], except QIDS at Week 1).

Conclusions: Significant improvements, considered meaningful by both patients and clinicians, in MDD symptom severity (QIDS), social and occupational functioning (SDS), and QoL (Q-LES-Q-SF) were observed for PGI-I/CGI-I-defined responders following AXS-05 treatment at Weeks 1, 2, and 6. These findings highlight the rapid and substantial benefits of AXS-05, resulting in meaningful improvements in symptoms, QoL, and functioning for individuals with MDD.

Keywords: Major Depressive Disorder (MDD), Patient Reported Outcomes, Rapid depression treatment

Disclosure: Axsome Therapeutics: Employee (Self)

P207. Impact of Social Defeat Stress on Microglia-Neuron Interactions in the Nucleus Accumbens

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Background: Chronic stress is a known risk factor for neuropsychiatric disorders, and it has been shown to alter neuron and myeloid cell structure and function in brain reward areas. The nucleus accumbens (NAc), a hub for integrating reward and motivation, exhibits molecular and cellular alterations that are found in postmortem tissue of patients with major depressive disorder and drive motivational deficits in rodents. Specifically, dendritic atrophy of dopamine receptor type 1 (D1), but not type 2 (D2) expressing medium spiny neurons (MSNs) is necessary and sufficient for stress-induced negative behavioral outcomes. Emerging evidence implicates microglia as potential mediators of neuronal atrophy and motivational deficits after social stress. Since microglia can interact with neurons and facilitate neuronal dysfunction and cell death, they are prime candidates for investigating the relationship between chronic stress and D1-MSN atrophy. Here we hypothesized that D1 dendritic atrophy would be associated with increased contact of D1-MSNs by microglia processes, thereby facilitating dendritic retraction and atrophy.

Methods: Male D1-CX3CR1-GFP and D2-CX3CR1-GFP mice were subjected to 10 days of Chronic Social Defeat Stress (CSDS), during which subjects were directly socially defeated by a male aggressor. CSDS was followed by a behavioral interaction test to determine low (avoids behavioral interaction) and high (approaches behavioral interaction) social interactors. Unstressed littermates were used as controls. 3D reconstruction analyses of D1 or D2 MSNs and adjacent microglia in the NAc were performed on confocal Z-stacks to quantify microglia morphology and contact at MSNs.

Results: A two-way ANOVA revealed that exposure to CSDS reduced the overall microglia contact at D1, but not D2, MSNs (total $n = 32$, $F(1, 28) = 5.866$, $p = 0.02$; Bonferroni Multiple Comparison Test, $p = 0.01$). However, the degree to which microglia contact MSNs in stressed animals negatively predicted CSDS-induced decreases in social interaction (Pearson Correlation, $r = -0.63$, $p = 0.03$). That is, animals displaying the greatest social avoidance showed the greatest microglia-D1 MSN contact. Interestingly, CSDS decreased the microglia density in the area occupied by D1 MSN dendrites (two-way ANOVA, total $n = 32$, $F(1, 28) = 9.252$, $p = 0.001$; Bonferroni Multiple Comparison Test, $p = 0.01$), yet the average volume of microglia somas (an index of activated microglia) in this region was increased by CSDS (one-way ANOVA, $F(2, 15) = 4.711$, $p = 0.03$) which also negatively predicted CSDS-induced social avoidance ($r = -0.58$, $p = 0.04$). Finally, reconstruction analyses of individual microglia within the region occupied by D1 MSNs revealed that animals that display low interaction, but not high interaction, to CSDS displayed reduced microglia complexity (one-way ANOVA, $F(2, 15) = 5.370$, $p = 0.02$).

Conclusions: Overall, these results add to the growing body of literature implicating microglia in maladaptive disease states, and future studies will focus on causally linking microglia alterations to anhedonia. Ongoing work seeks to not only further characterize neuron-microglia interactions in the context of social stress in both male and female mice, but also in the context of associated extracellular matrix proteins. Identifying microglia mechanisms contributing to altered neuronal dendritic morphology and negative affective behaviors can pinpoint novel therapeutic targets for stress-related disorders and improve current treatments.

Keywords: Chronic social stress, Nucleus Accumbens, microglia
Disclosure: Nothing to disclose.

P208. Zelquistinel, a Novel Nmdar Modulator with Rapid-Acting and Sustained Effects: Optimizing Dose and Dose Interval Based on Translational Measures of Synaptic Plasticity

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Background: NMDA receptor (NMDAR) modulation is an emerging rapid-acting pharmacological mechanism for enhancing synaptic plasticity to treat neuropsychiatric disease. The recent FDA approval of weekly-dosed esketamine (Spravato®), an NMDAR antagonist for depression, highlights the need for better understanding dose frequency dynamics for compounds that drive NMDAR-mediated plasticity. Zelquistinel is a novel orally bioavailable NMDAR positive allosteric modulator (PAM) that is currently being clinically investigated as a rapid acting antidepressant. Zelquistinel has been demonstrated to directly modulate long-term potentiation of synapses in hippocampal and mPFC slices. To assess a potential optimal dosing regimen for zelquistinel, we sought to systematically assess in vivo effects on homeostatic plasticity, duration of long-term plasticity and shift in threshold for plasticity (metaplasticity), and emotional learning following a single dose, daily repeated dosing, and once weekly dosing.

Methods: Adult male rats were implanted with intracranial recording and stimulation electrode in the medial prefrontal cortex (mPFC). Three groups of implanted rats and non-surgical controls were administered either zelquistinel as a single dose, 5 days of daily repeat dosing, or once weekly dosing for six weeks. Medial PFC excitatory field potentials evoked by mixed deep white matter inputs to layer II/III were recorded from freely behaving rats at baseline, and 1 hr, 24 hr and 1 week after dosing. Input/output curves (I/O) were generated during dosing, and 1 week after dosing I/O curves were measured, as well as long-term potentiation (LTP) induced by 6 Hz stimulation trains. Two weeks after dosing, I/O curves were recorded and LTP induced again. In a separate groups of rats, positive emotional learning was tested 1 and 2 weeks after single, daily or weekly dosing.

Results: A dose-response relation for zelquistinel was obtained in the MPFC-dependent positive emotional learning assay assessed 1 week post-dose. After a single dose, the 0.3 mg/kg PO dose was maximally effective ($F(4, 25) = 48.29$, $P < .05$; $P < .05$ Tukey post hoc test 0.3 mg/kg vs vehicle but not 0.3 mg/kg vs 3 mg/kg), while daily dosed animals exhibited an enhancement in PEL only at the lowest dose level of 0.003 mg/kg ($F(4, 25) = 27.05$, $P < .05$; $P < .05$ Tukey test 0.003 mg/kg vs vehicle) as compared to the single dose. Once weekly dose animals exhibited a similar PEL response as a single administration, but with increased benefit at lower doses ($F(4, 45) = 41.82$, $P < .05$; $P < .05$ Tukey post hoc test 0.003, 0.03, 0.3, 3 mg/kg vs vehicle). Similar results were seen, in measures of synaptic strength as measured by I/O curves ($F(3, 26) = 55.77$, $P < .05$). Importantly, weekly dosing led to a larger and longer lasting facilitation of LTP for 2 weeks (i.e., metaplasticity), as compared to a single dose or daily (1 week) dose ($F(3, 31) = 79.15$, $P < .05$; $P < .05$ weekly dosing vs vehicle or daily dosing). A similar shift in the dose response curve for single, daily and weekly dosing was observed with the metaplasticity assay as with the PEL and I/O curves. In addition, the metaplastic effect of zelquistinel was blocked by co-administration of isoflurane ($F(3, 22) = 57.56$, $P < .05$; $mP < .05$ Tukey test zelquistinel + Isoflurane pre-dosing vs isoflurane post-dosing), and not Isoflurane post-dosing or vehicle),

suggesting that the synaptic plasticity enhancing effects of zelquistinel are activity dependent.

Conclusions: Zelquistinel-mediated enhancement of in vivo plasticity and plasticity dependent behavior is both dose and dose frequency dependent. The increase in synaptic strength and learning seen with a single dose administration is a) lost by too frequent (daily) dosing, and conversely b) maintained or increased with a once-weekly dosing schedule. These data highlight the unexpected complexity and challenge of extrapolating synaptic plasticity effects from a single dose to clinical dosing strategies for plasticity enhancers. Zelquistinel is currently in a phase II clinical study for the treatment of depression in which low once-weekly doses will be given (Vitalize study).

Keywords: NMDA Receptor, Antidepressant, Synaptic Plasticity

Disclosure: Nothing to disclose.

P209. Multisite Prediction of Optimal Treatment Allocation Between Electroconvulsive Therapy and Ketamine

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Background: Clinicians lack clear guidelines to select the most effective antidepressant treatments for individual patients. Electroconvulsive therapy (ECT) and (es)ketamine are increasingly accessible and comparably effective treatments for treatment-resistant depression. We recently developed a machine learning adaptation of the Personalized Advantage Index (PAI) to predict optimal treatment allocation between ECT and (es)ketamine using pretreatment medical record data. Using a large, retrospective cohort of patients naturalistically assigned to ECT or (es)ketamine at McLean Hospital, we demonstrated that patients assigned to a treatment predicted optimal by our model had significantly reduced end-treatment depressive symptoms compared to those assigned a non-optimal treatment. Here, we evaluate the external validity of our model to predict optimal treatment assignment for individual patients from two interventional psychiatry clinics: The Universities of California, Los Angeles (UCLA) and San Diego (UCSD).

Methods: Data on ECT and (es)ketamine patients was gathered across three independent clinics: McLean (ECT $n = 2506$, IV or esketamine $n = 196$), UCLA (ECT $n = 44$, IV ketamine $n = 67$), and UCSD (ECT $n = 32$, esketamine $n = 66$). Depressive symptoms were measured pre- and post-treatment using the Quick Inventory of Depressive Symptomatology (QIDS) at McLean and UCLA and the Montgomery-Asberg Depression Rating Scale (MADRS) at UCSD, which was converted to QIDS scores. Propensity score matching (PSM) matched ECT and ketamine patients at McLean on pretreatment QIDS and age, yielding 196 patients per treatment ($N = 392$ total). Individual PAI scores were computed using leave-one-out cross validation with random forest regression (RFR) models trained to predict end-QIDS in $N-1$ patients. Two models were trained on the McLean cohort to include common data elements at the external sites including pretreatment QIDS, age, sex, medical and psychiatric comorbidities, medication history, and treatment type (ECT vs. ketamine). Two end-QIDS predictions were made for each held-out McLean patient: one using the patient's true treatment label and a counterfactual with the treatment label switched. The lowest end-QIDS prediction was deemed the patient's optimal treatment. We compared end-QIDS distributions between patients in predicted optimal and non-optimal treatments using a t-test. Interactions between predictors and treatment type were evaluated with Shapley values to identify

prescriptive measures. Model performance was based on the coefficient of determination (R^2) comparing predicted and real end-QIDS scores and permutation tests ($B = 1000$ resamples) determined the R^2 significance. RFR models trained on McLean patients with instance weighted domain adaptation to improve generalizability were used to predict treatment allocation for UCLA and UCSD patients.

Results: Baseline and end-QIDS, age, and sex did not differ between treatments at McLean after matching. The McLean-UCLA model predicted end-QIDS significantly ($R^2 = 0.15$, $p < 0.001$). Patients who received a predicted optimal treatment ($n = 199$, 50%) had significantly lower end-QIDS versus the non-optimal treatment group (mean difference [MD] end-QIDS = 1.07, $t = 2.27$, $df = 389.32$, $p < 0.05$, Cohen's D [CD] = 0.22). External validation at UCLA significantly predicted end-QIDS scores ($R^2 = 0.02$, $p < 0.001$) and patients who received a predicted optimal treatment had significantly lower end-QIDS versus the non-optimal group (MD = 2.24, $t = 1.87$, $df = 107.49$, $p < 0.05$, CD = 0.35) with a clinically significant difference identified in 46% of patients with the largest predicted advantages (MD = 3.76, $t = 2.05$, $df = 44.64$, $p < 0.05$, CD = 0.62). Patients with moderate depression, middle-aged patients, fewer prior antidepressant medications, or a history of lamotrigine or seroquel medication were predicted to have better outcomes with ketamine. Older patients, those with severe depression, more prior treatments, a history of substance abuse, or anxiety were indicated for ECT.

The McLean-UCSD model did not result in significant end-QIDS differences in McLean (MD = 0.61, $t = 0.78$, $df = 39.63$, $p > 0.05$, CD = 0.13). Adding inpatient status and psychotic symptom severity to PSM criteria yielded a trend-level effect of greater symptom reduction in the optimal treatment group (MD = 0.7, $t = 1.53$, $df = 370.71$, $p = 0.06$, CD = 0.15) and a significant prediction of end-QIDS ($R^2 = 0.16$, $p < 0.001$). External validation in UCSD patients showed greater trend-level symptom reductions in the optimal treatment group for the 75% of patients with the largest predicted advantages (MD = 1.81, $t = 1.61$, $df = 63.48$, $p = 0.055$, CD = 0.48). Patients with a personality disorder, self-harm symptoms, or alcohol abuse were indicated for ECT.

Conclusions: Strategies to perform prospective treatment allocation are critical to advance precision psychiatry. We validated our PAI model across two external clinics to predict optimal treatment allocation between ECT and (es)ketamine using medical record data. Within McLean and at external sites, patients who received a predicted optimal treatment had marginal to clinically significant differences in symptom reduction compared to those treated with a non-optimal treatment. We further identified factors predictive of optimal treatment allocation which could aid clinical decision-making. Future work will aim to improve model generalizability and predict treatment allocation across additional treatments.

Keywords: Major Depressive Disorder (MDD), electroconvulsive therapy, Ketamine/esketamine, Treatment Selection, machine learning

Disclosure: Nothing to disclose.

P210. AXS-05 in Major Depressive Disorder: Pooled Data From Two Six-Week Controlled Trials (GEMINI and ASCEND)

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Background: Many individuals experience enduring and burdensome tolerability problems with use of common antidepressant treatments (ADTs). Additionally, differences in treatment response

for patients with major depressive disorder (MDD) have been observed based on use of prior ADT, patient sex, and patient race. AXS-05 (dextromethorphan-bupropion) is a novel, oral N-methyl-D-aspartate (NMDA) receptor antagonist, sigma-1 receptor agonist, and aminoketone CYP2D6 inhibitor approved by the US Food and Drug Administration for the treatment of MDD in adults.

Methods: GEMINI and ASCEND assessed AXS-05 versus placebo and 105 mg bupropion, respectively, in 6-week trials of patients with MDD. In both, patients were administered AXS-05 or comparator once-daily for 3 days, then twice-daily. In the current post-hoc analyses, GEMINI and ASCEND data were pooled to compare data for AXS-05 across studies vs. comparator (placebo or bupropion). Safety analyses quantified treatment-emergent adverse event (TEAE) incidence by week, absolute prevalence (total number of patient days experiencing TEAE divided by total number of patient days), and duration of TEAEs. Efficacy was measured as change from baseline on the Montgomery-Åsberg Depression Rating Scale (MADRS) among patients stratified by prior use of an ADT in the current major depressive episode (MDE), patient sex, and patient race.

Results: Baseline and sociodemographic characteristics were generally similar across pooled AXS-05 and control groups. The most common TEAEs observed in $\geq 5\%$ of patients treated with AXS-05 were dizziness (17.1%), nausea (13.8%), headache (8.1%), diarrhea (6.7%), dry mouth (6.7%), anxiety (5.7%), somnolence (5.7%), sexual dysfunction (5.2%), and decreased appetite (5.2%). Of the TEAEs in $\geq 5\%$ of AXS-05 patients, most incidences were reported in Week 1, and the absolute prevalence ranged from 1.8% to 6.1%. All TEAEs in $\geq 5\%$ of AXS-05 patients resolved with a median duration of 2.5-16 days. Patients treated with AXS-05 showed reductions in MADRS total score at Weeks 1, 2, and 6 compared to control, irrespective of prior ADT use, sex, or race.

Conclusions: AXS-05 was safe and well-tolerated in the GEMINI and ASCEND trials. TEAEs occurring in the AXS-05 treatment group were low in prevalence and occurred early and with brief duration. AXS-05 exhibited consistent improvement in depressive symptoms compared to the control cohort across patients irrespective of prior ADT use in the current MDE, patient sex, and patient race.

Keywords: MDD, Tolerability, CNS Clinical Trials, NMDA antagonist

Disclosure: Axsome Therapeutics: Employee (Self)

P211. Prevalence and Correlates of Cognitive Impairment in Major Depression: Results From the Texas Resilience Against Depression (T-RAD) Study

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Background: Major Depressive Disorder (MDD) is a common, heterogeneous disorder that is a leading cause of disability worldwide. Cognitive deficits are a central component of depression that may reflect a specific subtype of the disorder, and are evident as early as childhood and in the first depressive episode. Cognitive impairment (CI) in MDD is associated with impairments in function, decreased likelihood of treatment response to standard treatments (such as SSRIs), persist in symptomatic remission, and increases risk for suicide. The actual prevalence of CI in MDD, however, is unknown. Rates of CI vary widely in studies (ranging from 30% to 60%) depending on the criteria used. Studies historically have used stricter criteria to define CI than regular psychometric criteria for a neurocognitive disorder (such as one standard deviation below normative data, and taking premorbid cognitive abilities into consideration), which means that prior estimates likely under-report the actual percentage of CI in MDD. Given that CI in MDD represents

a subset of MDD that has worse clinical and functional outcomes and poor response to treatment leading to a significant clinical unmet need, the current study seeks to estimate the prevalence of CI in MDD and to examine its clinical, functional, and demographic correlates.

Methods: Data were from 367 participants from the Texas Resilience Against Depression (T-RAD; NCT02919280) study, an ongoing natural history, longitudinal prospective study that follows individuals who have a past or current diagnosis of a depressive or bipolar disorder. Here, analyses focused on adults with a history of or current MDD (based on the MINI International Neuropsychiatric Interview) that were 18 years and older, at their first observation in which they completed the National Institute of Health Toolbox - Cognitive Battery (NIHTB-CB), which measures premorbid cognitive abilities (via a Crystallized Composite) and current cognitive functioning on tests of cognitive flexibility, inhibition, processing speed, working memory, and episodic memory. Cognitive Impairment was defined using the criteria set by Holdnack et al. (2017), with two or more cognitive scores approximately one standard deviation below premorbid cognitive abilities using demographically-adjusted T-Scores, which correct for age, gender, education, race, and ethnicity. Groups were compared on demographic and clinical measures, including the Patient Health Questionnaire - 9 item (PHQ-9), Generalized Anxiety Disorder - 7 item (GAD-7), Quick Inventory of Depressive Symptomatology - Clinician rated version (QIDS-C); quality of life, measured by the World Health Organization Quality of Life - Brief Version (WHO-QOL-BREF); functioning, as measured by the Work, Productivity and Activity Impairment (WPAI) and Work and Social Adjustment Scale (WSAS); and self-reported cognition, as measured by the Brief Assessment of Subjective Cognition scale (BASC). This study was powered to detect an effect size of $d = 0.29$, with two-sided alpha at $p = 0.05$ and power = 0.80. A False Discovery Rate was applied to protect against Type I error.

Results: Of 367 participants, 192 (52%) met criteria for cognitive impairment. Prevalence of CI was similar across all age groups and was similar in those with mild symptoms of depression (52%) and those with moderate-to-severe symptoms (57%). After controlling for multiple comparisons, participants with CI reported greater anhedonia (Cohen's $d = 0.32$, $P = 0.004$), worse overall functioning ($d = 0.29$, $P = 0.007$), greater physical health problems ($d = 0.27$, $P = 0.014$), more impairment in daily activities ($d = 0.31$, $P = 0.005$), and were less likely to be employed than those without CI (Odds Ratio = 0.45, $P < 0.001$). However, the groups did not differ in demographic characteristics, age of onset, number of episodes, or in current depression severity.

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Conclusions: These findings suggest that objectively determined cognitive impairment is present in half of all individuals with depression, regardless of current clinical severity. These impairments are present across the adult lifespan and are associated with worse quality of life and greater functional impairment. Given the high prevalence of CI in MDD, and that standard of care treatments are less effective in this population, this may explain modest outcomes in the treatment of depression in the full population.

Keywords: Major Depression Disorder, Cognition, Poor clinical outcome

Disclosure: Alto Neuroscience: Employee (Self)

P212. Sustained Benefits of Closed-Loop Transcranial Alternating Current Stimulation (CL-TACS) on Depression: A 12-Week Open-Label Study

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Background: Closed-loop transcranial alternating current stimulation (CL-tACS) has emerged as a promising therapeutic option for individuals with Major Depressive Disorder (MDD). By tailoring the stimulation to the individual brain state through concurrent recording and analysis of brain activity, it is hypothesized that clinical efficacy can be enhanced. Initial findings indicated improved depression symptoms up to two weeks following five days of alpha CL-tACS. However, the duration of symptom abatement has remained unstudied. This open-label study investigated the longer-term effects of alpha CL-tACS on depression severity and other clinical variables up to 12 weeks post-treatment.

Methods: This industry sponsored open-label clinical trial included 15 individuals with MDD (HDRS-17 > 8) to receive five consecutive days of bifrontal CL-tACS (4mA peak-to-peak) at individual peak alpha frequency (NCT05772702). Self-rating questionnaires of depression (QIDS), anxiety (STAI), anhedonia (SHAPS) and quality of life (Q-LES-Q-SF) were obtained on Day 1, Day 5, 1-week and 2-week follow-up and then biweekly up to 12-week follow-up. Additionally, clinician rated HDRS-17 was obtained at Day 1, Day 5 and 2-week follow-up. We conducted repeated measures ANOVA with ratings as the dependent variable and time as the independent variable. Planned contrasts, comparing each timepoint with Day 1, were performed afterwards.

Results: RM-ANOVA revealed a significant decrease of depression over time (QIDS: $p < 0.001$, $\eta^2 = 0.458$). Planned contrasts indicated a significant improvement at every timepoint, with sustained improvement in depression symptoms up to 12 weeks ($p = 0.003$, $d = 1.962$). Quality of life was significantly increased over time (Q-LES-Q-SF: $p = 0.001$, $\eta^2 = 0.230$), and was sustained up to the 12-week follow-up ($p = 0.003$, $d = 0.899$). Similarly, anhedonia also showed a significant reduction over time (SHAPS: $p = 0.006$, $\eta^2 = 0.291$), and subsequent planned contrasts revealed a significant reduction at the 12-week follow-up ($p = 0.017$, $d = 1.431$). State anxiety did not significantly decrease after CL-tACS (STAI, $p = 0.632$, $\eta^2 = 0.047$). Furthermore, depression symptoms obtained by clinician-administered HDRS-17 significantly decreased at the 2-week follow-up (HDRS-17: $p < 0.001$, $\eta^2 = 0.795$).

Conclusions: CL-tACS is a personalized, non-invasive brain stimulation technique that has shown promising results in reducing symptoms of depression and anhedonia and increasing quality of life for up to 12 weeks after a 5-day intervention. The large effect sizes observed at the 12-week follow-up highlight the potential of CL-tACS. The durability of these effects suggests that CL-tACS may contribute to the amelioration of the current depressive episode rather than merely providing short-term symptom relief. To build on these promising findings, a placebo-controlled randomized controlled trial is planned to further investigate the efficacy and broader therapeutic potential of CL-tACS.

Keywords: Depression, non-invasive brain stimulation, transcranial alternating current stimulation

Disclosure: Pulvinar Neuro LLC: Advisory Board (Self)

P213. Basmisanil, an $\alpha 5$ -GABAAR Negative Allosteric Modulator: Behavioral Effects and Underlying Neural Mechanisms in Mice

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Background: Rapid-acting antidepressants represent a promising treatment strategy for patients with stress-related disorders, including major depressive disorder (MDD). Chronic stress is associated with deficient inhibitory synaptic transmission onto glutamatergic neurons in the medial prefrontal cortex (mPFC) (Fogaça and Duman, 2019). Conversely, the rapid and sustained therapeutic effects of ketamine are mediated by enhanced synaptic plasticity and restoration of excitatory and inhibitory (E/I) levels in corticolimbic brain structures (Krystal et al., 2019). However, ketamine's abuse potential is an important safety concern. Our recent work demonstrates that Basmisanil, an $\alpha 5$ -GABAAR negative allosteric modulator, produces rapid and sustained enhancement of motivated and active behaviors in male mice, comparable to those induced by ketamine (Daher et al., 2024). Therefore, we aimed to investigate brain- and cellular-level mechanisms underlying Basmisanil's behavioral effects. Additionally, given the cumulative evidence suggesting that the chronic unpredictable stress (CUS) paradigm produces dysfunctions in cortical circuits similar to those observed in MDD subjects, we examined whether Basmisanil could reverse stress-induced maladaptive behaviors.

Methods: Experiment 1 identified brain areas activated upon single drug administration. C57BL/6 male mice ($n = 4/\text{group}$) were injected with vehicle (negative control, i.p.), ketamine (positive control, 10 mg/kg, i.p.) or Basmisanil (10 mg/kg, i.p.), and euthanized 1.5 h later. Brains were extracted, and slices containing the medial prefrontal cortex (mPFC), hippocampus (HPC) and basolateral amygdala (BLA) were analyzed by immunofluorescence against c-Fos. In experiment 2, we performed a dose-response curve to evaluate the behavioral changes induced by Basmisanil microinjected into the mPFC. C57BL/6 male mice ($n = 6-8/\text{group}$) were implanted bilateral cannulas in the mPFC for microinfusion (0.2 $\mu\text{L}/\text{side}$) of vehicle or Basmisanil (3, 10 and 30 μg) and underwent a battery of behavioral tests to evaluate the rapid and long-lasting effects of the drug (30 min, 24 h and 48 h post-administration). Additionally, ongoing virally mediated chemogenetic experiments are being conducted to investigate whether these effects are mediated by cell type-specific recruitment of CaMKII+ neurons in the mPFC. Experiment 3 examined the impact of Basmisanil (10 mg/kg, i.p.) on the rapidly recruited Akt-mTORC1 pathway and the late recruitment of glutamatergic and GABAergic function in the mPFC and HPC. C57BL/6 male mice ($n = 10-12/\text{group}$) were injected with vehicle (i.p.) or Basmisanil (10 mg/kg, i.p.), and euthanized 1 h or 24 h post-administration. Western blotting was conducted on synaptoneurosomes fractions of mPFC and HPC homogenates. In experiment 4, we assessed Basmisanil's potential to reverse stress-related behaviors. C57BL/6 male mice ($n = 8-10/\text{group}$) were subjected to the 21-day CUS with 2 stressors presented daily. Subsequently, mice were injected with vehicle (i.p.), ketamine (10 mg/kg) or Basmisanil (10 mg/kg, i.p.) and underwent behavioral tests to evaluate the recruitment of cortico-related circuits and motivated behaviors: novel object recognition test (NORT; 1 h and 24 h post-injection); female urine

sniffing test (FUST, 27 h); sucrose splash test (SUST, 48 h). As a control, non-stressed mice were also tested. All procedures were approved by the University of Rochester Committee on Animal Resources (UCAR) and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Results: In experiment 1, acute administration of Basmisanil increases c-Fos+ cells within the mPFC, with more significant expression in the infralimbic (IL) subregion. A similar increase in drug-induced c-Fos expression occurred within subregions of the HPC as well as the BLA. Ongoing experiments are determining cell type-specificity for these effects. In experiment 2, intra-mPFC Basmisanil (10 and 30 µg) produced fast and sustained modulation of behaviors relevant to coping strategies and motivation, similar to previous reports following intraperitoneal administration (Daher et al., 2024). Our preliminary chemogenetic findings suggest that these effects are mediated by disinhibition of glutamatergic neurons through decreased function of $\alpha 5$ -GABAA receptors in the mPFC. In experiment 3, Basmisanil produced a rapid (1 h) phosphorylation of proteins related to mTORC1 and GSK-3 β signaling in both the mPFC and HPC. In addition, Basmisanil increased the expression of proteins related to glutamatergic and GABAergic function 24 h post-treatment. In experiment 4, the 21-day CUS paradigm decreased the exploration time of a novel object in the NORT, which was attenuated by a single injection of Basmisanil 1 h and 24 h before testing. Data from additional tests are currently under analysis.

Conclusions: Our findings reveal that the mPFC, HPC and BLA are recruited upon administration of Basmisanil. Although the role of the HPC and BLA remains to be defined, the reduction of α -GABAA receptor function in the mPFC produces rapid behavioral responses in male mice. These responses may be associated with fast disinhibition of pyramidal neurons and enhanced expression of synaptic proteins. Importantly, putative pro-cognitive effects of Basmisanil will be further investigated.

Keywords: $\alpha 5$ -GABAA, Basmisanil, Depression, GABAA receptor, Stress

Disclosure: Nothing to disclose.

P214. New Antidepressant Use Among Latine Adults With Depression Before and During the COVID-19 Pandemic

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Background: The mental health effects of the COVID-19 pandemic were greater among US Latine individuals than white, non-Latine populations. Latine adults were also at higher risk of depressive symptoms and treatment interruptions. We conducted a retrospective study to assess antidepressant use before and during the COVID-19 pandemic by Latine adults with depression receiving care at a federally qualified health center

Methods: We conducted an observational, retrospective study of electronic health record (EHR) data from a federally qualified health center to assess new antidepressant use among Latine adults with depression. Participants were at least 18 years old, had a diagnosis of depression, and were prescribed a new antidepressant prior to the COVID-19 pandemic (February 2019 to February 2020) and during the COVID-19 pandemic (March 2020 to March 2021). We assessed reported depression symptoms, the antidepressant prescribed, dose titration, number of refills, follow up visits, and primary care provider notes for treatment effectiveness and medication side effects. Descriptive statistics were used to describe and compare results from each cohort.

Results: We screened all 447 EHR profiles; of these, less than half met inclusion criteria for a new antidepressant. Overall,

participants were diagnosed with unspecified major depressive disorder. Most participants were female, their average age was 43 years, and their preferred language of Spanish. The number of participants with a new antidepressant decreased by a one-third from 2019 to 2020. Interruptions in consistent access to providers, pharmacies, and behavioral health services were observed via canceled and reschedule visits and lack of renewed expired prescriptions. Rates of dose titration, adjunct medications, and antidepressant switching were low among both cohorts. Longer periods of time between follow up visits were reported during 2019 than 2020. Study limitations include missing data, lack of initial and follow up depression symptom assessment and antidepressant adherence. Data collection was managed using the REDCap tool. This study was exempt from University of Michigan Institutional Review Board review (HUM#00195619).

Conclusions: Despite reports of increased mental health effects of the COVID-19 pandemic among Latine adults, new antidepressant use decreased by a third between 2019 to 2020. Interruptions in PCP follow up visits, depression symptom monitoring, and behavioral health services may have contributed. Future studies involve outreach and preventive measures to connect Latine adults to depression care including education on antidepressant use and early discontinuation, nonpharmacological therapies, and access to online behavioral health services.

Keywords: Depression, COVID-19 pandemic, Antidepressant

Disclosure: Nothing to disclose.

P215. More May Not Be Better: Ketamine Dosing Frequency Modulates Synaptic Strength, Metaplasticity and Emotional Learning

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Background: Background: Major depression is a chronic disease with critical need for new pharmacologic treatment approaches. Traditional antidepressant medications (i.e. SSRIs) are taken daily since continuous target receptor occupancy is required for efficacy. Ketamine results in a rapid and sustained antidepressant response following a single dose where its pharmacodynamic responses, including reductions of depressive symptoms, far outlasts the drugs pharmacokinetics. In contrast to most antidepressant drugs, ketamine is typically administered intermittently, rather than every day. There is a dearth of data exploring the mechanisms underlying such dosing frequency. We sought to systematically assess in vivo effects of ketamine on synaptic plasticity, duration of such plasticity, and metaplasticity in the medial prefrontal cortex (mPFC) of rats following a single dose versus five daily doses. We used the mPFC-dependent positive emotional learning assay, as a behavioral measure of plasticity.

Methods: Methods: Male rats were implanted with intercranial recording and stimulation electrode in the mPFC. Implanted rats ($n = 6$ / group) were administered vehicle for 5 days, vehicle for 4 days and a single dose of (R,S) ketamine (10 mg/kg IP) on the 5th day, or ketamine (10 mg/kg IP) for 5 consecutive days. mPFC excitatory field potentials evoked by mixed deep white matter inputs to layer II/III were recorded from freely behaving rats at baseline, and 1 hr, 24 hr and 1 week after dosing. Input/output curves (I/O) were generated during dosing period, 1 week and weeks after the last dose. Long-term potentiation (LTP), induced by 6 Hz stimulation trains was measured one and two weeks after dosing. In a separate group of rats. The positive emotional learning test, which is both NMDA Receptor and medial prefrontal

cortex dependent, the effects of ketamine were tested 1 and 2 weeks after the last dose.

Results: Results: A dose-response relation for ketamine was obtained in the positive emotional learning assay assessed 7 days post-dose. In this behavioral assay, 10 mg/kg IP was optimal following a single dose ($F(5, 66) = 112.7, P < .05$; Tukey post hoc test 10 mg/kg vs vehicle, 1 or 3 mg/kg group $P < .05$), while a higher dose (30 mg/kg IP) induced sedation ($F(2, 15) = 293.9, P < .05$; Tukey post hoc test 30 vs 10 or vehicle, $P < .05$; Tukey post hoc test vehicle vs 10 $P > .05$). A single administration of 10 mg/kg ketamine IP enhanced in vivo mPFC synaptic strength 24 hours and 7 days post dose ($F(2, 15) = 300.5, P < .05$; Tukey test vs vehicle) and the magnitude of LTP ($F(2, 15) = 119.9, P < .05$; Tukey post hoc test vs vehicle) 7 days post dose. The enhancement of plasticity, metaplasticity and positive emotional learning was lost 14 days after dosing (all p 's $> .05$). In contrast, animals treated for 5 days with ketamine exhibited an increase in synaptic strength (increased slope of the I/O curve) 24 hours after the first dose, but had returned to baseline by 24 hours after the 5th dose ($P < .05$ Tukey post hoc test daily vs single or vehicle). Similar results were seen in the LTP metaplasticity assay 7 days after dosing, where the shift in threshold elicited by a single dose of ketamine had reversed after the 5th consecutive daily dose but were sustained following a single dose. Consistent with the effect on synaptic plasticity, positive emotional learning, which was enhanced by a single dose of ketamine, was reduced to below vehicle levels after the 5th daily dose ($P < .05$ Tukey post hoc test daily vs single or vehicle).

Conclusions: Conclusions: A single administration of ketamine enhances in vivo mPFC synaptic strength, shifts the threshold towards larger LTP (metaplasticity), and enhances emotional learning, with electrophysiological and behavioral effects lasting for 7 days. In contrast, daily repeat ketamine dosing for 5 days actively suppressed the increases in synaptic strength, metaplasticity, and enhancement in learning observed with single-dose administration. The active suppression of increased synaptic strength with frequent ketamine dosing (daily) may be due to a compensatory mechanism to prevent maladaptive plasticity. Our results support an event-driven pharmacology (EDP) model where a sustained response from a triggering dose of ketamine is due to a sustained increase in plasticity, synaptic strength, and metaplasticity. In EDP models, in which drug pharmacodynamic effects far outlast pharmacokinetics, dosing frequency may be a key determinant of efficacy. Such a dosing model has the advantage of a decrease in accumulating side-effect burden. These results suggest optimizing the potential efficacy of plasticity enhancing therapeutics will benefit from a systematic study for potential interactions of dose and dose interval following in vivo synaptic plasticity.

Keywords: (R,S)-ketamine, Synaptic Plasticity, Antidepressants, Memory and Learning

Disclosure: Gate Neuroscience: Consultant (Self). Recognify Lifesciences: Founder (Self). Alceptor Therapeutics: Founder (Self)

P216. Examining Barriers to the Achievement of Remission in Major Depressive Disorder

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Background: Millions of people each year experience episodes of major depressive disorder (MDD) despite the availability of therapies and approaches that hold the promise of reduced

symptoms and improved quality of life. With a growing number of approved antidepressants, it is critical for both providers and patients to be well-informed of treatment options and strategies to achieve optimal outcomes. The current study aims to identify patient and provider challenges in MDD care domains—including assessment, treatment, engagement in patient-centered strategies, and access—to inform educational approaches that bridge gaps and improve outcomes of individuals affected by MDD.

Methods: To evaluate patient and provider perceptions and challenges regarding MDD treatment, surveys were administered within companion patient-provider web-based learning activities (Web Apps). Customized Web Apps engaged providers and patients, respectively, in education on the treatment and management of MDD. The content for the Web Apps was informed by a steering committee comprised of psychiatrists and patients with MDD, identified via partnership with the Depression and Bipolar Support Alliance. To address access to care and treatment, the Web Apps were reviewed by the Academy of Managed Care Pharmacy Foundation. Between October 2023 and June 2024, 7,232 providers and 347 patients accessed the Web Apps. Surveys were completed by 1,073 providers (43% physicians, 5% physician assistants, 18% nurse practitioners, 9% pharmacists, 17% nurses, 8% other) and 112 patients.

Results: We found that while 60% of patients had been prescribed an antidepressant and nearly half (47%) had tried 3 or more in the last two years, and only 54% felt they were making progress (Likert 4/5) toward their MDD treatment goals. Moreover, both patients and providers reported gaps in their knowledge of newer and emerging treatment options (low to moderate understanding/familiarity [Likert 1/2/3]: patients 57% and providers 71%). Patients identified their top barriers to achieving remission as: finding the right therapist or provider (39%), inadequate response to medication/breakthrough symptoms (32%), and side effects from medications (30%). Providers reported their top challenges in providing ideal MDD care as: patient resistance to medication initiation (32%), difficulty determining if patients are experiencing residual symptoms or medication side effects (28%), and difficulty identifying which therapies will work best (26%). When examining gaps in assessment, only half (50%) of providers were likely (Likert 4/5) to use a depression screening tool during initial and follow-up appointments. Furthermore, only 33% of providers were highly confident (Likert 4/5) in evaluating and responding to residual symptoms and treatment-emergent side effects. When assessing patient experiences with shared decision-making, 81% of patients indicated they are involved in decisions regarding their care (Likert 4/5). However, < half of patients (46%) felt confident (Likert 4/5) in sharing treatment preferences and concerns with their provider. Barriers to accessing evidence-based treatments were also identified. < half of patients (47%) received antidepressants in < a week after being prescribed and 44% have had insurance coverage for their MDD medication(s) denied. Only 44% of patients reported that their health plan provides them with the information they need (Likert 4/5) and only 38% of providers had high (Likert 4/5) confidence in navigating patient access to MDD medications.

Conclusions: These findings underscore the need for patient and provider support in achieving MDD remission across several domains of care. These results can inform the development of targeted strategies to refine patient and provider approaches to symptom sharing and assessment, treatment decision-making, communication, and navigating access in MDD care to improve clinical outcomes.

Keywords: MDD, remission, antidepressants, Patient Reported Outcomes, patient-provider alliance

Disclosure: Sage Therapeutics, Inc.: Consultant (Self) Biogen: Consultant (Self). Takeda Pharmaceuticals U.S.A., Inc.: Consultant (Self)

P217. Intravenous Ketamine Versus Electroconvulsive Therapy for Non-Psychotic Treatment-Resistant Depression: A Cost-Effectiveness Analysis

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Background: Electroconvulsive therapy (ECT) is the gold-standard treatment for treatment-resistant depression (TRD), however its use is limited partly due to concerns about cognitive side effects. Evidence for the efficacy of IV ketamine (IVK) versus ECT for TRD beyond one month has only recently become available. No studies have examined the cost-effectiveness (i.e., costs and benefits measured as quality-adjusted life years-QALYs) of IVK versus ECT for TRD. Therefore, the objective of this study was to examine the cost-effectiveness of IVK versus ECT for TRD.

Methods: We developed a decision-analytic model to estimate the cost-effectiveness of IVK infusions relative to ECT for TRD. Using a decision-tree model, we compared the Quality Adjusted Life years (QALYs) and costs of IVK versus ECT over 29 weeks, from both healthcare and societal perspectives in the Canadian setting. We used deterministic and probabilistic sensitivity analyses to account for uncertainty. The model's time frame was based on the ELEKT-D trial, the most extensive and rigorous randomized clinical trial (RCT) to date comparing IVK and ECT. The ELEKT-D trial featured a 3-week acute treatment phase followed by a 6-month continuation phase. The model structure mirrored the ELEKT-D trial, categorizing participants as either having initiated treatment or not. We used the probabilities of treatment initiation, response, remission, and relapse for the ECT arm and risk difference comparing IVK to ECT from the ELEKT-D trial. Health related quality of life (HRQOL) was represented as utilities (i.e., 0-1 values, where 1 is equal to perfect health and 0 is equivalent to being dead), for TRD, response (i.e., responder non-remitters) and remission were obtained from Sapin et al. (2004). We estimated the costs of IVK and ECT procedures in a similar manner based on utilization of resources and current billing codes in the case of ECT or in the case of IVK, billing codes similar to those that we would expect to be in place if IVK was covered by the publicly funded health care payer in Ontario. All costs and utilities in the model were estimated to be for 29 weeks, which is the duration of the ELEKT-D RCT, the costs were inflated to 2023 values. This approach allowed us to estimate the cost-effectiveness of IVK infusions compared to ECT, considering the different treatment outcomes observed in the ELEKT-D trial.

Results: Compared to ECT, IVK resulted in higher HRQOL (incremental QALYs IVK vs. ECT: 0.024) and lower costs from both healthcare (incremental cost IVK vs. ECT: -\$1,674 CAD) and societal (incremental cost IVK vs. ECT: -\$3,410 CAD) perspective. From a healthcare perspective, the interval for the costs containing 95% of the simulations for IVK (\$1,309, \$2,234) did not overlap with the interval of costs (2506, 4482) suggesting a very high likelihood of cost-savings for the base case analysis. The Incremental Net Monetary Benefits (INMB) for IVK versus ECT from a health care perspective (\$2,874) and societal perspective (\$4,610) were both positive, which means IVK is the more cost-effective option from both perspectives, the probability that IVK was the most cost-effective option was 100% at all willingness to pay (WTP) thresholds-the intervals containing 95% of observations from the simulations for INMB estimated from a healthcare perspective were \$910-3,065, \$1,124-3,671, \$1,407-4,979, \$1,650-\$6,456, \$1,868-\$7,920 for a λ of \$10,000, \$25,000, \$50,000, \$75,000 and

\$100,000 respectively, suggesting a very high likelihood of being cost-effective. In one-way sensitivity analyses examining the INMB, varying all parameters across a range of values, the only INMB where IVK had a value cross 0 was when the healthcare cost of a IVK session was \$645 CAD or higher from a healthcare perspective and \$935 from a societal perspective. This suggests that IVK is the more cost-effective option from a healthcare and societal perspective for all other variable ranges or individual parameters. The health care costs of IVK or ECT and the risk difference for relapse and response of IVK versus ECT were the most important variables in one-way sensitivity analyses.

Conclusions: We found that IVK was cost-effective relative to ECT from both health care and societal perspectives. In deterministic sensitivity analyses, we varied relevant parameters (i.e., costs of treatment, treatment effects, etc.) from a health care perspective. IVK remained superior to ECT across all ranges of the parameters, except when IVK administration exceeded \$645 CAD per session. These results suggest that IVK is more cost-effective than ECT, primarily due to higher direct costs of ECT (i.e., more equipment costs, human resource requirements, and number of treatments) and associated indirect costs.

IVK provided additional QALYs while saving costs relative to ECT in the treatment of TRD. Health insurance plans that cover all the costs of ECT, should also consider covering costs of IVK treatment for TRD.

Keywords: Electroconvulsive therapy, IV ketamine, Cost-effectiveness, Decision tree

Disclosure: Nothing to disclose.

P218. Investigating the Impact of Histone Acetylation on Adolescent Depression

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Background: Major depressive disorder affects approximately 13% of adolescents in the United States. Although traditional antidepressant treatments can alleviate depressive symptoms in adolescents, they often result in low remission rates and carry potential adverse effects, such as suicidal thoughts. This highlights the need for novel pharmacotherapies for adolescent depression.

Methods: We conducted extensive bioinformatics analyses and drug repurposing screenings to identify drugs and compounds capable of reversing or mimicking the molecular signatures of adolescent depression using RNAseq data from the human dorsolateral prefrontal cortex. Additionally, to validate these results, we performed behavioral testing to assess the reversal of stress-induced maladaptive behaviors in adolescent male and female mice.

Results: Our study demonstrated that histone deacetylase inhibitors can reverse the molecular changes observed in adolescents with depression, indicating that increased histone acetylation affects gene expression. Notably, gene ontology analysis revealed significant downregulation of cholesterol metabolic and biosynthetic processes in adolescent patients compared to adults. Since cholesterol is a precursor for most steroid gonadal hormones, pathway enrichment analysis also showed downregulation in steroid biosynthesis. These results suggest that increased histone acetylation may lead to decreased synthesis of steroid gonadal hormones during adolescence, potentially increasing the risk of developing depression. To validate our findings, we subjected adolescent male and female mice to acute

foot-shock stress and assessed the development of anhedonia, social preference deficits, and anxiety-like behavior using the sucrose preference, social interaction, and open field tests. Stressed adolescent mice exhibited social interaction deficits and anxiety-like behaviors, which were reversed by administering a histone deacetylase inhibitor before the acute stress.

Conclusions: These findings indicate that targeting histone acetylation could represent a novel strategy for treating adolescent depression.

Keywords: Histone acetylation, Adolescent Depression, Bioinformatics

Disclosure: Nothing to disclose.

P219. Efficacy and Safety of a Single Administration of the NMDA Subunit 2B-Selective Negative Allosteric Modulator, BI 1569912, in People Living With Major Depressive Disorder: A Phase IB Randomized Controlled Trial

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Background: Although non-selective N-methyl-D-aspartate (NMDA) receptor antagonists, such as ketamine and esketamine, have shown efficacy in treating major depressive disorder (MDD) they are associated with multiple side effects, including dissociation and psychomimetic effects. Selective negative allosteric modulators of the NR2B-containing subunit of the NMDA receptor (NR2B NAMs) may offer efficacy without the side effects of non-selective NMDA receptor antagonists. BI 1569912 is an oral NR2B NAM currently in development for MDD.

Methods: In this Phase IB multicenter trial (NCT04937829) in the US, adults with moderate-to-severe MDD, and insufficient response to ongoing antidepressant monotherapy (selective serotonin reuptake inhibitors or serotonin and norepinephrine reuptake inhibitors), were randomized 1:1:1 to receive a single dose of oral BI 1569912 5 mg, 20 mg, or placebo during a 1-week inpatient stay. This was followed by a 1-week outpatient period and an end of study visit on Day 15. Efficacy was evaluated by maximum decrease from baseline in Montgomery-Åsberg Depression Rating Scale (MADRS) score at any day within a 7-day interval (primary), and by change from baseline (CFB) in MADRS total score on Days 1, 2, 4, 6, 8, 11, and 15 (exploratory). A MADRS response was defined as a CFB score reduction of $\geq 50\%$, and a remission was defined as a MADRS score ≤ 10 , at individual time points, assessed on Days 1, 2, 4, 6, 8, 11, and 15 (exploratory). Proof of clinical principle for BI 1569912 was defined as an improvement in MADRS total score of ≥ 3 points at any time point and ≥ 2 points at a neighboring time point, at any day within a 7-day interval, versus placebo. Efficacy was also assessed by CFB in Leuven Affect and Pleasure Scale (LAPS) subscales on Days 1, 2, 4, 6, 8, 11, and 15 (exploratory). MADRS response and remission data were analyzed using a logistic regression model, with baseline total score and treatment as fixed effects.

Safety was assessed from start of treatment to last visit (Day 15) and included: monitoring adverse events (AEs), including severe and serious AEs, drug-related AEs (DRAEs) (primary), and AEs of special interest (AESI); Clinician Administered Dissociative States Scale (CADSS) scores at baseline and Days 1 and 15; and assessment of psychedelic effects (indicative of potential for drug abuse and dissociation over time) on the Bowdle-Visual Analog Scale (B-VAS; at baseline, Days 1, 2, and 15).

Results: Participants (N = 59) were randomized and treated as follows: placebo (n = 19), BI 1569912 5 mg (n = 20), and 20 mg (n = 20). Demographic and baseline characteristics were generally balanced between groups. Median age (range) was 54.0 (18–65) years, 54.2% of participants were female, and mean (standard deviation) MADRS total score at baseline was 34.6 (5.8) points.

The adjusted mean (standard error) maximum decrease from baseline in MADRS within a 7-day interval was similar between groups, at -19.3 (2.3), -16.8 (2.2), and -19.9 (2.2) for placebo, BI 1569912 5 mg, and 20 mg, respectively. The 3.4–4.9 point improvement in MADRS total score on Days 2, 4, and 6 after treatment with BI 1569912 20 mg, versus placebo, supported proof of clinical principle. A higher proportion of participants treated with BI 1569912 20 mg achieved MADRS response at Days 2, 4, and 6 (50.0%, 45.0%, and 50.0%) compared with placebo (21.1%, 10.5%, and 21.1%). MADRS remission on Days 2, 4, and 6 was also achieved by a higher proportion of participants treated with BI 1569912 20 mg (20.0%, 35.0%, and 40.0%) than placebo (10.5%, 10.5%, and 21.1%). Logistic regression analysis also showed that, on Days 2, 4, and 6, the predicted odds of achieving a response for participants receiving BI 1569912 20 mg were higher than the odds for participants receiving placebo, with odds ratios (95% confidence interval [CI]) of 3.58 (0.83, 15.48), 6.70 (1.16, 38.53), and 3.58 (0.86, 14.94). In addition, the predicted odds of achieving remission for participants receiving BI 1569912 20 mg were higher than the odds for participants receiving placebo, with odds ratios (95% CI) of 1.78 (0.27, 11.85), 4.22 (0.71, 25.09), and 2.27 (0.51, 10.02) at Days 2, 4, and 6, respectively. Results on the LAPS showed participant-centered dimensions of depression improved in all treatment arms, including placebo.

The proportion of participants with ≥ 1 AE, from start of treatment to Day 15, was 31.6%, 35.0%, and 15% for the placebo, BI 1569912 5 mg, and 20 mg groups, respectively. The most common AEs in those receiving BI 1569912 were constipation (n = 2), increased blood pressure (n = 2), and headache (n = 2). DRAEs were reported in 1 participant in the placebo group, 3 in the BI 1569912 5 mg group, and 1 in the 20 mg group. In BI 1569912-treated participants, DRAEs included constipation (n = 2), and flatulence, increased blood pressure, decreased appetite, dizziness, and dry skin (n = 1 for all). No severe or serious AEs, or AESI were reported. In participants treated with BI 1569912, no clinically relevant dissociation per CADSS scores was observed, and no signs or symptoms of human abuse potential were indicated from B-VAS assessment.

Conclusions: BI 1569912, an oral NR2B NAM in development for the treatment of MDD, was well tolerated with no dissociation and no clinically relevant signs of human abuse potential. Efficacy assessments showed that proof of clinical principle was met, and study findings support the progression to a Phase II trial of BI 1569912 (NCT06280235) in adults with MDD.

Funding: Boehringer Ingelheim (1447-0003).

Keywords: MDD, NR2B NAM, BI 1569912

Disclosure Abbvie, Atai, Biogen, Bristol-Myers Squibb, Clexio, Denovo Biopharma, Daiichi Sankyo, Janssen, Neurocrine, Perception Neuroscience, Sage Pharmaceuticals, Seelos Pharmaceuticals, Usona Institute, XW Labs: Consultant (Self). Biohaven Pharmaceuticals, Boehringer Ingelheim International, EMA Wellness, Embark, Freedom Biosciences, Gilgamesh, Merck, Novartis, Relmada Therapeutics, Tetricus, Transcend Therapeutics: Advisory Board (Self). Biohaven Pharmaceuticals: Founder (Self). Janssen, Merck: Contracted Research (Self). Relmada Therapeutics, Biohaven Pharmaceuticals: Stock / Equity - Publicly Traded Company (Self). Freedom Biosciences, Tetricus: Stock / Equity - Privately Held Company (Self)

P220. Improvement in Cognitive Control Following rTMS of the DLPFC in Major Depressive Disorder: Evidence From Eye-Tracking

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Background: Repetitive transcranial magnetic stimulation (rTMS) targeting the dorsolateral prefrontal cortex (DLPFC) has proven to be an effective treatment for refractory major depressive disorder (MDD). However, the precise mechanisms through which rTMS improves mood symptoms are not fully understood. One potential explanation is that rTMS may alleviate depressive symptoms by enhancing cognitive control abilities, which are often impaired in individuals with MDD.

Methods: To address this question, we recruited patients with MDD scheduled for rTMS and demographically matched non-depressed control participants. Intermittent theta-burst stimulation targeting the left DLPFC was administered over 20 daily sessions. We used the interleaved pro/anti-saccade task (IPAST) to assess cognitive control abilities. In pro-saccade trials, participants looked at a peripheral stimulus, providing a measure of processing speed. In anti-saccade trials, participants suppressed the reflex to look at a peripheral stimulus and instead looked away, providing a measure of cognitive control.

Results: A total of 29 individuals with MDD (mean age = 44.9 years, 48% female) completed the IPAST before and after a 4-week course of rTMS. Additionally, 19 non-depressed control participants (mean age = 38.9 years, 58% female) completed the task at corresponding time points. Mixed ANOVAs were used to assess between- and within-group differences in pro- and anti-saccade reaction time (SRT).

As expected, pro-saccade SRT did not differ between groups ($F(1,46) = 0.20$, $p = 0.66$) or over time ($F(1,46) = 0.64$, $p = 0.43$), and there was no group-by-time interaction ($F(1,46) = 0.00$, $p = 0.99$). However, as expected, anti-saccade SRT was significantly longer in individuals with MDD compared to controls ($F(1,46) = 5.98$, $p = 0.02$) and decreased significantly over time across participants ($F(1,46) = 11.58$, $p = 0.001$). The group-by-time interaction approached significance ($F(1,46) = 2.36$, $p = 0.08$), with anti-saccade SRT decreasing to a greater extent in individuals with MDD receiving rTMS compared to control participants.

Conclusions: These findings suggest that rTMS targeting the left DLPFC specifically enhances cognitive control abilities without impacting other cognitive domains, such as processing speed. This suggests that improvements in cognitive control may be a key mechanism through which rTMS contributes to the reduction of depressive symptoms in individuals with MDD.

Keywords: repetitive transcranial magnetic stimulation (rTMS), Depression, cognitive control, Cognition

Disclosure: Nothing to disclose.

P221. NV-5138, an Orally-Administered, Investigational Rapid-Acting Antidepressant, Acutely Alters Prefrontal Cortical Neuronal Responses in Mice: Comparison to Ketamine and Rapastinel

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Background: NV-5138 is a rapid-acting antidepressant (RAAD) candidate currently undergoing Phase 2 clinical trials. NV-5138 has

shown no evidence of psychotomimetic effects and is thought to work by activating mechanistic target of rapamycin complex 1 (mTORC1) through a novel intracellular mechanism. RAADs that engage NMDA receptors (ketamine and rapastinel), must activate mTORC1 in the prefrontal cortex (PFC) to demonstrate efficacy in preclinical models. We evaluated the acute impact of NV-5138 on PFC neuronal activity relative to ketamine and rapastinel.

Methods: In vivo cellular Ca^{2+} imaging was performed in male c57bl/6j mice in 3 separate experiments. Mice received a unilateral intra-PFC microinjection of a viral vector containing a genetically encoded Ca^{2+} sensor (AAV1-CaMKII-GCaMP6f) with transgene expression confined to excitatory neurons. One-week post-surgery, mice were implanted with a lens probe for in vivo imaging during freely moving behavior. Experiments were conducted 3 weeks after lens implant. All procedures were performed in accordance with the USDA Animal Welfare Act, the conditions specified in the Guide for the Care and Use of Laboratory Animals, and were approved by an Institutional Animal Care and Use Committee. Mice were fully habituated to wearing the miniscope, handling, the open field chamber (OFC), and route of drug administration prior to experiments. For each experiment recordings were conducted at timepoints following drug administration that are consistent with target engagement in the brain using clinically relevant doses. Within each experiment, all mice received each dose of drug and vehicle. In experiment 1, mice ($n = 8$) were administered vehicle or NV-5138 (160 mg/kg, PO) and a 15-minute recording occurred 1-hour post dose. In experiment 2, mice ($n = 20$) were administered vehicle or ketamine (3, 10, or 30 mg/kg, IP) and a 10-minute recording occurred immediately after dosing. In experiment 3, mice ($n = 8$) were administered vehicle or rapastinel (10 or 30 mg/kg, SC) and a 10-minute recording occurred 30 minutes post dose. For each experiment, Ca^{2+} imaging variables (average spike rate, active cell count, average interneuronal activity positive pairwise correlation, drug modulation index [fraction of neurons with up- or down-modulated event rates relative to baseline]), as well as average locomotor velocity were measured. Data were analyzed using either linear mixed-effects models with condition (baseline/post treatment) and dose as fixed effects, and the interaction term (condition x dose) used to assess significance at each dose level or Wilcoxon non-parametric paired t-test as appropriate; $p < 0.05$ was considered significant. All metrics were then incorporated in a principal component analysis (PCA) plot to visualize distinct drug profiles and overlapping clusters. All between-drug comparisons were qualitative in nature.

Results: NV-5138 (160 mg/kg) and ketamine (10 mg/kg) increased the average locomotor velocity (~2-fold over vehicle, $p < 0.01$ and $p < 0.05$, respectively), whereas ketamine (30-mg/kg) produced strong hyperlocomotion (~6-fold over vehicle, $p < 0.001$). NV 5138 showed a strong trend towards increasing the average spike rate ($p = 0.05$) without changing the number of active cells, whereas ketamine 10- and 30-mg/kg reduced both metrics (all $p < 0.05$). NV 5138 increased the proportion of up-modulated neurons (Wilcoxon stat=3, $p < 0.05$) without impacting the proportion of down-modulated neurons (Wilcoxon stat=11, $p = 0.38$), whereas ketamine conferred opposite effects, increasing the proportion of down-modulated neurons ($p < 0.01$, all doses) without impacting the proportion of up modulated neurons ($p > 0.25$, all doses). NV-5138 did not impact the magnitude of positively correlated cell pairs ($p = 0.74$), whereas ketamine decreased the magnitude at both 10 mg/kg ($p < 0.05$) and 30 mg/kg ($p < 0.001$). Rapastinel had no effects on any metrics at either dose. PCA analysis showed distinct clustering of NV-5138 compared to all doses of ketamine and rapastinel, whereas rapastinel overlapped with vehicle.

Conclusions: NV-5138, ketamine, and rapastinel have shown to be efficacious in traditional preclinical assays of depression-like behavior. Thus, evaluating distinctions in mechanism of action

requires a more sensitive approach with potentially improved translational relevance. Our experiments show that orally-administered NV-5138 produces a unique profile of acute behavioral and PFC neural circuit responses compared to ketamine and rapastinel. Ketamine acutely and dose-dependently reduced neuronal responses while increasing locomotor activity, particularly at the high doses (30 mg/kg) whereas NV-5138 generally conferred opposite effects on neuronal activity and modestly increased locomotor activity. Interestingly, NV-5138 increased the proportion of neurons that were activated relative to baseline, suggesting activity-dependent modulatory properties. Rapastinel was ineffective on all metrics in this integrated neurobehavioral assay. Our results show that NV-5138, which activates mTORC1 via an intracellular mechanism, produces disparate neurobehavioral outcomes relative to drugs like ketamine and rapastinel which activate mTORC1 via NMDA modulation.

Keywords: Medial Prefrontal Cortex (mPFC), calcium imaging, Novel Rapid Antidepressant

Disclosure: Supernus Pharmaceuticals: Employee (Self)

P222. Ketamine for Suicidal Behavior in Treatment-Resistant Depression: A Comparative Analysis of Antisuicidal and Antidepressant Effects

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Background: Previous research has indicated that intravenous ketamine's acute antisuicidal effects are more pronounced than its antidepressant properties. This study aimed to investigate this differential impact in patients with chronic suicidal ideation. Participants were enrolled in a clinical trial designed to explore a potential intervention for extending ketamine's duration of treatment response.

Methods: Participants received an open-label ketamine infusion (0.5 mg/kg) over 40 minutes as part of an ongoing randomized clinical trial (ClinicalTrials.gov Identifier: NCT04116528). After 48 hours, they were allocated to receive either dose-escalated buprenorphine therapy (0.2-0.8 mg, QD) or a placebo for four weeks. Individuals who were in a unipolar or bipolar type II major depressive episode (MDE) of at least eight weeks duration, with a history of at least one failed antidepressant treatment and suicidal ideation (Beck Scale for Suicidal Ideation [BSSI] score ≥ 6), were eligible to participate. The Montgomery-Åsberg Depression Rating Scale (MADRS), Hamilton Depression Rating Scale (HAMD-17), and BSSI were used as assessment tools before infusion and on day 3.

Results: Forty individuals in an MDE with suicidal ideation participated in the study. The average age of the participants was 37.2 years (SD = 10.9), 75% were female, and only one subject was diagnosed with bipolar disorder type II. The MADRS total score decreased from 34.87 to 21.58 ($p < .0001$). Among the participants, 37.5% met the response criteria, defined as a reduction in the MADRS total score of at least 50%, and 25% achieved remission, defined as a total score of 10 or less. The HAMD-17 also reflected significant improvements. The total HAMD-17 score decreased from 23.7 to 14.85 ($p < .0001$). In this scale, 35% of participants met the response criteria, which is defined as a reduction of at least 50% in the total score, and 17.5% achieved remission, which is defined as a total score of 7 or less. In addition, the BSSI scores substantially decreased from 15.1 to 7.47 ($p < .0001$). In terms of response, 52.5% of participants achieved a reduction of at least 50% in their BSSI total scores.

Regarding individual scores, the MADRS reported sadness (Item 2) score decreased significantly from an average of 4.3 to 2.58 ($p < .0001$), with 45% of participants experiencing a reduction of at least 50% in this score. Suicidal ideation (Item 10) also showed a significant decrease, with scores dropping from 3.7 to 1.71 ($p < .0001$), and 60% of participants achieved a reduction of at least 50% in this score. When analyzing the total MADRS score excluding Item 10 (suicidal ideation), the score decreased from 31.17 to 19.87 ($p < .0001$), with 35% of subjects showing a reduction of at least 50% in symptoms.

With respect to the HAMD-17 individual scores, depressed mood (Item 1) decreased from an average of 3.2 to 2.0 ($p < .0001$), with 42.5% of the participants experiencing a reduction of at least 50% in this score. Suicidal ideation (Item 3) scores decreased from 2.22 to 0.90 ($p < .0001$), with 72.5% of participants showing a reduction of at least 50% in this score. When considering the total HAMD-17 score without Item 3 (suicidal ideation), the score decreased from 21.47 to 13.95 ($p < .0001$), with 30% of subjects achieving a reduction of at least 50% in symptoms.

Conclusions: Ketamine infusion significantly improved both depressive symptoms and suicidal ideation scores at day 3, with a larger effect on suicidal ideation. These findings in an open-label context, combined with the relative safety of IV ketamine infusion, support its potential as a valuable intervention for suicidal ideation. The randomized phase will further explore the persistence of this difference and whether it is influenced by the extended activation of opioid receptors.

Keywords: Ketamine, Depression, Treatment Resistant Depression, Bipolar Depression, Suicidal Behavior

Disclosure: Nothing to disclose.

P223. Evaluation of Anti-depressant Effect of Tirzepatide on Unpredictable Chronic Mild Stress Induced Swiss Albino Mice: An Experimental Study

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Background: Depression is a prevalent mental disorder affecting approximately 300 million people worldwide and is projected to become a leading cause of global burden in the next decade. Current antidepressant therapies, such as SSRIs, SNRIs, and TCAs, often exhibit limited efficacy, delayed onset of action, high relapse rates, and significant side effects. These limitations underscore the urgent need for novel therapeutic strategies. Tirzepatide, a GLP-1 and GIP receptor agonist approved for type-2 diabetes and obesity, has shown potential neurobiological effects that may benefit depression. This study aims to evaluate the antidepressant effect of Tirzepatide in a well-characterized Unpredictable Chronic Mild Stress (UCMS) model of depression, focusing on its impact on behavioral and biochemical parameters associated with depressive states.

Methods: Following Institutional Animal Ethics Committee (IAEC) approval, the study was conducted using 40 Swiss Albino mice (25-30 grams, either sex), which were randomly divided into five groups: Normal Control, Disease Control (UCMS + saline), Positive Control (UCMS + Fluoxetine 10 mg/kg), Test Group 1 (UCMS + Tirzepatide 10 nmol/kg), and Test Group 2 (UCMS + Tirzepatide 25 nmol/kg). UCMS was administered over four weeks to induce depressive-like states, followed by group-specific drug administration for an additional two weeks. Behavioral assessments were conducted using the Forced Swim Test (FST) and Tail Suspension Test (TST), measuring the duration of immobility as an indicator of depressive behavior. Biochemical analyses were performed to quantify hippocampal levels of Brain-Derived Neurotrophic Factor (BDNF), serotonin (5-HT), and interleukin-6

(IL-6) using ELISA kits. Data were analyzed using SPSS Version 27, employing appropriate statistical tests.

Results: The results demonstrated a significant improvement in FST, TST, and BDNF values in the Fluoxetine and Tirzepatide groups compared to the Disease Control group ($p < 0.05$). However, no significant difference was observed between the Fluoxetine and Tirzepatide groups ($p > 0.05$), indicating a lesser antidepressant effect of Tirzepatide compared to Fluoxetine.

Conclusions: Tirzepatide (25 nmol/kg) showed improvement in depressive symptoms but was not as effective as Fluoxetine. Further elucidation of its role as an antidepressant is needed, and studies exploring combination therapy may provide a promising avenue for future research.

Keywords: GLP-1 receptor agonist, Tirzepatide, Neurobiological effects, Depression model

Disclosure: Nothing to disclose.

P224. Multichannel Individualized Stimulation Therapy (MIST): Precision Through Computational Modeling and Multitargeted Stimulation

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Background: Despite its efficacy in alleviating mood symptoms, the practice of electroconvulsive therapy (ECT) faces challenges, especially in balancing therapeutic efficacy with cognitive side effects. Traditionally, ECT uses standardized electrode placements, such as right unilateral or bitemporal, with a fixed, high current amplitude (typically 800 mA or 900 mA). The present stimulation technique generally produces a widespread distribution of strong electric fields throughout the entire brain rather than targeting specific neural circuits. The lack of discrimination between different brain regions can result in undesirable cognitive impairments, such as memory loss or confusion. Furthermore, this 'one-size-fits-all' approach does not account for interindividual differences in head anatomy, resulting in variable stimulation intensities and, consequently, inconsistent clinical outcomes. A significant obstacle in optimizing ECT is the absence of stimulation approaches and technologies capable of precisely targeting the brain structures implicated in the therapeutic effects while avoiding areas associated with cognitive side effects.

Methods: We have developed an advanced Multichannel Individualized Stimulation Therapy (MIST) system, designed to deliver personalized, multifocal stimulation with a wide range of customizable parameters. This system leverages cutting-edge computational electric field modeling and optimization algorithms, enhancing its ability to precisely target specific neural circuits and to accommodate individual anatomical variations. The stimulation device is equipped with 15 independently programmable isolated current sources, supporting stimulation using up to 16 electrodes on the scalp, providing a highly flexible platform to shape the induced electric field in the brain. The system utilizes biphasic pulses delivered via an H-bridge circuit topology. To ensure both safety and efficacy, dynamic impedance measurements are conducted continuously, adjusting the voltages in real-time in response to changing tissue properties to maintain constant current. This feature is crucial for maintaining optimal current delivery throughout the treatment session. The MIST system is designed with patient safety as a priority, operating on battery power to electrically isolate the patient. The stimulation automatically stops when an electrode disconnection is detected. It also includes independent current monitors and a sophisticated front-end amplifier unit, which collectively gather and display

critical physiological data, including EEG, ECG, and EMG signals. This integrated monitoring allows clinicians to observe patient responses in real-time.

Results: We conducted comprehensive testing of the MIST device across a full spectrum of output stimulation parameters to ensure robust performance. The stimulator can deliver a full range of adjustable stimulation parameters with a pulse amplitude up to 900 mA, pulse width up to 1.5 ms, pulse-pair frequency up to 120 Hz, and stimulus train duration up to 8 s. The upper limits for the stimulation parameters are presently predicated on existing commercial ECT devices, with an energy limit of 100 J per pulse train on an assumed patient load of 220 ohms. The MIST hardware is controlled by real-time software written in LabVIEW (National Instruments) that allows user-defined stimulation protocols, including conventional ECT and more complex protocols. Additionally, MIST current pulse waveforms are better formed than those of existing ECT devices: amplitudes are more stable and accurate during the on-phases, and edges are sharper and cleaner during transition-phases. This waveform integrity is a significant improvement in stimulation dosage consistency.

Conclusions: By utilizing up to 16 head-mounted electrodes and a variety of stimulus pulse sequences, the MIST system can provide more targeted, and patient-specific stimulation. Additionally, the MIST system can be used for targeting distinct brain circuits associated with various psychiatric indications which may involve different patterns of aberrant circuitry, necessitating tailored stimulation approaches. The innovative MIST system facilitates clinical studies that enhance understanding of the mechanisms underlying seizure therapy for depression.

Keywords: electroconvulsive therapy, Neurotechnology, Depression

Disclosure: Nothing to disclose.

P225. The Role of Immunometabolic Dysregulation in the Link Between Depression and Obesity

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Background: Major depressive disorder (MDD) and obesity (OB) are two highly prevalent health issues that often co-occur and substantially contribute to morbidity and mortality worldwide. Comorbid patients with MDD and OB might be particularly prone to treatment resistance and have an elevated risk of developing serious medical illnesses and functional disabilities. Although the biological mechanisms that drive the link between MDD and OB are not fully understood, converging evidence suggests that both conditions might share dysregulation of immunometabolic pathways. However, prior studies have primarily examined these conditions separately, leaving gaps in our understanding of their combined effects on immunometabolic dynamics.

Methods: This ongoing cross-sectional study examines immunometabolic dysregulation in 4 groups: 1) non-obese MDD patients (MDD+/OB-); 2) non-depressed OB patients (MDD-/OB+); 3) comorbid patients with MDD and OB (MDD+/OB+); and 4) non-depressed and lean healthy controls (MDD-/OB-). Participants underwent a clinical interview and blood tests to evaluate systemic (i.e., C-reactive protein (CRP), interleukin (IL)-6, tumor necrosis factor (TNF)- α , glucose, hemoglobin A1c (HbA1c), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol), cellular (i.e., absolute number of non-classical monocytes) and molecular (i.e., monocytic mRNA expression of IL-6, TNF- α , lysosomal acid lipase) immunometabolic markers. Subjects aged 18-65 years were recruited from the Department of Psychiatry and the Division of Psychosomatic Medicine at

Charité – Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany. MDD was diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5); obesity was determined by a body mass index ≥ 30 kg/m². The main exclusion criteria were bipolar affective disorder, schizoaffective disorder, schizophrenia, cognitive impairment, substance disorder, acute and chronic medical illnesses (besides obesity), pregnancy, and postpartum period. Group differences were tested with ANOVA, followed up by post-hoc Dunnett's test, when applicable.

Results: As of August 2024, a total of 154 subjects (mean age = 39.1 years, 68% female) have completed the study, with group sizes as follows: MDD + /OB- (n = 40), MDD/OB+ (n = 34), MDD + /OB+ (n = 40), and MDD-/OB- (n = 40). The groups showed no significant differences in sociodemographic parameters, including sex, age, and smoking status. As hypothesized, analysis of systemic immunometabolic markers revealed significant group differences in CRP, IL-6, TNF- α , glucose and HDL levels. Post-hoc tests confirmed that, compared to non-depressed and lean healthy controls (MDD-/OB-), immunometabolic dysregulation was most pronounced in the comorbid group (MDD + /OB +), as evidenced by higher levels of CRP, IL-6, TNF- α and glucose as well as lower levels of HDL. However, there were no significant group differences in LDL and HbA1c levels.

Conclusions: Our findings suggest that immune and metabolic alterations may be particularly pronounced in comorbid patients with MDD and OB. This immunometabolic dysregulation in comorbid patients may be a potential target for pharmacological interventions aimed at reducing inflammation and metabolic dysregulation in addition to traditional antidepressant treatments. Whether immunometabolic dysfunction is linked to clinical outcomes such as treatment resistance or functional decline remains to be determined in longitudinal studies. Our ongoing further analyses of cellular and molecular markers will provide additional insights into the complex interplay between MDD, OB and immunometabolic functions.

Keywords: Depression, Obesity, Immunometabolism

Disclosure: Nothing to disclose.

P226. Towards a Person-Centered Approach to Reconceptualizing Mood Disorders Using Latent Profile Analysis of Symptom Dimensions

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Background: Three nosologies -- the DSM-5,(1,2), the Hierarchical Taxonomy of Psychopathology (HiToP) (3) and Research Domain Criteria (RDoC) (4,5) -- all have embraced dimensional models of psychopathology. However, mood disorders require attention to multiple dimensions. Depression involves both negative valence and low levels of positive valence, (6-8) whereas classic manic states involve the opposite (low negative valence and high positive valence), and mixed states involve simultaneous elevations of both. (9) DSM and ICD(10) classify mood disorders using lifetime history of these episodes. However, thresholds separating hypomania, mania, depression, and mixed states have been guided more by convention (11) than data. Latent profile analysis (LPA) offers an empirical framework to use dimensional mood scores to identify clusters of youth with similar current mood states. (12) Modeling both positive and negative valenced scores should identify groups with distinct profiles, including classically depressed, classically hypomanic/manic, and various mixed mood state profiles. This project applies LPA to a well-validated measure with broad coverage of both mood dimensions, comparing

classification consistency against psychiatric diagnoses and mood states.

Methods: Secondary analysis of semi-structured interview ratings of N = 817 youths age 5-18 years presenting for outpatient mental health services in Ohio. (13) Consensus diagnoses¹⁴ combined KSADS interviews with family psychiatric history and prior treatment history. LPA used 20 facets from the Parent General Behavior Inventory (15) with two continuous factors to cover manic and depressive symptoms, implemented in Latent Gold. Clusters were compared to DSM diagnoses and current mood state using accepted CDRS-R (16) and YMRS (17) thresholds.

Results: Model comparison statistics (BIC, entropy, minimum cluster size) indicated a 9-profile solution (n = 40 to 158). Clusters were strongly associated with both consensus diagnoses, X²(32 df)=251.57, and with interview ratings of mood state, X²(32 df)=230.96, p < .000005.

Conclusions: When both positive (manic) and negative (depressive) symptom parcels were used in LPA, clusters emerged that showed distinct profiles combined with strong associations with research interview ratings of mood states and diagnoses. If these replicate, they provide an empirical approach to classification, potentially improving early detection of mood disorder types. Future work should test how profile definitions using items from short forms, instead of parcels, calibrate against other definitions, and also examine longitudinal patterns of profiles using growth mixture modeling. (18)

Keywords: Depressive Mood, evidence-base assessment, psychiatric measurement

Disclosure: Nothing to disclose.

P227. AMPA Receptors Modulate Increased Dopamine Neuronal Activity Induced by the Negative Allosteric Modulator of GABAA Receptor L655,708

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Background: There has been a push for the development of novel fast-acting medications to treat major depressive episodes, primarily after the advent of ketamine shown to have a rapid antidepressant effect in the clinic. Presumably by blocking NMDA receptors on gamma-aminobutyric acid (GABA) interneurons in the prefrontal cortex (PFC), ketamine indirectly increases glutamatergic AMPA receptor throughput.

Negative allosteric modulators of GABAA (GABA-NAMs) receptors bind with high affinity at their $\alpha 5$ subunit located primarily on glutamatergic pyramidal neurons. By acting directly on these neurons, they induce their disinhibition thereby promoting enhanced excitatory glutamatergic transmission. Interestingly, the $\alpha 5$ -preferring GABA-NAM L655,708 has been shown to exert rapid and sustained antidepressant-like action in rats subjected to chronic stress and displaying anhedonia.

While some studies have investigated the behavioral effects of GABA-NAMs, limited work has been done to study their functional activity. It is hypothesized that crosstalk between glutamatergic and monoaminergic systems are important in the antidepressant-like response and that differential modulation of these systems by the negative allosteric modulator of GABAA receptor L655,708 underlie induction and maintenance of rapid and sustained antidepressant response.

Methods: Experiments were performed in male Sprague-Dawley rats. Animals were anesthetized with chloral hydrate and mounted on a stereotaxic frame. *in vivo* electrophysiological recordings of 5-HT, NE and DA neuronal activity were carried out, in the dorsal raphe nucleus (DRN), locus coeruleus (LC) and ventral

tegmental area (VTA), respectively, within 2 hours after a single administration of vehicle or L655,708 (3 mg/kg; intraperitoneal) in at least 5 rats. Neuronal activity was also determined 24 hours after injection of L655,708 for 5-HT and DA neurons, the latter was also assessed after one week post-administration. Neuronal firing was analyzed for changes in spike frequency and burst activity as well as the number of spontaneously active neurons per track in the VTA (population activity). Statistical comparisons were done using two-tailed *t* test.

Results: A single administration of L655,708 30 minutes prior to electrophysiological recordings did not significantly alter 5-HT (Vehicle: 1.2 ± 0.2 Hz; L655,708: 1.8 ± 0.3 Hz), NE (Vehicle: 2.2 ± 0.2 Hz; L655,708: 2.5 ± 0.4 Hz) and DA neuronal firing (Vehicle: 3.7 ± 0.1 Hz; L655,708: 4.1 ± 0.7 Hz). When assessed 24 hours following injection of L655,708, DA neuron burst activity determined as percentage of spikes occurring in burst was not significantly altered by L655,708 (Vehicle: $21\% \pm 4$; L655,708: $31\% \pm 3$). However, there was a significant 3-fold enhancement in the number of spontaneously active DA neurons compared to vehicle administration (Vehicle: 1.3 ± 0.1 Hz; L655,708: 3.3 ± 0.1 , $p < 0.001$). In addition, their firing rate, was also significantly increased (Vehicle: 3.6 ± 0.1 Hz; L655,708: 4.5 ± 0.1 Hz; $p = 0.006$). This increase in population activity and firing rate was abolished after the administration of flumazenil, a competitive GABAA receptor antagonist. Additionally, a similar attenuating effect took place after the administration of the AMPA receptor antagonist NBQX. Strikingly, a sustained increase in the number of spontaneously active DA neurons was still present one week following injection of L655,708 (Vehicle: 1.0 ± 0.1 ; L655,708: 2.4 ± 0.2 ; $p = 0.002$). However, no significant change in their firing rate was observed (Vehicle: 3.7 ± 0.2 Hz; L655,708: 5.3 ± 0.6 Hz). For the DRN, no significant change in firing rate or burst activity was observed 24 hours following injection of L655,708.

Conclusions: L655,708 caused a significant increase in the population activity of DA neurons 24 hours and one week after injection but not after 30 minutes. This result is in accordance with data showing that this drug produced an antidepressant-like effect in the FST at one-day and one week post-treatment, but not acutely [1]. The reversal of these effects with the administration of NBQX and flumazenil suggests, respectively, that L-655-708 exerts this effect by modulating AMPA receptors and through action on the benzodiazepine site of the GABAA receptor. Since the $\alpha 5$ subunit of latter receptor was shown to be located only in PFC/ventral hippocampus, these effects could be a consequence of an indirect effect of L655,708 on DA neurons through these two forebrain regions, via $\alpha 5$ subunit of GABAA receptors controlling glutamatergic afferents that regulate neuronal activity of monoaminergic neurons. Ketamine and L655,708 can enhance the population activity of DA neuron, but ketamine does it acutely and it dissipates after 24 hours, whereas that of L655,708 becomes apparent only 24 hours. In contrast, the enhancing effect of L655,708 is sustained for one week with a single injection, whereas this action of ketamine requires multiple injections and lasts only for 3 days [2,3]. In summary, these results support the possibility that GABA NAMs could exert rapid and sustained antidepressant activity, through a modulation of DA neuronal activity by AMPA receptors.

Keywords: Negative allosteric modulator of GABAA receptor, Dopamine, Norepinephrine, Serotonin, depression

Disclosure: Nothing to disclose.

P228. Dorsal Attention – Salience Network Coupling Moderates the Impact of Drug-Assignment Beliefs on Mood Improvement

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Background: Treatment expectancies play a key role in the response to antidepressants. Despite the well-established, strong antidepressant placebo effect, little is understood of the neural mechanisms. Our recent findings suggest that during placebo administration, greater attention processing and salience attribution to contextual cues (e.g., placebo) are encoded in the dorsal attention network (DAN) and the salience network (SN), respectively, whereas internally focused mood regulation and perception are encoded in the default model network (DMN). If confirmed, enhanced connectivity between the DAN-SN-DMN facilitates the switch from greater DAN-attention and SN-salience attribution to contextual cues to DMN-mediated mood regulation, contributing to the formation of attention-expectancy-mood interactions that lead to antidepressant placebo effects. However, the extent to which these neural effects persist at rest, even after placebo administration, and predict long-term belief-mediated mood responses remains unknown. To test this hypothesis, we examined whether baseline resting state functional connectivity (rsFC) after an acute experimental manipulation of antidepressant placebo effects predict long-term placebo responses.

Methods: Fifty-four psychotropic-free patients with major depressive disorder underwent an 8-week double-blind, randomized, placebo-controlled trial of a selective serotonin reuptake inhibitor ($n = 25$) or placebo ($n = 29$). Participants completed baseline structural and resting-state functional MRI. Depressive symptoms were assessed weekly with the Quick Inventory of Depressive Symptomatology (QIDS) as well as the patient's drug-assignment belief. Resting state scans were processed with standard modules in SPM. Whole-brain rsFC was calculated for 7 predefined regions of interest, and network-to-network connectivity was calculated for the Yeo definitions of DMN, SN, and DAN. We employed *t*-tests and mixed effects models to assess the effects of drug/placebo assignment, assignment belief, and neuroimaging measures on depressive symptoms over 8 weeks. Region-to-voxel analyses employed the Statistical non-Parametric Mapping toolbox for multiple comparisons correction (10,000 permutations, cluster-forming threshold of $p = 0.001$, cluster-wise inference to control family-wise error at $\alpha = 0.05$).

Results: 43 participants with high-quality resting state scans (22 escitalopram, 21 placebo) were included in the analysis. In the drug-assigned group, 13 ended the study with a drug-assignment belief and 9 with a placebo-assignment belief; in the placebo-assigned group, 11 ended the study with a drug-assignment belief and 9 with a placebo-assignment belief (and 1 missing belief measurement). Assigned groups did not differ on baseline QIDS ($t_{41} = 0.75$, $p = 0.459$), total change in QIDS ($t_{42} = -0.80$, $p = 0.427$), or QIDS trajectory ($t = 0.06$, $p = 0.952$), but those who ended the study believing they were on the drug showed a greater reduction in QIDS trajectory ($t = 2.18$, $p = 0.029$); however this effect was not significant after controlling for baseline QIDS ($t = 1.29$, $p = 0.196$). Assigned groups did not differ on baseline rsFC for any region across all networks of interest, but the drug-assignment belief group had lower bilateral insula to premotor rsFC than the placebo-assignment belief group. Further, the relationship between SN-DAN rsFC and QIDS was moderated by assignment belief, both measuring week-by-week belief and final belief. Subjects who believed they were receiving an antidepressant had a negative association between baseline SN-DAN rsFC and overall QIDS while the placebo-assignment belief group had a positive association ($t = 2.07$, $p = 0.039$); for final belief, this effect is driven by the drug-assignment group ($t = -1.98$, $p = 0.048$). Additionally, lower DMN-SN rsFC in the placebo group was associated with a greater reduction in QIDS over time ($t = 2.00$, $p = 0.046$).

Conclusions: These findings underscore the pivotal role of DAN-SN coupling in shaping antidepressant placebo effects, serving as a moderator for the impact of drug-assignment beliefs on mood improvement, both acutely and over time. Greater DAN-SN coupling after placebo administration may contribute to the encoding of

antidepressant expectancies through increased attention to the treatment cue, contributing to overall mood improvement.

Keywords: Depression, Placebo Response, Randomized-Controlled Trial, Resting State Functional Connectivity

Disclosure: Nothing to disclose.

P229. Neural Correlates of an Eeg Biomarker Predictive of the Antidepressant Response to ALTO-300 in Patients With Major Depression

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Background: Major Depressive Disorder (MDD) is a complex and heterogeneous condition. Despite this heterogeneity, treatment selection is not tailored to individual characteristics, leading to modest treatment efficacy. Identifying specific sub-population for targeted treatment through objective biomarkers may enhance treatment outcomes for patients. Previously, we demonstrated the benefit of this approach by identifying, and prospectively replicating, a machine learning-derived biomarker based on resting-state electroencephalography (rsEEG), in response to the ALTO-300 treatment. This biomarker indexes the predictability or irregularity of an EEG time series. Here, we sought to better understand the functional relevance of this biomarker. We hypothesized that irregularity of rsEEG could reflect changes in underlying neural network dynamics, as measured through EEG functional connectivity. Specifically, we proposed that more connected networks would exhibit higher synchronous activity and thereby higher regularity. Thus, we explored the relationship between the biomarker and functional connectivity in rsEEG.

Methods: Pre-treatment EEG data from four independent datasets involving MDD patients were used to check for consistency of findings (ALTO-300: N = 123, BEAM: N = 867, iSPOT-D: N = 726, ALTO-100: N = 205). The rsEEG features were calculated from 19 channels during the eyes-closed condition (2–8-minute recordings), corresponding to the condition used to calculate the biomarker. Functional connectivity was calculated at the sensor level using orthogonalized power envelope connectivity (PEC) method from the channel used to calculate the biomarker to all other channels. For each of these connectivity features, the partial correlation of the feature with the ALTO-300 biomarker, controlling for age and sex, is reported. The same analysis was repeated in the cortical space in the BEAM dataset, which had a denser montage (64 channel), to spatially localize the connectivity relationships.

Results: The sensor-level connectivity analysis revealed that the strongest correlation was centered around the midline frontal Fz channel across all four datasets in the expected direction (higher irregularity predicts lower connectivity). Additionally, datasets with larger sample sizes exhibited stronger correlations. The partial correlation of Fz PEC to the biomarker in different datasets are as follows: ALTO-300 ($r = -0.42$, $p = 1.9e-6$), BEAM ($r = -0.49$, $p = 2.8e-54$), iSPOT-D: ($r = -0.57$, $p = 3.6e-63$), ALTO-100 ($r = -0.18$, $p = 0.009$). Source analysis further revealed that the strongest correlation with the biomarker was in the connectivity with the medial prefrontal cortex, consistent with the scalp location of the Fz electrode.

Conclusions: Medial prefrontal connectivity at the sensor and/or source levels is inversely correlated with the biomarker across all the datasets tested, reflecting high consistency in the findings, and generalizing across EEG hardware. These results provide brain network functional insights into the meaning of the ALTO-300

predictive EEG biomarker currently being used for patient selection in an ongoing large Phase 2b study (NCT05922878).

Keywords: MDD, precision psychiatry, EEG, Biomarker

Disclosure: Alto Neuroscience: Employee (Self)

P230. Safety and Tolerability of Lumateperone 42 Mg for the Treatment of Major Depressive Disorder: A Pooled Analysis of 2 Randomized Placebo-Controlled Trials

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Background: Major depressive disorder (MDD) is common, complex, and disabling. Current treatment options have tolerability concerns that affect medication adherence (e.g. weight gain, metabolic disturbances, and sexual dysfunction). Lumateperone is an FDA-approved antipsychotic indicated to treat schizophrenia and depressive episodes associated with bipolar I or bipolar II disorder. The efficacy and safety of lumateperone 42 mg adjunctive to antidepressant therapy (ADT) was demonstrated in two Phase 3, randomized, double-blind, placebo-controlled studies (Study 501 [NCT04985942]; Study 502 [NCT05061706]) in patients with MDD with inadequate ADT response. A pooled analysis of these studies evaluated the safety and tolerability of lumateperone 42 mg adjunctive to ADT.

Methods: Data were pooled from the 2 studies that enrolled males and females (18–65 years) who met DSM-5 criteria for MDD with inadequate response to 1 to 2 courses of prior ADT ($< 50\%$ improvement on the Antidepressant Treatment Response Questionnaire). Eligible patients had Montgomery-Asberg Depression Rating Scale (MADRS) Total score ≥ 24 , Clinical Global Impression Scale-Severity (CGI-S) score ≥ 4 , and Quick Inventory of Depressive Symptomatology-Self Report-16 item (QIDS-SR-16) score ≥ 14 . Patients were randomized 1:1 to 6-week oral placebo or lumateperone 42 mg adjunctive treatment to ADT. Safety assessments including adverse events (AEs), vital signs, laboratory parameters, and extrapyramidal symptoms (EPS) were analyzed descriptively.

Results: The safety population comprised 964 patients (placebo, 481; lumateperone, 483) and 91.4% completed treatment. Treatment-emergent AEs (TEAEs) occurred in 45.1% of patients in the adjunctive placebo group and 68.1% in the adjunctive lumateperone group. TEAEs that occurred in $\geq 5\%$ of patients receiving adjunctive lumateperone at twice adjunctive placebo rate were dizziness, dry mouth, somnolence, nausea, and fatigue. Serious TEAEs were rare, occurring in 1 patient per group and not considered related to treatment (placebo, joint dislocation; lumateperone, polypectomy). Rates of AEs leading to treatment discontinuation were 0.8% (placebo) and 8.7% (lumateperone). No patients died during the study.

Mean changes from baseline to end of treatment were minimal and similar for body mass index (both groups 0.0 kg/m²), waist circumference (placebo, -0.3 cm; lumateperone, -0.2 cm), and weight (placebo, 0.0 kg; lumateperone, -0.1 kg). Potentially clinically significant ($\geq 7\%$) weight gain occurred in 1.3% of the placebo + ADT group and 0.4% of the lumateperone + ADT group. There were no clinically relevant increases at Day 43 in prolactin or metabolic parameters in the adjunctive lumateperone group. There were no notable changes in extrapyramidal symptoms as assessed by the Abnormal Involuntary Movement Scale, Barnes Akathisia Rating Scale, and Simpson-Angus Scale.

Conclusions: In this pooled analysis of 2 trials, lumateperone 42 mg adjunctive to ADT was generally safe with minimal cardiometabolic, prolactin, and EPS risks. These results suggest

that lumateperone may be a well-tolerated treatment option for patients with MDD with inadequate ADT response.

Keywords: major depressive disorder, lumateperone, adjunctive treatment, clinical trial, safety

Disclosure: Intra-Cellular Therapies, Inc.: Employee, Stock / Equity - Publicly Traded Company (Self)

P231. iPSC-Derived Neurons From Treatment-Resistant Depression Display Time-Dependent Partially Overlapping Responses to (2R,6R)-HNK and Psychedelics

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Background: A key focus in neuropsychiatric research is developing therapies for treatment-resistant depression (TRD), which is typically characterized by a lack of adequate response to one or more classes of antidepressants (Fava et al., 2003). Replicating TRD in animal models is particularly challenging, which has led to a shortage of effective antidepressant therapies developed in recent years (Planchet et al., 2019). Innovative in vitro models, such as neurons derived from induced pluripotent stem cells (iPSCs) of individuals with the condition of interest, may enhance our ability to model molecular diagnostic differences and predict treatment response. While iPSC-models have captured interest in other neuropsychiatric disorders, there is a striking paucity of research which utilizes this in vitro approach to investigate the effects of novel therapeutics in depression (McNeill et al., 2020). With the discovery of subanesthetic ketamine as a rapid-acting antidepressant, a surge in research has followed to discover both its mechanism of action and other similar therapeutics. These include ketamine's own metabolite (2R,6R)-hydroxynorketamine (HNK) and serotonergic psychedelics (Johnston et al., 2023). The aim of this exploratory study was to assess the response of iPSC-derived neurons from a TRD population to potential fast-acting antidepressants, as well as their inherent differences to healthy control cell lines.

Methods: iPSCs were reprogrammed through typical Sendai virus protocols at the NIH iPSC core from healthy control (HC) fibroblasts ($n = 5$) and peripheral mononuclear blood cells of TRD participants enrolled in clinical trial at the NIH clinical center. All subjects were female. Following STEMCellITM protocols, cells were differentiated to primary cortical neurons over 10 weeks. TRD samples were treated with (2R,6R)-HNK (1uM), lysergic acid diethylamide (LSD) (10uM), and Psilocybin (10uM) for 6 and 24 hours. After respective treatment, neurons were collected for RNA-sequencing. VariancePartition was used to define variables to be included as fixed (diagnosis, time, and treatment) or random effects (individual and batch). Analysis of the RNA counts was processed with dream, a modified version of limma/voom analysis for assessing drug-treatment effects (Hoffman et al., 2021). Hallmark gene sets from the bulk RNA-sequencing dataset were annotated through Zenith and corrected for multiple comparisons.

Results: iPSC-derived neurons from TRD participants had many significantly decreased pathways in comparison to healthy controls, including mTORC1 ($FDR < 0.001$) and PI3K-Akt-mTOR signaling ($FDR = 0.007$), as well as protein secretion ($FDR < 0.001$), glycolysis ($FDR = 0.002$), and adipogenesis ($FDR = 0.003$). In comparison to vehicle (acetonitrile) at 6 hours, (2R,6R)-HNK had a significant decrease in the hedgehog signaling pathway ($FDR = 0.036$) and non-significant increases in the interferon alpha and gamma responses. Psilocybin increased epithelial mesenchymal transition ($FDR = 0.003$), E2F targets ($FDR = 0.003$), and G2M

checkpoint signaling ($FDR = 0.039$). LSD significantly decreased MYC targets ($FDR < 0.001$), and increased interferon alpha ($FDR = 0.014$) and gamma ($FDR = 0.019$) responses.

At 24 hours, (2R,6R)-HNK increased mTORC1 ($FDR = 0.001$) and PI3K-Akt-mTOR signaling ($FDR = 0.041$) while decreasing E2F targets ($FDR = 0.001$) and G2M checkpoint signals ($FDR = 0.001$). Similarly, psilocybin increased mTORC1 signaling ($FDR < 0.001$), cholesterol homeostasis ($FDR < 0.001$), and adipogenesis ($FDR < 0.001$) and decreased G2M checkpoint signals ($FDR < 0.001$). Interestingly, it still had indicators of increased inflammatory signaling through interferon alpha responses ($FDR < 0.001$) and TNF-alpha signaling ($FDR < 0.001$). Finally, LSD increased mTORC1 signaling ($FDR < 0.001$), cholesterol homeostasis ($FDR < 0.001$), adipogenesis ($FDR < 0.001$), and protein secretion ($FDR < 0.001$) and other metabolism-related pathways.

Conclusions: At 6 hours, the hallmark gene set patterns suggested an increase in inflammatory response and cell survival mechanisms. However, pathways affected at 24 hours were more indicative of increases in protein translation and excitatory synaptic signaling. In addition, the hallmark pathways more closely reflected those of healthy control cell lines, suggesting a potential return to function. These mechanisms provide further insight into how potential rapid-acting antidepressants may affect cellular and synaptic changes to improve depressive symptomatology. The challenges in translating animal and in vitro models of depression to human conditions have hindered successful drug discovery. However, neurons derived from iPSCs of TRD participants exhibit similar treatment-response to those previously recorded in animal models and human patients, including upregulations in mTORC1 signaling in a time-dependent manner (Aleksandrova and Phillips, 2021). This similarity could offer deeper insights into the mechanisms of fast-acting antidepressants, enhancing the relevance of in vitro models and advancing future drug discovery efforts. Future research should include the expansion of further cell lines, functional assays such as electrophysiology, and comparisons between actual clinical response to changes observed in the cell lines.

Keywords: Depression, Induced pluripotent stem cells (iPSCs), (2R,6R)-hydroxynorketamine, Psychedelics

Disclosure: Nothing to disclose.

P232. Adjunctive Lumateperone in Patients With Major Depressive Disorder: Results From an Additional Randomized, Double-Blind, Phase 3 Trial

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Background: Lumateperone is an FDA-approved antipsychotic for schizophrenia and bipolar depression. This Phase 3, randomized, double-blind, placebo-controlled, multicenter trial (NCT05061706) investigated adjunctive lumateperone 42 mg in patients with major depressive disorder (MDD) with inadequate antidepressant therapy (ADT) response.

Methods: Eligible male and female outpatients (18-65 years old) met DSM-5 criteria for MDD with inadequate response to 1-2 prior ADT courses ($< 50\%$ improvement on the Antidepressant Treatment Response Questionnaire) and had Montgomery-Asberg Depression Rating Scale (MADRS) Total score ≥ 24 , Clinical Global Impression Scale-Severity (CGI-S) score ≥ 4 , and Quick Inventory of Depressive Symptomatology-Self Report-16 item (QIDS-SR-16) score ≥ 14 . Patients were randomized to 6-week placebo or lumateperone 42 mg adjunctive treatment to ADT. Primary and key secondary 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. Additional efficacy measures included response ($\geq 50\%$ MADRS Total score decrease) and remission (MADRS Total score ≤ 10), based on a logistic regression model. Change from baseline in QIDS-SR-16 Total score was evaluated using an analysis of covariance. Safety assessments included adverse events (AEs), vital signs, laboratory parameters, and extrapyramidal symptoms.

Results: Of 480 patients (placebo, 238; lumateperone, 242), 89% completed treatment. The mean age was 46 years, 70% were women, and 95% were White. Primary and key secondary endpoints were met with significant improvement for adjunctive lumateperone vs placebo from baseline to Day 43 in MADRS Total score (least squares mean difference vs placebo [LSMD] = -4.5 ; effect size [ES] = -0.56 ; $P < .0001$) and CGI-S (LSMD = -0.5 ; ES = -0.51 ; $P < .0001$). Rates of MADRS Total score response (placebo, 25%; lumateperone, 40%; $P < .01$) and remission (placebo, 14%; lumateperone, 25%; $P < .01$) were significantly greater with lumateperone vs placebo at Day 43. Adjunctive lumateperone significantly improved self-reported depressive symptoms at Day 43 vs placebo, as measured by QIDS-SR-16 Total score (LSMD = -2.2 ; $P < .0001$). Adjunctive lumateperone was relatively well tolerated, consistent with prior studies. The most common treatment-emergent AEs with lumateperone ($\geq 5\%$ and twice placebo) were dizziness, somnolence, dry mouth, nausea, diarrhea, and fatigue. AEs were mostly mild or moderate.

Conclusions: Lumateperone 42 mg adjunctive to ADT demonstrated robust, clinically meaningful efficacy over adjunctive placebo to ADT and was generally safe and well tolerated, indicating lumateperone is a promising treatment as adjunctive therapy to ADT to treat MDD in adults.

Keywords: major depressive disorder, lumateperone, adjunctive treatment, clinical trial

Disclosure: Intra-Cellular Therapies, Inc.: Employee, Stock / Equity - Publicly Traded Company (Self)

P233. Depression Severity and Efficacy Outcomes: Post Hoc Analyses From a Phase 3 Trial of the Novel NMDAR Antagonist Antidepressant, Esmethadone, for the Adjunctive Treatment for MDD With Inadequate Response to Standard Antidepressants

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Background: Major depressive disorder (MDD) trials are rendered complex by the lack of objective diagnostic tests to confirm the diagnosis of MDD. Depression severity may enhance diagnostic accuracy. The Montgomery-Åsberg Depression Rating Scale (MADRS) is a 10-item diagnostic questionnaire used to measure the severity of depressive episodes; scores of 35 or above indicate severe depression. REL-1017 (esmethadone) is a novel N-methyl-D-aspartate (NMDA) receptor uncompetitive antagonist under development for the adjunctive treatment of MDD in patients with inadequate response to standard antidepressants. It is hypothesized that esmethadone may improve MDD by restoring impaired neural plasticity caused by tonic NMDAR hyperactivation. In a recently completed Phase 3 study (NCT04688164), esmethadone did not meet the primary efficacy endpoint for the difference between esmethadone and placebo at Day 28 for mean change from baseline in MADRS10: Intent-to-Treat (ITT) analysis ($n = 227$): mean difference 2.3; $p = 0.154$, effect size = 0.21; Per protocol (PP)

analysis ($n = 198$): mean difference 3.1; $p = 0.051$, effect size = 0.29. In the same study, post-hoc analyses in the subgroup of patients with severe depression (baseline MADRS ≥ 35) showed statistically significant efficacy and a robust effect size*. Sensitivity analyses may enhance the reliability of post-hoc primary analyses by showing that the results are consistent across multiple severity cut off points.

Methods: We performed sensitivity analyses at different severity cut off points (31, 32, 33, 34, 35, 36, 37) for the ITT and the PP populations. We modeled the change from baseline individually in each treatment arm by smoothing splines of the MADRS baseline value using all data.

Results: MADRS ≥ 31 , ITT ($n = 181$): MD 3.5; $p = 0.0587$, ES = 0.31

MADRS ≥ 31 , PP ($n = 157$): MD 4.5; $p = 0.0165$, ES = 0.40

MADRS ≥ 32 , ITT ($n = 169$): MD 3.9; $p = 0.0467$, ES = 0.33

MADRS ≥ 32 , PP ($n = 145$): MD 4.9; $p = 0.0119$, ES = 0.44

MADRS ≥ 33 , ITT ($n = 156$): MD 4.8; $p = 0.0188$, ES = 0.41

MADRS ≥ 33 , PP ($n = 133$): MD 6.0; $p = 0.0034$, ES = 0.53

MADRS ≥ 34 , ITT ($n = 135$): MD 5.3; $p = 0.0184$, ES = 0.44

MADRS ≥ 34 , PP ($n = 117$): MD 6.3; $p = 0.0049$, ES = 0.55

*MADRS ≥ 35 , ITT ($n = 112$): MD 6.9; $p = 0.006$, ES = 0.57

MADRS ≥ 35 , PP ($n = 98$): MD 7.9; $p = 0.0015$, ES = 0.68

MADRS ≥ 36 , ITT ($n = 96$): MD 6.7; $p = 0.0123$, ES = 0.56

MADRS ≥ 36 , PP ($n = 83$): MD 7.8; $p = 0.0029$, ES = 0.69

MADRS ≥ 37 , ITT ($n = 75$): MD 7.9; $p = 0.0117$, ES = 0.65

MADRS ≥ 37 , PP ($n = 63$): MD 8.8; $p = 0.0051$, ES = 0.75

Results (b):

The mean change from baseline for MADRS in esmethadone and placebo arms was stable at around -12 to -15 points in both arms up to a baseline MADRS score of 35. Above this value, the curves separated. In the placebo arm, change from baseline for MADRS remained stable at a value of -12. In the esmethadone arm, a linear decrease in change from baseline for MADRS change was observed from -12 at a MADRS baseline score of 35 to -30 at a MADRS baseline score of 45.

Conclusions: The previously reported results achieved with esmethadone in patients with baseline MADRS ≥ 35 in the ITT and PP populations are supported by these results and overall trend obtained at different severity cutoff points (MADRS 31, 32, 33, 34, 36 and 37) and by the smoothing spline analysis modeling the change from baseline individually in each treatment arm. These sensitivity analyses support the reliability of the results in severely depressed patients at the clinically meaningful post-hoc severe depression cut off point (baseline MADRS ≥ 35). We hypothesize that patients with severe depression may suffer from a different pathophysiology compared to patients with milder depression and may therefore uniquely respond to uncompetitive NMDAR antagonists targeting restoration of impaired neural plasticity. A complementary hypothesis is that the post-hoc severity analysis using baseline MADRS ≥ 35 enhances MDD diagnostic accuracy by potentially removing from the analysis patients with transient reactive depression mimicking MDD and "professional patients" without MDD that simulate MDD symptoms, in order to enter the trial.

Keywords: Major Depression Disorder, Antidepressants, NMDA receptor antagonist

Disclosure: Relmada Therapeutics: Employee (Self)

P234. Precision Psychiatry Using Human Induced Pluripotent Stem Cell (hiPSC) Derived Neurons: Assessing Synaptic Connectivity as a Biomarker for Drug Response

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Background: The treatment of major depressive disorder (MDD) remains a significant clinical challenge due to the variability in patient responses to antidepressant medications. Current methods of selecting antidepressants are largely trial-and-error, leading to prolonged periods of ineffective treatment. To address this issue, we have developed a model using human induced pluripotent stem cell (hiPSC) derived neurons to predict patient-specific antidepressant efficacy.

Methods: Patients suffering from Major Depressive Disorder were assessed according to Quick Inventory of Depressive Symptomatology (QIDS) as part of the STAR*D clinical trial. Patients selected for assessment were balanced for gender. Patients with greater than a 50% reduction in QIDS score were considered to be responders, and those with lower were non-responders. Samples from patients were converted into hiPSC using Sendai Virus and differentiated into cortical neurons. These neurons were treated with commonly prescribed antidepressants. Synaptic connectivity was assessed by confocal microscopy. Key neuronal plasticity markers, such as synaptic density, dendritic branching, and synaptic protein levels, were measured to determine the overall synaptic connectivity.

Results: Cortical neurons were generated from MDD patients, confirmed by FACS staining and immunohistochemistry. MDD responders versus MDD non-responders were compared, demonstrating significant changes in synaptic plasticity in response to antidepressants in patient-derived neurons derived from responder versus non-responder patients. Specifically, pre and post-synaptic connectivity were addressed using analysis of PSD95 and synapsin, spine morphology and dendritic branching. Features were identified that correlate QIDS response with improved synaptic connectivity. These biomarkers provide a quantifiable metric for predicting patient-specific responses to antidepressants.

Conclusions: This study highlights the potential of hiPSC-derived neurons as a tool to match optimal treatment for antidepressant selection. By utilizing synaptic connectivity as a biomarker, our model offers a promising approach to tailor antidepressant treatment to individual patients, potentially reducing the trial-and-error period and improving clinical outcomes. Future directions include expanding this model to include a broader range of antidepressants and refining the biomarkers for even more precise predictions.

Keywords: Anxiety and Depression, hiPSCs, Pharmacogenomic-guided treatment recommendations

Disclosure: NeuroKaire: Founder (Self)

P235. Spatially-Resolved Multiomic Profiling of the Human Hippocampus to Investigate Subfield and Cell-Type Specific Gene Regulatory Disruptions in Major Depressive Disorder

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Background: Major depressive disorder (MDD) presents with high risk of suicide, great social/economic burden, and poor treatment response. Cumulative research on the hippocampus (HPC) in MDD point to structural atrophy and functional connectivity alterations that are either the result of MDD or integral to its etiology. Disrupted neurogenesis, increased neuroinflammation, cell death, altered synaptic maintenance, and neuroplasticity may be underlying those changes. The link of MDD with recent and early life stress suggests epigenetic mechanisms such as chromatin remodeling which can open/close sites for transcription factor (TF) binding. Previous attempts at molecular profiling have been

limited to bulk tissue sequencing (seq) but recent advances in single-nucleus (sn) technologies allow co-profiling cell type-specific chromatin accessibility (ATAC) and transcriptome (RNA) to identify alterations in MDD. Nevertheless, sn approaches lack cytoplasmic, dendritic, and anatomical information.

Methods: Here we used Chromium Single-Cell Multiome and Visium Spatial Gene Expression assays (10x Genomics) to perform sn RNA and ATAC-seq and capture extranuclear RNA in the context of multicellular anatomical architecture. Hippocampal tissue was obtained from Brain Collections of the New York State Psychiatric Institute and Columbia University and the National Institute for Mental Health. Psychological autopsy and toxicology exams showed donors had died by sudden death with short agonal state, and had no alcohol or substance use disorder, nor history of treatment with psychotropic drugs. We included 27 subjects aged 28 to 74, males and females, 12 neurotypical controls (CTRL) and 15 untreated MDDs (uMDD), males and females. For both the multiome and the Visium assays, we had 2-3 replicates per subject. 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 currently more than 350,000 nuclei (average 6,728 nuclei/sample) and 70,817 spots (average 3,079/sample) retained after quality control. Preprocessing and quality control was performed via Seurat and Signac pipelines. Multiome data was batch corrected and integrated using Harmony followed by Weighted Nearest Neighbor (WNN) analysis. Visium data was batch corrected and integrated using canonical correlation analysis. Cell type clustering of Multiome data, and spatial clustering of Visium data, were both performed with a Louvain clustering algorithm. For both Multiome and Visium, differential expression modeling of uMDD vs. CTRL was performed on pseudo-bulked samples using the limma-voom pipeline with age, RIN, PMI, and batch, (and # spots for Visium) as covariates. TF footprint analysis on the Multiome data was performed following the Tobias pipeline. For Spatially Resolved Transcriptomics (SRT) data analysis and visualization we used the Semla pipeline.

Results: Integrating sn multiomics with SRT revealed both consistent and divergent differentially expressed genes in uMDD vs. CTRL. Consistent differential expression was observed with snRNA-seq data in granule neurons (GNs) and Visium data in the granule cell layer (GCL). Pseudo-bulked GCL Visium data show lower ABCA5, ENOX1, DNAJC15, GFOD1, and PRKCB, and higher GABBR1, CLPSL1, CPNE8, EPCAM, HLA-DQB1 and LAMP5 expression in uMDD, consistent with data in GNs (all adjusted p-values < 0.046). Some of these transcripts (GFOD1, GABBR1, PRKCB) exhibit spatial gradients with RNA trafficking from the GCL to the outer molecular layer. For oligodendrocytes, snRNA-seq showed increased FAM221A in MDD, and in Visium we found this enrichment to be specific to a white matter subcluster. snATAC-seq data showed less chromatin accessibility for HLA-DQB1 in oligodendrocyte precursor cells and more open chromatin for HLA-DQB1 in GNs in MDD. We imputed TF binding sites with footprint analysis either at the genes or their cis regulatory elements for the above genes, suggesting differential binding of the following TFs in MDD: lower SP5 for ABCA5, lower YY1 for PRKCB, higher FOS/JUN complex for HLA-DQB1, lower GF11/GF11B for LAMP5, and higher POU-class TFs for FAM221A.

Conclusions: This study is the first to integrate cell type-specific transcriptomic and epigenomic with spatially resolved transcriptomics data from uMDD and CTRL. Consistency of DEGs in both nuclear and extranuclear datasets suggests differences in MDD that are due to epigenetic or transcriptional regulation, rather than post-transcriptional regulation. DEGs indicate dysfunctions in GN pathways involved in calcium-activity dependent intracellular signaling (PRKCB, CPNE8) and cell adhesion (EPCAM); electron-transport (DNAJC15, GFOD1, ENOX1); GABAergic signaling (GABBR1, LAMP5); lipid metabolism (ABCA5, CLPSL1); and exogenous antigen presentation at the cell surface (HLA-DQB1). Differentially bound TFs have known roles in cellular development and differentiation, while FOS/JUN is known to be an neuronal

activity-regulated TF complex. These findings point to molecular underpinnings of HPC anatomical and functional disruption in MDD and potential treatment targets. More work is needed to confirm MDD molecular profiling by increasing sample size, imputing gene regulatory networks of the above-mentioned TFs, and including female samples in our analyses.

Keywords: Major Depressive Disorder (MDD), Hippocampus, spatial transcriptomics, Transcriptomics, epigenomics

Disclosure: Nothing to disclose.

P236. [11C]SL25.1188 Total Distribution Volume, Index of Monoamine Oxidase B Level and Marker of Astroglialosis, After COVID-19 With Ongoing Depressive and Cognitive Symptoms

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Background: After acute COVID-19, persistent depressive and cognitive symptoms occur in ~5% of cases (termed COVID-DC). Theoretical models of COVID-DC suggest astroglialosis is important but measures primarily indicative of astroglialosis have not been studied in COVID-DC.

Methods: In this case-control study, positron emission tomography (PET) is used to determine if [11C]SL25.1188 total distribution volume ([11C]SL25.1188 VT), an index of monoamine oxidase B (MAO-B) density and marker of astroglialosis is elevated in COVID-DC. [11C]SL25.1188 VT was measured in primary regions of the prefrontal cortex (PFC), anterior cingulate cortex (ACC), hippocampus, dorsal putamen (DP) and ventral striatum (VS) in 21 COVID-DC cases, 21 healthy controls, and 21 additional controls with major depressive episodes of major depressive disorder and no history of COVID-19. Depressive and cognitive symptoms were measured with psychological and neuropsychological tests.

Results: Linear mixed effects model found an effect of group on [11C]SL25.1188 VT with greater [11C]SL25.1188 VT in COVID-DC compared to healthy across prioritized regions ($F_{1,40} = 14.19$; $p = 0.001$). When compared to participants with current MDE and no history of COVID-19, [11C]SL25.1188 VT was greater in COVID-DC in VS ($U = 140$; $p = 0.04$), DP ($U = 123$; $p = 0.01$), and hippocampus ($U = 130$; $p = 0.02$). MDE severity, measured by the Beck Depression Inventory-2 negatively correlated with [11C]SL25.1188 VT in PFC, ACC, DP and VS, $r = -0.55$ ($p = 0.02$) to -0.48 ($p = 0.04$). More recent acute COVID-19 correlated with greater [11C]SL25.1188 VT, $r = 0.61$ ($p = 0.003$) to 0.74 ($p = 0.0001$), reflecting higher values since predominance of the omicron variant.

Conclusions: This is strong evidence that MAO-B labelled astroglialosis occurs throughout grey matter regions in COVID-DC. The inverse relationship between [11C]SL25.1188 VT and symptom severity raises the possibility of a protective role for astroglialosis in COVID-DC, such as the anti-viral role of astroglialosis in combatting infection. Magnitude of astroglialosis in COVID-DC is greater since emergence of the omicron variant.

Keywords: PET Imaging, COVID-19, Major Depression Disorder, neuroinflammation, astroglialosis

Disclosure: Nothing to disclose.

P237. Unraveling the Gene Regulatory Role of MDD-Associated Genetic Variants in Response to Stress

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Background: MDD emerging following exposure to adversity may be biologically and genetically distinct from MDD without such exposures. This distinction is supported by unique biological changes observed in patients with a history of early adversity (see talk C. Nemeroff) and genetic evidence from studies like those conducted with the UK Biobank (Coleman et al., 2020). Glucocorticoids (GCs), or stress hormones have been shown to be important mediators of the impact of adversity on disease risk. To better understand the interaction between stress and genetic variants in MDD, we aimed to functionally characterize SNPs in linkage disequilibrium (LD) with 102 independent loci found to be associated with MDD at genome-wide significance (Howard et al., 2019) and identified those altering the molecular response to stress.

Methods: Using a massively parallel reporter assay (STARR-seq), we examined the regulatory element activity of 13116 SNPs in linkage disequilibrium (LD) with the 102 independent SNPs associated with MDD. These SNPs had an R^2 value of ≥ 0.5 and were within 500 kb of an independent SNP. For each SNP, we assessed allele-dependent regulatory element activity, resulting in over 26000 sequences being analyzed. We tested these sequences both at baseline and after activating the glucocorticoid receptor using GCs (dexamethasone) in an osteoblastoma cell line. MPRAalyze, which employs a generalized linear model, was used to quantify regulatory element activity.

Results: From the 13116 genetic loci tested, over 3900 show regulatory element activity ($FDR < 0.05$) with 1072 elements showing a significant response to GCs ($FDR < 0.05$). These are referred to as dexamethasone regulatory elements (DREs). These DREs are distributed across all chromosomes and are enriched in glucocorticoid receptor-binding regions (ChIP-seq, permutation p -value < 0.01). Based on positional mapping, we identified an association of these DREs to 220 protein-coding genes expressed across over 53 tissues. Of the DREs, 240 showed a significant allele-dependent transcriptional response ($FDR < 0.05$) to GCs. These variants are enriched in open chromatin sites specific for glutamatergic neurons as compared to open chromatin sites in other brain cell types (Fisher's Exact test; $FDR = 0.0035$).

Conclusions: These findings suggest that genetic variants influencing the molecular impact of GCs represent a significant portion of those associated with MDD at genome-wide significance. Mapping these GC-responsive variants in patients with MDD could identify a stress-susceptible subtype characterized by distinct biological mechanisms and comorbidities. This insight could help in preventing and managing the physical and psychological manifestations of the disease and provide new targets for developing more individualized treatment approaches.

Keywords: Depression, gene regulation, glucocorticoid receptor

Disclosure: Nothing to disclose.

P238. Lumateperone Treatment for Major Depressive Episodes With Mixed Features in Major Depressive Disorder and Bipolar I or Bipolar II Disorder: A Post Hoc Analysis of Anhedonia

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Background: Anhedonia is common in mood disorders, occurring in $\approx 70\%$ of patients with major depressive disorder (MDD) and $\approx 50\%$ of patients with bipolar depression. Heightened levels of anhedonia in mood disorders are associated with more severe and

recurrent depressive illness, a greater risk for suicidal ideation, and poorer treatment response compared with lower levels of anhedonia.

Lumateperone is an FDA-approved antipsychotic indicated to treat schizophrenia and depressive episodes associated with bipolar I or bipolar II disorder. In a randomized, double-blind, placebo-controlled trial (Study 403; NCT04285515) lumateperone 42 mg demonstrated efficacy over placebo with a favorable safety profile in patients with MDD or bipolar depression with mixed features. This post hoc analysis of Study 403 investigated the efficacy of lumateperone 42 mg in improving measures of anhedonia in patients with MDD or bipolar depression with mixed features.

Methods: Eligible males and females (18-75 years) had DSM-5 diagnosed MDD with mixed features or bipolar I or II disorder with mixed features and were experiencing a major depressive episode (MDE; Montgomery-Asberg Depression Rating Scale [MADRS] Total score ≥ 24 and Clinical Global Impression Scale-Severity [CGI-S] score ≥ 4) and had a Young Mania Rating Scale Total score 4-16 (inclusive). Patients were stratified by MDD or bipolar disorder diagnosis and randomized 1:1 to 6-weeks lumateperone 42 mg or placebo. This analysis evaluated anhedonia in the overall combined MDD/bipolar depression population, the individual MDD population, and the individual bipolar depression population. Assessments included change from baseline in MADRS anhedonia factor score and its individual MADRS item components (apparent sadness, reported sadness, concentration difficulties, lassitude, and inability to feel) using a mixed-effects model for repeated measures in the modified intent-to-treat (mITT) population.

Results: In the combined MDD/bipolar depression population, 385 patients received treatment and 383 (placebo, 191; lumateperone, 192) were included in the mITT population. The majority (89.4%) of patients completed treatment. Compared with placebo, lumateperone significantly improved MADRS anhedonia factor score as evidenced by reduction in score from baseline to Day 43 in all 3 populations: combined MDD/bipolar depression population (least squares mean difference vs placebo [LSMD], -3.4 ; 95% CI -4.56 to -2.26 ; effect size [ES], -0.63 ; $P < .0001$), MDD individual population (placebo, $n = 92$; lumateperone, $n = 92$; LSMD, -3.4 ; 95% CI -4.97 to -1.76 ; ES, -0.63 ; $P < .0001$), and bipolar depression individual population (placebo, $n = 99$; lumateperone, $n = 100$; LSMD, -3.3 ; 95% CI -4.89 to -1.67 ; ES, -0.61 ; $P < .0001$). In all 3 populations, significantly greater ($P < .01$) reductions in change from baseline of MADRS anhedonia factor score occurred by Day 15 and persisted throughout the study in lumateperone-treated patients.

In the combined MDD/bipolar depression population, all the individual MADRS items that comprise the anhedonia factor score significantly improved with lumateperone vs placebo on Day 43 relative to baseline, including apparent sadness (LSMD, -0.9 ; 95% CI -1.14 to -0.60 ; ES, -0.68 ; $P < .0001$), reported sadness (LSMD, -0.8 ; 95% CI -1.05 to -0.50 ; ES, -0.59 ; $P < .0001$), concentration difficulties (LSMD, -0.4 ; 95% CI -0.71 to -0.16 ; ES, -0.34 ; $P < .01$), lassitude (LSMD, -0.6 ; 95% CI -0.94 to -0.36 ; ES, -0.48 ; $P < .0001$), and inability to feel (LSMD, -0.7 ; 95% CI -0.97 to -0.47 ; ES, -0.62 ; $P < .0001$). Lumateperone treatment was generally safe and well tolerated and consistent with prior studies.

Conclusions: Lumateperone 42 mg demonstrated efficacy in improving a range of symptoms related to anhedonia and was generally well tolerated in patients with MDD with mixed features or bipolar depression with mixed features.

Keywords: major depressive disorder, bipolar depression, lumateperone, anhedonia

Disclosure: Intra-Cellular Therapies, Inc.: Employee, Stock / Equity - Publicly Traded Company (Self)

P239. Novel Single-Session Behavioral Activation With Delta-Beta Transcranial Alternating Current Stimulation for Depression: Results From a Double-Blind Randomized Clinical Trial

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Background: Behavioral activation (BA), an evidence-based therapy for major depressive disorder (MDD), produces reductions in depression even following a single-session of treatment. This is of particular importance, given the barriers to treatment in patients with MDD (e.g., cost, travel, time). Transcranial alternating current stimulation (tACS) modulates brain states and has significant potential to augment interventions for depression. Of particular interest is delta-beta tACS for the treatment of underlying symptoms of MDD. Specifically, delta-beta coupling in the frontal cortex has been shown to be negatively associated with anhedonia and effort exertion; positioning delta-beta tACS as a potentially advantageous augmentation to goal-directed interventions such as BA. Despite the evidence supporting these approaches, the combination of single-session BA and delta-beta tACS has not yet been evaluated as a potential accelerated treatment for MDD. As such, this double-blind randomized clinical trial evaluated whether delta-beta tACS increased the efficacy of single-session BA in individuals with MDD.

Methods: A total of $N = 30$ (Mage=36, SD = 15; 70% female) participants who met criteria for current MDD based on the DSM-5 were included in the present study. Participants were randomized into one of two groups: 1) tACS with BA or 2) sham tACS with BA. Delta-beta tACS targeted left dorsolateral prefrontal cortex and left motor cortex. Cross-frequency delta-beta or active sham stimulation was delivered during the final 30 minutes of the BA intervention. Depression and anhedonia were assessed via clinician-rated measures including the Hamilton Depression Rating Scale (HDRS) and the novel clinician-rated Snaith-Hamilton Pleasure Scale (SHAPS-C), as well as self-report measures including the Inventory of Depression and Anxiety Symptoms (IDAS) and SHAPS. Assessment was completed at baseline, two-weeks following treatment, and one-month following treatment. Repeated-measures ANOVAs were used to assess change in depressive and anhedonia symptoms. Group differences in responders and remitters were also assessed. Preregistration of the full clinical trial is available at NCT05693922.

Results: Results revealed a significant effect of time on the HDRS $F(1,28) = 46.04$, $p < .001$, $\eta^2 = 0.326$ such that depression scores decreased significantly over time, but no significant difference for condition over time for the HDRS emerged $F(1,28) = 0.94$, $p = .341$, $\eta^2 = .010$. This pattern of results was the same across the SHAPS-C such that there was a significant effect of time on SHAPS-C $F(1,28) = 15.93$, $p < .001$, $\eta^2 = .080$; showing a decrease in anhedonia over time, but no significant difference for condition over time for the SHAPS-C $F(1,28) = 0.08$, $p = .776$, $\eta^2 < .001$. Self-reported measures, showed a significant effect of time for of depression (IDAS Dysphoria $F(1,28) = 14.52$, $p < .001$, $\eta^2 = .117$) and anhedonia (IDAS Well-Being: $F(1,28) = 13.72$, $p < .001$, $\eta^2 = 0.121$; SHAPS: $F(1,28) = 5.21$, $p < .05$, $\eta^2 = .033$), but no effect of condition ($ps > .05$). In the verum group, 33% of participants ($n = 5$) were categorized as "responders" to treatment as indicated by a decrease of at least half of the baseline HDRS score at follow-up. In contrast, 13% of participants ($n = 2$) in the sham group were categorized as "responders". Regarding remitters (e.g., a reduction of the HDRS to < 8 at follow-up), 27% of participants ($n = 4$) remitted in the

verum group versus 6% of participants ($n = 1$) remitted in the sham group. A chi-square analysis was conducted, however, no significant differences between groups emerged for remitters ($p = .141$) or responders ($p = .195$).

Conclusions: To our knowledge, findings from this double-blind randomized clinical trial are the first to examine tACS combined with single-session psychotherapy. Treatment gains in self-reported reductions of depression and anhedonia were consistent with single-session BA studies. Results from this study also demonstrated clinician-rated decreases in depression and anhedonia symptoms; a novel contribution to the field. Moreover, the current trial provided initial feasibility data for this augmented treatment approach – suggesting that the combination of delta-beta tACS with single-session BA is feasible. Further, although not statistically significant, augmentation with delta-beta tACS showed greater numerical decreases in clinician-rated depression over time. In future studies, it may be beneficial to recruit a larger sample and extend the amount of time participants received the stimulation across multiple days in conjunction with modularized BA.

Keywords: tACS, Major Depressive Disorder (MDD), Behavioral Activation, Clinical Trial, Single Session

Disclosure: Nothing to disclose.

P240. Identifying Biomarkers for Predicting Cognitive Outcomes in Patients Undergoing Electroconvulsive Therapy for Treatment-Resistant Depression

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Background: Electroconvulsive therapy (ECT) is an effective treatment for depression, but it has notable drawbacks, including cognitive impairment. Currently, there are no known biomarkers to predict cognitive outcomes following ECT. This study aimed to measure biomarkers such as DTI-based free water (FW), a potential indicator of neuroinflammation or edema) and FW-corrected FA (FA-t) in depressed patients undergoing ECT. We examined the relationship between these measures and cognitive outcomes post-ECT.

Methods: Male and female patients with a clinical indication for ECT ($n = 33$) were recruited through Zucker Hillside Hospital (ZHH) in New York. Cognitive assessments included the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). Diffusion MRI data were preprocessed using QSIprep (v0.17.0). Diffusion tensor fitting and derivation of free water (FW) were performed with DiPy tools. Tract-based spatial statistics (TBSS) were used for whole-brain white matter skeletonization for each patient, with specific regions of interest derived using the ENIGMA white matter skeleton atlas. Paired t-tests compared baseline and follow-up timepoints, while Pearson's correlations assessed associations between continuous variables. Analyses were performed using SPSS Statistics v. 28 (IBM).

Results: At baseline, whole-brain FW was lower ($p = 0.01$) and whole-brain FA-t was higher ($p < 0.001$) in healthy controls ($n = 31$) compared to patients ($n = 33$). Higher FW was negatively associated with total RBANS scores ($p < 0.05$) at baseline in patients. Additionally, there was a significant reduction in the RBANS cognitive domain of 'delayed memory' from pre- to post-ECT ($p < 0.001$) in patients. Post-ECT delayed memory scores were negatively associated with baseline FW levels ($p = 0.04$).

Conclusions: Our findings indicate that diffusion tensor imaging (DTI) measures, especially free water (FW), are significantly associated with cognitive impairment in patients undergoing ECT. By identifying and understanding these biomarkers of

cognitive outcomes, we aim to enhance our ability to predict and manage the cognitive side effects of ECT. This research could lead to more personalized treatment strategies, optimizing ECT's efficacy while mitigating its cognitive risks.

Keywords: electroconvulsive therapy, treatment resistance, free water, DTI, Cognition

Disclosure: Nothing to disclose.

P241. Sex-Specific Regulation of Stress Susceptibility by the Astrocytic Gene Htra1

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Background: Major depressive disorder (MDD) represents a significant public health issue, with its prevalence and impact exacerbated by the recent COVID-19 pandemic. MDD displays a notable sexual dimorphism, with females being more susceptible and experiencing more severe symptoms than males. While neuronal mechanisms in MDD have been extensively studied, the role of astrocytes, particularly in the nucleus accumbens (NAc), is gaining attention. This study investigates the astrocyte-specific gene HTRA1 and its sexually dimorphic regulation in the context of chronic stress and MDD.

Methods: We analyzed the transcriptional profiles of astrocyte-specific genes in the NAc from postmortem brain tissue of individuals with MDD and from mice exposed to chronic variable stress (CVS). Using viral constructs, we overexpressed (OE) and knocked down (KD) Htra1 expression specifically in NAc astrocytes in male and female mice. Behavioral responses to CVS were assessed through a series of tests. We examined D1- and D2-MSN activity using ex-vivo slice electrophysiology and in vivo fiber photometry. Furthermore, we evaluated extracellular matrix (ECM) remodeling by analyzing perineuronal net (PNN) intensity and BCAN protein expression levels.

Results: HTRA1 was identified as a differentially regulated astrocyte-specific gene in the NAc, with sexually dimorphic expression and opposite regulation in response to MDD and CVS in males and females. Astrocytic KD of Htra1 induced stress susceptibility in male mice but resilience in females, while OE induced resilience in males and susceptibility in females. Manipulating Htra1 expression in NAc astrocytes modulated D1- and D2-MSN activity in a sex-specific manner. Changes in HTRA1 levels inversely correlated with BCAN expression and PNN intensity, suggesting a role in ECM remodeling.

Conclusions: Our findings highlight the critical role of astrocytic HTRA1 in regulating stress susceptibility and its potential involvement in MDD pathophysiology. The sex-specific effects of HTRA1 manipulation on behavioral responses and neuronal activity underscore the importance of considering sex differences in developing targeted therapies for mood disorders. This study provides new insights into the interplay between astrocytes, the ECM, and neuronal circuitry, paving the way for novel, sex-specific therapeutic approaches for MDD.

Keywords: Major Depression Disorder, Acute and Chronic Stress, extracellular matrix, Sexual Dimorphism, Astrocyte

Disclosure: Nothing to disclose.

P242. Dysfunctional Basal Forebrain Coordination of Intrinsic Brain Networks in Depressive and Anxiety Disorders

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Background: Altered connectivity both within and between the default mode and salience networks have been observed across anxiety and affective disorders. However, recent work has highlighted the importance of subcortical regions, including subdivisions of the basal forebrain, in coordinating the activity of these networks. This suggests that dysfunction in basal forebrain subregions may be driving changes in intrinsic brain network functioning, however, limited work has examined this relationship in humans.

Methods: Using ultra-high field (7T) functional magnetic resonance imaging, we examined the resting-state effective connectivity of basal forebrain subregions in a transdiagnostic group of 70 individuals with depressive and anxiety disorders and 78 healthy controls. We explored the connectivity of basal forebrain divisions with regions of the salience network (left and right anterior insula and dorsal anterior cingulate) and default mode network (ventromedial prefrontal cortex, posterior cingulate cortex, and left and right inferior parietal lobule).

Results: Clinical participants showed wide-spread altered connectivity from the nucleus basalis of Meynert to the default mode network, including increased inhibitory connectivity to the ventromedial prefrontal cortex, posterior cingulate cortex, and bilateral inferior parietal lobules. Increased inhibitory connectivity was also observed from the ventral pallidum to the posterior cingulate cortex and bilateral inferior parietal lobule.

Conclusions: Given the nucleus basalis of Meynert's role as a key cholinergic hub, these findings indicate that further investigation into this circuitry's response to anticholinergic antidepressants, such as scopolamine, could enable more targeted interventions.

Keywords: Anxiety and Depression, Ultra-high field MRI, Effective Connectivity

Disclosure: Nothing to disclose.

P243. Corticotropin-Releasing Factor Selectively Enhances the Synaptic Effects of Ketamine Metabolite (2R,6R)-Hydroxynorketamine at the Hippocampal Synapses

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Background: Major depressive disorder is associated with structural and functional changes in mood-related brain regions, such as the hippocampus. The hippocampal has several important synapses including the entorhinal cortex (EC) perforant path (PP) to granule cell (GC) synapse, mossy fiber to CA3 synapse, Schaffer collateral CA3 (SC) to CA1 synapse, and the direct EC temporammonic (TA) to CA1 synapse. Rapid-onset antidepressants like ketamine and its bioactive metabolite, (2R,6R)-hydroxynorketamine (HNK), are thought to exert their effects by enhancing excitatory synaptic transmission and plasticity. Additionally, the stress hormone corticotropin-releasing factor (CRF) enhances neuroplasticity. In this study, we examined the synaptic effects of a combination of HNK and CRF.

Methods: We combined electrophysiological recordings of field excitatory postsynaptic potentials and pharmacological approaches in mouse hippocampal slices to investigate the effect

of HNK alone and a combination of HNK and CRF on the synaptic transmission at the hippocampal synapses. Transverse hippocampal slices were prepared from male and female C57BL/6J mice. Paired student's t test was used for one factor and one treatment experiments for within-group comparison. One-way analysis of variance (ANOVA) was used for experiments with one factor and more than two treatments.

Results: HNK alone potentiated the SC-CA1 synapse ($n = 8$, $p = 0.0045$). Regardless of the test concentration, HNK did not potentiate PP-GC or TA-CA1 synapses (PP-GC; $n = 6$, $p = 0.2146$, TA-CA1; $p = 0.7874$). A combination of CRF and HNK significantly potentiated TA-CA1 synapse but not PP-GC synapse (PP-GC; $n = 7$, $p = 0.7381$, TA-CA1; $n = 7$, $p = 0.0426$). This synergistic synaptic potentiation at TA-CA1 synapses was NMDAR-independent and it was associated with a significant decrease in pair pulse ratio indicating a presynaptic mechanism ($n = 7$, $p = 0.0340$). Inhibition of CRF receptor 1, but not 2, blocked CRF/HNK-induced potentiation (Veh; $n = 6$, CP-154; $n = 6$, A-2B; $n = 6$; ordinary one-way ANOVA). Unlike the SC-CA1 synapse, inhibition of cyclic adenosine monophosphate (cAMP) did not block CRF/HNK-induced potentiation at TA-CA1 synapse (Veh; $n = 6$, SQ 22536; $n = 7$, CRF/HNK; $n = 6$ and CRF/HNK/SQ; $n = 7$, ordinary one-way ANOVA). The CRF/HNK mediated potentiation of the TA-CA1 synapse was long-lasting. For all the experiments, similar results were obtained in both sexes.

Conclusions: These experiments revealed a synapse-specific function of CRF in enhancing the synaptic effect of HNK in the hippocampus, therefore, providing a novel insight into our understanding of the potential synaptic mechanisms by which HNK may exert its rapid onset antidepressant effect. Approaches combining manipulations that increase CRF combined with ketamine and/or HNK may have utility in the treatment of depression.

Keywords: Ketamine, (2R,6R)-hydroxynorketamine, Hippocampus, Antidepressant, Corticotropin-releasing factor (CRF)

Disclosure: Nothing to disclose.

P244. Effects of Electroconvulsive Shock on Hippocampal Granule Neuron Structure, Function, and Transcriptome

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Background: Therapeutic use of electroconvulsive shock (ECS) therapy is particularly effective for the remission of treatment-resistant depression. Yet the question of how ECS produces these powerful effects remains unanswered. Structural MRI of patients with depression reveals an increase in dentate gyrus volume after ECS treatment, as compared to volume before treatment, and the increase in dentate gyrus volume correlated with symptom improvement. Like other more common forms of antidepressant treatment such as fluoxetine, ECS has been shown to increase neurogenesis in the hippocampal dentate gyrus of rodent models. Yet the question of how ECS-induced neurogenesis supports improvement of depressive symptoms remains unknown. Here we use a mouse model to examine the effects of ECS on granule neuron structure, function, and RNA transcriptome. First, we test the hypothesis that the presence of immature granule neurons is necessary for the therapeutic effects of ECS. We then use slice electrophysiology to ask how activity in immature granule neurons affects activity of mature granule neurons in ECS vs Sham subjects. We then use single nucleus RNA sequencing to transcriptomically profile granule neurons after ECS or Fluoxetine treatment. Finally, we use immunohistochemistry and electron microscopy to profile immature axon terminals in the dentate gyrus.

Methods: Mice were exposed to chronic corticosterone to induce a stressed phenotype. ECS was delivered every other day for 10 sessions in the experimental group, while Sham mice received isoflurane anesthesia and ear clip placement identical to the ECS group, yet no current was delivered to Sham mice. To ablate neurogenesis, X-irradiated mice received three sessions of x-ray exposure targeting the hippocampus bilaterally, while Sham mice received anesthesia only. For slice electrophysiology, we used a tamoxifen inducible nestin-creERT2 line crossed with a cre-dependent channelrhodopsin (ChR)-YFP to allow us to specifically activate immature granule neurons. We then measured electrophysiological responses in mature granule neurons. We also used this transgenic cross to label immature cell terminals in electron microscopy studies, as well as a synaptophysin-YFP transgenic to quantify terminals in the dentate gyrus. For RNA-sequencing experiments, we sorted for NeuN expressing cells in order to limit our exploration to neurons. Antidepressant-like behavior was assessed by the novelty suppressed feeding test and the forced swim test.

Results: We found that that ECS-induced neurogenesis is necessary to improve depressive-like behavior of mice exposed to chronic corticosterone. Using slice electrophysiology, we found that optogenetic stimulation of immature granule neurons produces a hyperpolarization in mature granule neurons. We identified that this hyperpolarization requires the activation of mGluR2, as pharmacological blockage of mGluR2 eliminates the effect. Consistent with this finding, we observed reduced expression of the early immediate gene cFos in ECS vs sham subjects. This result indicates that ECS amplifies the effect of a direct, inhibitory connection between immature and mature granule neurons. Evidence of this direct connection is present in our investigation of axon terminals in the dentate gyrus. We also found that ECS increases terminals present in the granule cell layer. By contrast, we found that ablation of neurogenesis reduces expression of terminals in the dentate gyrus. Using single nucleus RNA sequencing, we reveal major transcriptomic shifts in granule neurons after treatment with ECS+Cort or fluoxetine+Cort vs Cort alone. We identify a population of immature cells which has greater representation in both ECS+Cort and fluoxetine+Cort treated samples vs Cort alone. We also find global differences in ECS- vs fluoxetine-induced transcriptomic shifts.

Conclusions: Together, our findings highlight the role of immature granule neurons as mediators of the therapeutic effects of ECS. We show that ECS increases the ability of immature cells to suppress activity of mature cells. Our findings also reveal the importance of mGluR2 in the suppression of mature granule neuron activity and as a potentially powerful therapeutic target.

Keywords: electroconvulsive therapy, dentate gyrus, adult hippocampal neurogenesis, mGlu 2/3, Mouse models

Disclosure: Nothing to disclose.

P245. Relationships Between Pro- and Anti-Inflammatory Cytokines, Cortical Excitability, and Ketamine Effects in Treatment-Resistant Depression

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Background: Elevated levels of inflammatory markers including proinflammatory cytokines might confer risk for treatment-resistant major depression (TRD) and resistance to treatment with conventional antidepressants, but not lack of response to rapid-acting treatments such as ketamine. In addition, neurophysiological recordings of gamma (30-58 Hz) power—a proxy measure of

cortical excitability—have emerged as a promising biomarker of TRD and ketamine response. We were thus interested in the relationships between pro- and anti-inflammatory cytokine concentrations, magnetoencephalography (MEG) gamma power, and antidepressant response to ketamine.

Methods: Data were drawn from a double-blind, placebo-controlled clinical trial of ketamine's mechanisms of action (NCT00088699). Thirty-five unmedicated TRD patients and 25 healthy control (HC) participants consented to study participation, provided plasma samples, and underwent MEG scanning.

Plasma samples were obtained at baseline and 4 hours post-infusion (ketamine and placebo) and were analyzed for cytokine concentrations using a BioRad 27-plex multiplex magnetic bead assay. Cytokines with over 50% out of range values were removed from the analysis. Cytomod was used to cluster cytokines into co-signaling modules via unsupervised hierarchical clustering performed over 1000 bootstrapped subsets of the cohort (with replacement). The optimal number of clusters (K) was determined using the Tibshirani "gap statistic" scores and were calculated based on the mean of all standardized cytokine values within a given module. Module allegiance at baseline (K = 5) was retained to calculate post-infusion scores in the same manner.

One or two 10-minute eyes-closed resting-state MEG datasets were collected at baseline and 6-to-9 hours following infusions. In addition, T1-weighted magnetic resonance imaging scans were collected from each participant for MEG data coregistration. Synthetic aperture magnetometry (SAM), a linearly constrained minimum-variance beamformer algorithm, was used to project gamma power over the entire rest period, normalized by the wideband (2-100 Hz) sum of squared images. Resultant root-mean-square gamma power images were warped to Talairach space, and linear mixed-effects models implemented in AFNI (<https://afni.nimh.nih.gov/>) were used to test for main effects of plasma module, diagnosis (TRD, HC), session (baseline, placebo, ketamine), and all two- and three-way interactions. We specifically focused on main effects of plasma module and plasma-by-diagnosis, plasma-by-session, and plasma-by-diagnosis-by-session interactions that survived cluster-level correction of $p < 0.05$.

Results: Two plasma modules demonstrated significant associations with gamma power in the linear mixed effects models - modules 1 and 5. Module 1 included interferon gamma (IFN- γ), interleukin (IL)-1 receptor antagonist (IL-1Ra), and monocyte chemoattractant protein-1 (MCP-1), while module 5 included IL-8, IL-10, and IL-13. For plasma module 1, significant negative associations between gamma power and plasma values in regions of the frontal, anterior, and medial temporal lobes including orbitofrontal cortex, insula, and hippocampus were found. There was also a significant plasma-by-diagnosis interaction in overlapping regions of cortex, where TRDs demonstrated negative associations between plasma values and gamma power while HCs did not. For plasma module 5, significant plasma-by-session and plasma-by-diagnosis-by-session interactions were found. Probing the interactions, we found significant positive associations between gamma power and plasma values at baseline in TRD patients within the medial temporal lobes bilaterally, including regions of the hippocampus and ventral striatum. Similar associations were not found for HCs at baseline or for TRDs and HCs following placebo and ketamine infusions.

Conclusions: Two principal findings were uncovered. First, higher levels of plasma module 1 (IFN- γ , IL-1Ra, and MCP-1) were associated with reduced baseline gamma power in orbitofrontal cortex, insula, and hippocampus, primarily in TRDs. In response to inflammation, infection, or injury, classical (M1) activation of monocyte/macrophages is driven primarily by IFN- γ , leading to a pro-inflammatory phenotype marked by the expression of cytokines including MCP-1, tumor necrosis factor alpha (TNF α), IL-1 β , and others. To counterbalance this activity, plasma module 5 cytokines (including IL-10 and IL-13), along with IL-4 and IL-6

are classically secreted, polarizing macrophages to an anti-inflammatory (M2a) phenotype, promoting tissue repair, angiogenesis and resolution of pro-inflammatory cascades. The second principal finding of widespread positive associations between plasma module 5 cytokines and baseline gamma power across the medial temporal lobes and regions of striatum bilaterally in patients with TRD may be reflective of intact, counterregulatory feedback. While canonically pro-inflammatory, IL-8 is potentially angiogenic, enhances nerve growth factor (NGF) secretion in response to brain injury, and potentiates prefrontal synaptic transmission in response to painful stimuli. In patients with TRD, higher levels of immunoregulatory (M2) feedback along with compensatory effects of IL-8 may thus confer heightened synaptic efficacy independent of ketamine response.

Keywords: cytokines, treatment-resistant-depression, magnetoencephalography, (R,S)-ketamine

Disclosure: Nothing to disclose.

P246. Resilient Specific Sex-Conserved Transcriptomic Networks in the Nucleus Accumbens Following Chronic Social Defeat Stress in Mice

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Background: Major depressive disorder (MDD) is a leading cause of disability and a leading contributor to suicide according to the World Health Organization. Chronic stress is a primary risk factor for MDD and is modeled in rodents using the chronic social defeat stress (CSDS) paradigm. Importantly, this paradigm allows for identifying animals across a continuum of responses from those that develop depression-like behavioral abnormalities, termed susceptible, to those that maintain mostly normal behavioral function, termed resilient. This approach has proven to be highly useful but has been mostly examined in male mice, leaving the examination of female mice understudied. Given that depression is more prevalent in women, it is crucial to investigate potential sex-specific molecular mechanisms underlying susceptibility vs. resilience.

Methods: To address this gap, we conducted RNA-sequencing on female mice subjected to an adapted model of CSDS and identified transcriptional changes associated with the susceptibility-resilience spectrum across multiple brain regions. Initial comparison of this new dataset with published findings on male mice replicated earlier findings of striking sexual dimorphism in adaptations associated with susceptibility or with resilience in female vs. male mice in the brain regions studied. Despite this sexual dimorphism, we identified a cluster of genes uniquely upregulated in the NAc of resilient mice that overlapped ~40% across sex. To further investigate this convergence, we performed Weighted Gene Co-Expression Network Analysis (WGCNA) on both resilient male and female mice. After the creation of a co-expression network, we compared gene modules (comprised of genes with similar co-expression) across sex to identify convergent modules across sexes in resilient mice.

Results: We found a pair of modules that display 25% percent overlap, the highest of all possible module combinations across sex. Additionally, both modules have the highest enrichment for differentially expressed genes that are upregulated in the NAc of resilience mice. Furthermore, within these gene modules, two key driver genes, *Gprn1* and *Stx1a*, were predicted to regulate other

module genes and positively correlate with behavioral resilience in both sexes. To elucidate the role of these key driver genes, we are currently investigating the consequences of viral manipulation within medium spiny neuron subtypes of the NAc in both male and female mice prior to CSDS. Bilateral overexpression of *GPRIN1* or *STX1a* in all NAc neurons of male mice induces a pro-resilient effect and ongoing research is characterizing effects on transcriptional, neuronal, and circuit function that underlie the promotion of behavioral resilience.

Conclusions: This study provides novel insight into the molecular mechanisms underlying stress resilience and offers valuable guidance for future efforts in antidepressant drug discovery. Our findings highlight the importance of considering sex-specific factors in understanding depression and developing targeted interventions.

Keywords: Chronic social stress, Stress-resilience, Depression

Disclosure: Nothing to disclose.

P247. Smaller Putamen Volumes are Associated With Familial Depression in an Infant Population

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Background: Major depressive disorder (MDD) is a chronic psychiatric disorder associated with significant impairment and increased morbidity and mortality. MDD often runs in families, yet underlying mechanisms are incompletely understood. The putamen, part of the cortico-striatal-thalamic circuits that influence reward learning and motivation, is increasingly indicated as having a role in MDD.

Family history is one of the strongest indicators of depression risk. Previous studies have tried to identify neurobiological makers of depression risk through structural MRI analysis of individuals with and without a family history of depression. A study from 9- and 10-year-old children found that parental depressive history was related to a smaller right putamen volume (Pagliaccio et al., 2020). A further study designed to elucidate how putamen structure and function increase depression risk again demonstrated reduced putamen volumes in 30-year-old adults at risk for MDD. (Talati et al., 2022).

Infant neuroimaging, including structural MRI, is an emerging area of study with the potential to provide early biomarkers of neurodevelopmental processes. The human brain undergoes extraordinary growth during the fetal period – growth that may be influenced by a wide range of potential prenatal exposures that may confer long-term effects on the child's subsequent cognitive and social behaviors. Despite the importance of the prenatal period, human research into this neurodevelopmental period has been limited by methodological challenges, such as isolating the influence of pre- vs post-natal exposures. Infant neuroimaging is helping to address these challenges by allowing investigators to assess brain structure and function with minimal post-natal influences. This may help establish the role of genetic predisposition versus the impact of rearing as influenced by a parent or family with MDD.

Methods: T2-weighted MRI data of infants of a post-menstrual age (PMA) between 36 and 47 weeks were obtained from the NIH study "Maternal adversity, inflammation, and neurodevelopment: How intergenerational processes perpetuate disadvantage in a low-resource setting" (5R01MH121070-04). FSL (version 6.0.7.1, Smith et al., 2004) was used to remove the skull and *fnias.ai* (version 1.0.1, Wang et al., 2022) was used to segment subcortical

structures, including the putamen. MINI and PHQ-9 scores were used to determine maternal history of MDD and PHQ-9 was used to determine paternal history of MDD. Low-risk infants are defined as those with no parental history of MDD and high-risk infants defined as those with a parental history of MDD in either or both parents. Python (version 3.9) library statsmodels (version 0.14.1, Seabold et al., 2010) was used to perform linear regression between infant putamen volumes (dependent variable) and parental history of MDD (independent variable) with PMA and sex as covariates. Infants of mothers with postpartum depression as indicated by the Edinburgh Postnatal Depression Scale (EPDS) but no prior history of MDD were excluded. Infants with a left, right, or total putamen volume 2.5 standard deviations outside of the mean were also excluded.

Data from $n = 155$ infants of both sexes were included.

Results: Infants at high risk for MDD had a significantly lower total (sum of left and right) putamen volume than those at low risk ($n = 155$ infants, $p < 0.05$ via linear regression). Post-hoc analysis similarly showed smaller volumes in the left putamen in high-risk infants ($n = 155$ infants, $p < 0.05$ via linear regression).

Conclusions: Now in a third, independent cohort comprised of infants, we have demonstrated that individuals at high familial risk for MDD have lower putamen volumes. As this is a dataset with minimal effect of postnatal influences, this indicates that lower putamen volumes may be congenital, rather than developmental, and may serve as a potential biomarker for risk of MDD, raising the possibility of early intervention in at-risk individuals.

Keywords: Brain Based Markers for Depression, infant MRI, subcortical volume

Disclosure: Nothing to disclose.

P248. An Open Label Study of COMP360 (Synthetic Psilocybin) in Treatment Resistant Depression With Chronic Active Suicidal Ideation

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Background: Despite high prevalence and morbidity from suicidal ideation, most clinical trials exclude participants who report suicidal thoughts. With early evidence of psychedelic efficacy in depression, we explored whether patients with chronic active suicidal ideation could benefit without worsening.

Methods: Fifteen adults (10 Males/5 Females) age: (avg 37.2 SD 6.15) with treatment resistant depression chronic active suicidal ideation were recruited to be dosed with a single 25 mg dose of synthetic psilocybin (COMP360) after withdrawal from psychotropic medication. All subjects had standard psychological preparation and 3 post dose integration sessions. Subjects were followed for 12 weeks. The primary outcome measures were the MADRS and Modified Scale for Suicidal Ideation (MSSI) at three weeks post dose. Response was defined as a MADRS score reduction of 50% since baseline and remission as a score < 10 . MSSI response was defined a score of < 6 and remission a score of 0.

Results: At three weeks after dosing, 46.7% met remission and 53.3% met response criteria on MADRS and 40% met remission criteria and 73.3% met response criteria for their MSSI score. At 12 weeks 26.7% were depression remitters and 46.7% were responders, 26.7% were MSSI remitters and 73.3% were responders.

The fixed effect of treatment (time) on MSSI scores in the overall mixed-effects model was robust [$F(3, 26) = 16.2$, $p = 0.000006$]. MSSI scores at each post-dosing time point were significantly reduced relative to baseline (Week 3 least-squares mean change = -12.9 , 95% CI = -19.2 , -6.6 , Cohen's $d = 1.92$ $p = 0.00005$; Week 12, least-squares mean change in MSSI = -12.1 , 95% CI = -18.6 , -5.6 ,

Cohen's $d = 1.75$, $p = 0.0002$). MADRS scores showed similar outcomes.

Change in MSSI and Change in MADRS were significantly correlated at Weeks 3, and 12. Week 3: Pearson $r = 0.719$, 2-tailed $p = 0.003$. Week 12: Pearson $r = 0.615$, 2-tailed $p = 0.02$.

There were no unexpected adverse events. No subjects exhibited suicidal behaviors during the post dose period.

Conclusions: Most subjects reported significant improvement in chronic depressive and suicidal ideation symptoms with promising durability. Improvements in suicidal ideation were closely correlated with depression improvement. Further exploration into the efficacy of psychedelic therapy within this poorly served psychiatrically ill population is warranted.

Keywords: psilocybin, psychedelics, treatment resistant depression, suicidal ideation, clinical trial

Disclosure: Compass Pathways: Grant (Self). LivaNova: Consultant, Neuronetics, Sage Therapeutics (Self). Genomind: Advisory Board (Self)

P249. Rapid Hippocampal Synaptic Potentiation Induced by Ketamine Metabolite (2R,6R)-Hydroxynorketamine Persistently Primes Synaptic Plasticity

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Background: Ketamine is a remarkable antidepressant that rapidly alleviates depression symptoms hours after a single infusion, and this relief lasts for days. However, ketamine's dissociative effects and abuse liability necessitate the development of therapies that mimic its acute and sustained therapeutic outcomes with fewer side effects. Prior preclinical studies suggest that ketamine metabolite (2R,6R)-hydroxynorketamine (HNK) fits this profile, and has begun phase II clinical trials for the treatment of depression. While hypotheses have been proposed to explain how ketamine and its metabolites initiate their rapid antidepressant-relevant effects, it is unclear how sustained therapeutic actions are promoted following drug elimination. We hypothesized that rapid potentiation of hippocampal neurotransmission evoked by HNK activates N-methyl-D-aspartate (NMDAR)-dependent synaptic metaplastic (priming) mechanisms to persistently favor plasticity formation.

Methods: We used in vitro and ex vivo hippocampal slice electrophysiology to develop a model that distinguishes mechanisms rapidly activated after HNK treatment from those responsible for persisting effects hours later. To study in vitro HNK effects, we incubated slices collected from male and female mice with HNK for 60 min followed by washout for 35 min or 3 hr. To study the sustained effects of HNK ex vivo, mice were treated with HNK and sacrificed for electrophysiology experiments 24 h later. Primary outcomes assessed were input/output (I/O) excitatory postsynaptic potential slope, paired-pulse facilitation (PPF), and long-term potentiation (LTP) at the Schaffer collateral-CA1 synapse.

Results: Incubation with HNK enhanced presynaptic-mediated synaptic transmission 35 min after 10 μM HNK washout as indicated by enhanced basal I/O responses (HNK main effect: $P = 0.023$; Stim Intensity $\times$ HNK: $P = 0.0045$) and reduced PPF ($P = 0.0057$) while also reducing LTP magnitude ($P = 0.0002$). Ex vivo ($P < 0.0001$) and in vitro ($P = 0.0053$) LTP measured 3 hr after 10 mg/kg HNK administration or 10 μM HNK exposure, respectively, was enhanced whereas I/O and PPR were unaffected ($P > 0.05$). Preincubation with the NMDAR antagonist D-APV (50 μM) neither blocked HNK's effects 30 min after exposure nor exerted effects on its own (I/O: HNK main effect: $P = 0.008$, HNK $\times$ D-APV: $P = 0.77$; PPF: HNK: $p < 0.0001$, HNK

x D-APV: $P = 0.34$; LTP: HNK: $P < 0.0001$; HNK x D-APV: $P = 0.62$). Following incubation with HNK, subsequent incubation with D-APV prevented the lowered threshold for LTP measured 3 hr later (HNK: $P = 0.0058$; D-APV: $P = 0.0439$; D-APV x HNK: $P = 0.0207$) without effects of either treatment on I/O or PPR ($P > 0.05$). The rapid effects of HNK were blocked by preincubation with 10 μM pan-adenylyl cyclase (AC) inhibitor SQ22536 (I/O: HNK: $P = 0.020$, HNK x SQ22536: $P = 0.0044$; PPF: HNK: $P = 0.019$; HNK x SQ22536: $P = 0.045$; LTP: HNK: $P = 0.048$; HNK x SQ22536: $P = 0.016$) or AC1 selective inhibitor, 1 μM ST034307 (I/O: HNK: $P = 0.0147$; ST034307: $P = 0.2716$; ST034307 x HNK: $P = 0.0409$; PPF: HNK: $P = 0.0446$; ST034307: $P = 0.0323$; ST034307 x HNK: $P = 0.0320$; LTP: HNK: $P = 0.0477$; ST034307: $P = 0.0298$; ST034307 x HNK: $P = 0.0036$). However, preincubation with a selective AC5 inhibitor, 10 μM NK80, did not prevent HNK's rapid synaptic effects (I/O: HNK: $P = 0.0054$; NKY80: $P = 0.7685$; NKY80 x HNK: $P = 0.7312$; PPF: HNK: $P < 0.0001$; NKY80: $P = 0.6295$; NKY80 x HNK: $P = 0.8059$; LTP: HNK: $P < 0.0001$; NKY80: $P = 0.6295$; NKY80 x HNK: $P = 0.8059$). Strikingly, 10 μM cell permeable PKA inhibitor H89 pretreatment prevented the rapid effects (I/O: HNK: $P = 0.023$; HNK x H89: $P = 0.0082$; PPF: HNK: $P = 0.049$; HNK x H89: $P = 0.021$; LTP: HNK: $P = 0.0006$; HNK x H89: $P = 0.043$) and sustained effects of HNK (I/O: HNK: $P = 0.3963$; H-89: $P = 0.2291$; H-89 x HNK: $P = 0.9112$; PPF: HNK: $P = 0.6292$; H-89: $P = 0.1040$; H-89 x HNK: $P = 0.5352$; LTP: $P = 0.0454$; H-89: $P = 0.0348$; H-89 x HNK: $P = 0.0047$), suggesting that HNK-elicited potentiation is required for sustained synaptic priming.

Conclusions: We developed a slice incubation model with fidelity to the HNK's rapid in vitro effects detected during bath application and sustained ex vivo effects to reveal that PKA activity-dependent potentiation of neurotransmission shortly after HNK exposure was required for sustained plasticity priming. AC1, but not AC5, activity was required for the rapid effects of HNK. Further, the induction of synaptic potentiation did not require NMDAR activity, but NMDAR activity was necessary to maintain synaptic priming. We conclude that approaches such as our incubation model can be leveraged to reveal novel druggable mechanisms that engage temporal-sensitive signaling cascades mediating the progression from acute to sustained therapeutic effects of rapid-acting antidepressants.

Keywords: (2R,6R)-hydroxynorketamine, (R,S)-ketamine, Synaptic Plasticity, CA3 metaplasticity

Disclosure: Nothing to disclose.

P250. Investigating the Role of m6A-Mediated Epitranscriptomic Modifications in Depression: A Postmortem Brain Study

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Background: Increasing evidence suggests that fine-tuning of transcriptional regulation by gene x environment interaction is central to the etiology of major depressive disorder (MDD). In this regard, the concept of post-transcriptional gene regulation through "epitranscriptomic" mechanism is gaining traction, indicating their involvement not only in the regulation of transcript abundance but also their ability to impact maturity, stability, localization, and, most importantly, availability of "select" gene transcripts to protein translation despite varying transcription rates. This regulatory mechanism has the distinct ability to quickly respond to external stimuli, fine-tune protein accessibility, and execute localized control, which is critical to stimulus-dependent gene expression. In this study, we examined if m6A methylation-based mRNA modification and consequent expression of genes participate in MDD pathogenesis.

Methods: Dorsolateral prefrontal cortex (dlPFC) from 49 non-psychiatric controls and 49 MDD subjects were used to measure m6A methylation. Nuclei from dlPFC were enriched to perform RNA extraction. Two hundred nt long random RNA fragments were produced, and fragmented RNAs were denatured. For methylated RNA immunoprecipitation, the anti-m6A antibody was used for cross-linking. cRNAs and immunoprecipitated RNAs were amplified and labeled separately with Cy5 and Cy3 fluorescent dyes. Raw intensities of Cy5-labeled RNA and Cy3-labeled RNA were normalized with an average log2-scaled spike-in RNA control. Differentially, m6A-methylated RNAs were identified by filtering with the fold change and two-tailed Student's t-test. Fold-change ≥ 1.5 or ≤ 0.7 and $P < 0.05$ were used to determine the differentially m6A methylated RNAs. Gene Ontology (GO) term enrichment analyses were performed for biological processes, cellular components, and pathways.

Results: Based on 53135 probe sets, 309 transcripts were identified as significantly hypermethylated in the MDD compared to the control group. On the other hand, 9174 transcripts were significantly hypomethylated. Chromosomes 9, 13, 16, and 22 were found to code low to moderate amounts of hypermethylated transcripts. Most of the methylation sites were associated with the coding region (CDS) of mRNA transcripts in contrast to the two untranslated regions (5' and 3'UTR) flanking the CDS. Sequence motif analyses revealed the enrichment of the m6A consensus motif RRACH in all samples. Based on sequence conservation patterns, two high consensus motifs, AAGA and ACCCA were identified. Prediction of GO, based on the identified genes using the Panther classification system, identified significant functional enrichment (following FDR correction and Fisher exact test) for neuron development, trans-synaptic signaling, synaptic transmission, axonogenesis, neuron projection development, and nervous system development. KEGG analysis further revealed pathways, including signal transduction, immune system processing, and protein degradation. These pathways represented diverse molecular roles related to MDD, which included olfactory transduction, neuroactive ligand-receptor interaction, and signaling pathways such as PI3K-Akt, MAPK, Wnt, TNF, and nuclear factor-kappa B (NF- κ B).

Conclusions: The present study, for the first time, reports the phenomenon of RNA methylation modification in the context of MDD. It also unfolds the potential impact of this modification in understanding the impeded function of transcripts involved in neural plasticity-related pathways.

Keywords: Epigenetics, Epitranscriptomics, Human postmortem brain, Microarray, Depression

Disclosure: Nothing to disclose.

P251. Profiling Transcriptomic Changes Within the Perivascular Space of the Dorsolateral Prefrontal Cortex (dlPFC) in Major Depressive Disorder (MDD)

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Background: Major depressive disorder (MDD) is a prevalent mental health condition characterized by severe social, occupational, and cognitive impairment. Pathological features associated with MDD, such as vascular dysfunction and systemic inflammation, suggest a compromised blood-brain barrier (BBB). This compromised BBB may allow peripheral proinflammatory molecules to penetrate the brain, contributing to neuroinflammation and exacerbating depressive symptoms. Previous studies have

indicated that vascular and inflammatory changes are present in MDD, but the specific mechanisms and cell-type specific effects remain unclear. In this study, we aimed to investigate stress-related transcriptomic signatures and signals of BBB hyperpermeability in the MDD brain, with a particular focus on cell-type specific variations.

Methods: Two layers of omics data from the dorsolateral prefrontal cortex were utilized. Bulk RNA sequencing was performed on tissue samples from 154 individuals with MDD and neurotypical controls (NC), and differentially expressed genes were spatially registered to the cortical layers. Single nucleus RNA-seq data were derived from three distinct datasets of cortical areas from 152 samples (MDD and NC). Upon in silico filtering, the specialized cell types of the perivascular unit were clustered and annotated. Differential gene expression was analyzed using a negative binomial model and pathway analysis was performed to functionally annotate the profile of each cell type.

Results: Bulk RNA sequencing revealed dysregulation in the main stress response in MDD highlighted by the upregulation of FKBP5 (pFDR = 0.05) and downregulation of CRH (pFDR = 0.01), and an overall registration of DEGs in the leptomeningeal layer. Pathways significantly enriched were related to immune processes, reorganization of the extracellular matrix (ECM) and vascular dysfunction. Zooming in single cell resolution (~16k nuclei) we detected distinct cell type specific profiles associated with MDD. Within the capillary cells, EPHA4 was downregulated, while in venous and arterial endothelial cells we observed downregulation of ADCY2 and CNTNAP5, respectively. Functional annotation of such expression changes were linked to diseases of immune system and overall dysregulated cell adhesion processes and inflammatory molecules. Interestingly, transcriptional MDD-associated changes in the immune cell types detected, i.e. macrophages, were linked to endothelial cell apoptotic processes and ECM dysregulation, tying together the structural and immune units of the perivascular space and hinting at BBB vulnerability.

Conclusions: The perivascular space in MDD is highly restructured and carries stress signatures. These observations hint towards hyperpermeability of the BBB which would allow proinflammatory components from the periphery to enter the brain and potentially aggravate disease progression.

Keywords: Depression, single cell RNA-Seq, Blood-Brain-Barrier, brain microvascular endothelial cells

Disclosure: Nothing to disclose.

P252. DLX-159: A Novel, Next Generation, Non-Hallucinogenic Neuroplastogen With the Potential for Treating Neuropsychiatric Diseases

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Background: Cortical synaptic deficits are a key component of many neuropsychiatric disorders, and treatments that promote structural and functional neuroplasticity have demonstrated efficacy in treating these disorders. Recently, psychedelic drugs, including the dissociative anesthetic ketamine, have shown efficacy in humans for treating multiple neuropsychiatric disorders. The clinical effects of these compounds are hypothesized to be due to their ability to promote rapid and enduring effects on structural neuroplasticity, and as such, these compounds have

been classified as psychoplastogens. Unfortunately, the dissociative experiences, hallucinations, and/or cardiotoxic properties of psychoplastogens will limit their widespread clinical use, which has led to the development of non-hallucinogenic analogues known as neuroplastogens. We designed a novel neuroplastogen, DLX-159, to have similar efficacy as psychoplastogens without their undesirable effects.

Methods: The effects of DLX-159 were examined in a series of in vitro, ex vivo, and in vivo assays relevant to neuroplasticity and the treatment of major depressive disorder. All experimental protocols in animal studies were approved by the appropriate Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Results: DLX-159 showed significant effects on structural and functional neuroplasticity in in vitro and ex vivo assays. In a phenotypic screen of neurite outgrowth in primary rat cortical neurons, DLX-159 produced significant increases in both neurite length and branching; these effects were blocked by pre-treatment with the 5HT2A/2C antagonist ketanserin and the mTOR inhibitor rapamycin. In a second in vitro assay using primary rat hippocampal cell cultures, DLX-159 significantly increased synaptogenesis. DLX-159 also exhibited antidepressant-like activity in rodents. Similar to ketamine, DLX-159 exhibited antidepressant-like effects in the rat forced swim test (FST), 24 hours after a single administration, with a decrease in immobility and an increase in swimming. These antidepressant-like effects of DLX-159 and ketamine in the FST were maintained up to at least 3 days after a single administration. Similar to ketamine, 24 hours after a single IP administration, DLX-159 reversed the depression-like phenotype in the rat FST caused by 4 weeks of administration of IFN-alpha. In electrophysiology experiments, 24 hours after a single IP administration of a behaviorally relevant dose, DLX-159 significantly increased the cumulative peak amplitude of excitatory post-synaptic currents (EPSCs) recorded from layer-V, medial prefrontal cortex (mPFC) pyramidal neurons in rat brain slices suggesting increases in synaptic strength. In an ex vivo measure of structural plasticity using Golgi staining, 24 hours after a single IP administration of a behaviorally relevant dose, DLX-159 significantly increased dendritic spine density of layer V mPFC pyramidal neurons. In the mouse head-twitch assay, DLX-159 did not significantly elevate head twitches of male C57BL/6J mice compared to vehicle while the psychedelic DOI did. In a rat quantitative electroencephalography (qEEG) study, DLX-159 administration led to increased power in the delta and theta band frequencies. As a similar signature of change in delta and theta power was seen in Phase 1 studies of our clinical compound DLX-001, this assay may provide biomarker translatability.

In studies relevant to cardiotoxicity, no signal was identified for DLX-159 in cardiovascular safety pharmacology studies at relevant concentrations/doses and DLX-159 was shown to be an antagonist at 5-HT2B receptors. In pharmacokinetic studies, DLX-159 was shown to be orally bioavailable and brain penetrant. Results will be presented for the affinity and functional activity of DLX-159 at a broad range of receptors and enzymes.

Conclusions: DLX-159 is an orally active, brain-penetrant small molecule that promotes structural and functional neuroplasticity without the hallucinogenic or cardiotoxic properties of psychedelic compounds. Preclinical data suggests that it will have rapid and enduring antidepressant effects in humans without inducing hallucinations or psychotomimetic behavior.

Keywords: Neuroplasticity, Major Depression Disorder, drug discovery/development

Disclosure: Delix Therapeutics: Employee (Self). Proniras, Freedom Biosciences: Advisory Board (Self)

P253. Sex Differences in Cortical Cell Types Underlie Sex-Specific Alterations Associated With Major Depressive Disorder

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Background: In addition to sex differences in major depressive disorder (MDD) incidence, there are also sex differences in MDD clinical phenotypes. Women with MDD are more likely to experience hyperphagia and hypersomnia, and more likely to have comorbid anxiety. Men with MDD are more likely to lose weight and have insomnia, and more likely to have a comorbid substance use disorder. However, little is known about the underlying biology of sex-specific clinical features. Evidence suggests unique transcriptional signatures of MDD, including in subgenual anterior cingulate cortex (sgACC). However, previous studies were performed in bulk tissue and were therefore unable to resolve cell type-specific differences. In a study which examined cell type-specific alterations in MDD, men and women were run separately on different platforms, preventing direct comparisons across sex.

Methods: We performed single nucleus RNA- and ATAC-seq of sgACC (N=18; 50% female) to examine cell type-specific alterations in MDD. High numbers of healthy nuclei were sorted using the Chromium Nuclei Isolation Kit with RNase Inhibitor (~1x10⁵ nuclei/sample). Nuclei were indexed (10X Chromium Next GEM Single Cell Multi-ome ATAC + Gene Expression platform), libraries prepared (Chromium Next GEM Single Cell 3' Kit v3.1), and sequenced (NovaSeq 6000). snRNA-seq libraries were sequenced on 2 separate S4 Flow Cells to target at least 50,000 reads/nuclei (10,000 nuclei/sample). snATAC libraries are sequenced using Illumina NovaSeq 6000 S4 flow cells (100,000 paired-end reads/nuclei). Raw gene counts for each subject were integrated within an annotated cell type to create a cell type-specific pseudobulk profile. EdgeR was used to normalize gene counts, estimate variance, and fit a linear model for subject-level covariates (Diagnosis+Sex+Age+pGDR, where pGDR is the pseudobulk detection rate and fraction of non-zero gene counts). We calculated false discovery rate (FDR) within each cell type comparison and between all cell type comparisons (adjusted $p < 0.05$ considered differentially expressed genes (DEGs)). Threshold-free rank rank hypergeometric overlap (RRHO) was used to assess concordance of alterations between men and women.

Results: We first probed for main effects of MDD, finding similar numbers of DEGs across major cell types (~475 DEGs/cell type). However, when we examined sex-specific alterations, microglia had the most robust alterations in men and women, but the genes were largely distinct in men and women. Sex-specific effects of MDD within individual cell types were confirmed using threshold-free RRHO analyses. Given the sex-specific alterations in the context of MDD, we asked whether cell type-specific profiles differed in controls. Indeed, there were large numbers of DEGs in each cell type, with microglia exhibiting 3 times as many genes that differed as a function of sex than other cell types (microglia: 387 DEGs vs. < 100 DEGs in other cell types).

Conclusions: These results suggest men and women with MDD exhibit distinct cell type-specific alterations. Some sex-specific alterations may be explained by underlying differences between men and women that are independent of disease. These underlying sex differences in neurotypical subjects may put one sex at increased risk (e.g., increased risk in women) and/or protect the other sex from developing MDD (e.g., reduced risk in men).

Keywords: sex differences, Postmortem Brain Tissue, Depression
Disclosure: Nothing to disclose.

P254. Repetitive Neuronal Activations Regulate Cellular Maturation State via Nuclear Reprogramming

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Background: Neural stimulations, such as electroconvulsive therapy (ECT) and repetitive transcranial magnetic stimulation (rTMS), are highly effective clinical interventions for a broad spectrum of psychiatric disorders, including depression and schizophrenia. However, their mechanism of action at the cellular level remains poorly understood.

Methods: We challenged neurons in vivo with a stimulation protocol similar to human ECT that could trigger cellular changes. Specifically, we modelled ECT with REPetitive OPTogenetic Stimulation (REPOPS) to the dentate gyrus (DG) of mice expressing ChR2 in granule cells (GCs) (POMC-Cre::ChR2-EYFP mice). This method allowed us to stimulate GCs with a controlled frequency and duration and accurately study the resultant cellular effects confined to a specific target cell population in isolation.

Results: We observed ECT-like behavioral effects, including decreased depression (tail suspension test, $P < 0.05$; forced swim test; $P < 0.05$) and increased locomotion (openfield test, $P < 0.05$). At the cellular level, we found dematuration to a long-term stable state, which can last for more than one month, defined by changes in nuclear structure, gene expression patterns resembling the G2/M phase of the cell cycle, and altered neural coding of navigational information. Moreover, knockout of the G2/M master regulator Cyclin B rescued some of behavioral and cellular effects.

Conclusions: Our findings demonstrate that ECT-like brain stimulation triggers plasticity of the cellular state, revealing a form of stimulus-regulated nuclear reprogramming with potential clinical utility.

Keywords: Antidepressant, dentate gyrus, Hippocampus, optogenetics, calcium imaging

Disclosure: Nothing to disclose.

P255. Neurocognitive Effects of Repeated Intravenous Ketamine in a Large Cohort of Patients With Treatment-Resistant Depression: A Secondary Analysis of the Ketamine Arm of the Elekt-D Trial

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Background: Ketamine, an N-methyl-D-aspartate antagonist, is a novel, interventional therapy for treatment-resistant depression, potentially offering an alternative to existing treatments such as electroconvulsive therapy. Despite robust evidence of subanesthetic dose intravenous (IV) ketamine's anti-depressant and anti-suicidal effects, the cognitive effects of ketamine in multidose protocols remain under researched. The present study investigates cognitive effects of IV ketamine in a sample of adults with treatment-resistant depression, collected as part of a well powered, multisite clinical trial (ELEKT-D study; ClinicalTrials.gov Identifier: NCT03113968).

Methods: Participants received a total of six IV ketamine treatments over 3 to 5 weeks, at a dose of 0.5mg/kg over 40 minutes. Participants completed a comprehensive cognitive battery prior to beginning treatment, and again at their end of treatment visit. The battery included the Montreal Cognitive Assessment (MoCA) to assess overall cognitive functioning, Hopkins Verbal Learning Test Revised (HVLTR) to evaluate verbal episodic memory and learning, Controlled Oral Word Association Test (COWAT) to measure verbal fluency, and the Stroop Color and Word Reading Test (Stroop) to assess selective attention and cognitive flexibility. North American North American Adult Reading Test-35 (NAART) was administered at visit 1 to provide and estimation of pre-morbid ability. A self-report scale (Squire Memory Complaint Questionnaire) was also completed at baseline and end of treatment. Changes in mood were assessed using the Quick Inventory of Depressive Symptomatology Self Report. Least Square Means Regressions were used to compare the change in mean performance from baseline to end of treatment for each cognitive measure. Linear regression analyses were used to test for possible relationships between QIDS-SR-16 depression severity and the change in cognitive performance. Age, education, NAART-determined intelligence, and sex were used as adjusted covariates.

Results: The analytic sample comprised 195 patients (104 female, mean age of 45.60 years $\pm$ 14.83) with severe baseline depression (mean QIDS-SR-16 = 17.9). There was significant improvement between pre-treatment and end of treatment performance for both the COWAT ($p < 0.001$, Cohen's $d = 0.47$) and the Stroop interference task ($p < 0.001$; Cohen's $d = 0.40$). No associations between depression severity and cognitive flexibility were observed, suggesting that these cognitive improvements were at least partially independent of improvements in depression severity. Subjective memory, as measured by the SMCQ, was also found to improve ($p < 0.001$; Cohen's $d = 0.94$) and was significantly associated with improvement in self-rated depression severity. No changes in HVLTR or MoCA performance were observed.

Conclusions: We found no evidence of cognitive impairment associated with multi-dose ketamine therapy for use in this population. There was significant improvement in cognitive flexibility, which may afford opportunities to combine ketamine with psychotherapeutic or cognitive restructuring approaches to improve long-term outcomes. To our knowledge, this analysis represents the largest cohort of patients with treatment-resistant depression treated with IV ketamine.

Keywords: (R,S)-ketamine, Neurocognitive functioning, Depression

Disclosure: Janssen: Contracted Research (Self). OUI Therapeutics: Contracted Research (Self). LivaNova: Advisory Board (Self)

P256. Association Between Sleep and Fatigue in Depression: Role of Depression Severity, GABA and Glutamate

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Background: Sleep disturbances and fatigue are commonly reported in major depressive disorder (MDD). It is unclear whether fatigue is solely a result of poor sleep or an independent symptom within MDD. One way to disentangle these effects is to examine the relationship between levels of neurotransmitters responsible for sleep and arousal (gamma-aminobutyric acid [GABA] and glutamate) and sleep/fatigue in MDD.

Methods: Participants with MDD ($N = 76$) were antidepressant naïve or medication-free for at least three weeks before imaging. Two GABA-edited MEGA-PRESS Magnetic Resonance Spectroscopy (1H-MRS) acquisitions in the anterior cingulate cortex (ACC) were collected. GABA and Glx (combination of glutamate and

glutamine) concentrations were quantified from an average difference spectrum, with preprocessing using Gannet and spectral fitting using TARQUIN. Participants completed sleep questionnaires, and depression was assessed with the Hamilton Depression Rating Scale. The Wilcoxon rank sum test or Kruskal-Wallis test was used to examine the marginal difference in continuous variables between levels of fatigue. Mediation effects of depression severity, GABA, and Glx concentrations on the relationship between sleep duration and fatigue were evaluated using multivariable logistic regression models with fatigue as the dependent variable. Bayes Factor hypothesis testing was used to examine the strength of evidence. The analysis was repeated with sleep efficiency as the main predictor.

Results: No significant relationship was observed between sleep duration/efficiency and fatigue. However, depression severity was significantly associated with fatigue (p -value = 0.0087). Further, depression severity was found to have a significant mediation effect on the relationship between sleep duration and fatigue. Specifically, for every additional hour of sleep duration, the risk of feeling fatigued nearly every day decreased by 11% via an indirect mediating effect of decreased depression severity. GABA and Glx concentrations were not significantly associated with fatigue and did not mediate the effects of sleep duration/efficiency on fatigue.

Conclusions: This study is the first to examine GABA and Glx concentrations in relation to depression, fatigue, and sleep. Different from what has been reported in non-depressed individuals, fatigue severity may be independent of sleep duration and quality and instead related to MDD severity/neurobiology. Therefore, interventions aimed at depression improvement in MDD may be more successful in reducing the debilitating effects of fatigue than those aimed at improving sleep.

Keywords: fMRI, depression, rTMS, prefrontal cortex, fatigue, GABA and Glutamate, sleep and fatigue

Disclosure: Nothing to disclose.

P257. Medication Adherence Following Weighted Multi-Gene Pharmacogenomic Testing in Real-World Insurance Claims Data From Patients With Major Depressive Disorder

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Background: Approximately 50% of patients with major depressive disorder (MDD) discontinue their antidepressant medication within 6 months, which increases risk of relapse. Clinical application of pharmacogenomic (PGx) testing may improve adherence to medications that have clinically actionable gene-drug interactions. In this study, we assessed medication adherence and discontinuation in patients with MDD who received a weighted multi-gene PGx test compared to patients who did not receive this PGx test using insurance claims data.

Methods: Adult patients with MDD who received PGx testing between 5 January 2015 and 30 September 2021 were de-identified and linked with administrative claims data from Optum Labs Data Warehouse. Patients with PGx testing were propensity-matched for baseline demographics and comorbidities to non-PGx tested controls with MDD who were switched to a new psychiatric medication. Medication adherence (defined as the proportion of days covered [PDC]) and discontinuation (defined as a ≥ 45 -day gap in medication fills) were assessed for the new medication during the 180 days following the medication switch. A patient with PDC ≥ 0.8 was considered adherent.

Results: A total of 6,224 patients with PGx testing (72.9% female, mean age = 44.8 years) were matched with 6,224 controls

(73.1% female, mean age = 43.8 years). Patients who received PGx testing had a higher average PDC (controls: 52%, PGx-tested: 62%, $p < 0.001$), were more likely to be adherent (controls: 34%, PGx-tested: 45%, $p < 0.001$), and were less likely to have medication discontinuation (controls: 60%, PGx-tested: 49%, $p < 0.001$).

Conclusions: In this study, PGx testing was associated with improved medication adherence among patients with MDD.

Keywords: Major Depressive Disorder, Pharmacogenomic Testing, Medication adherence, Claims database

Disclosure: Myriad Genetics, Inc.: Employee (Self). Myriad Genetics, Inc.: Stock / Equity - Publicly Traded Company (Self). Acadia Pharmaceuticals: Stock / Equity - Publicly Traded Company (Self)

P258. Elevated Nuclear Circulating Cell-Free DNA (ccf-NDNA) Levels in Older Adults With Depression

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Background: Circulating cell-free DNA (ccfDNA) are small fragments (~166bp) of DNA that circulate freely in various biofluids and at low concentrations (~1-20ng/mL) in healthy individuals. It can be released through multiple mechanisms, including apoptosis, necrosis, NETosis, and active secretion, providing a “snapshot” of different cellular physiological and pathological processes. Studies evaluating ccfDNA as a biomarker of depression have primarily focused on ccfDNA of mitochondrial origin (ccf-mtDNA), with inconsistent findings across studies, however there is limited information regarding ccf-DNA from nuclear origin (ccf-nDNA) in this condition. This study aims to evaluate changes in ccf-nDNA in older adults with major depression by analyzing its concentration and size distribution in plasma.

Methods: This pilot study included 24 older individuals, comprising individuals with major depression ($n = 13$) and healthy controls ($n = 11$). The two groups were matched for age and sex. We extracted ccf-nDNA from archived plasma samples and performed next-generation paired-end short-read sequencing. Nuclear-mapped reads were quantified, and the size distribution of ccf-nDNA fragments was assessed.

Results: Older adults with major depression had significantly higher levels of mapped ccf-nDNA reads compared to healthy controls ($p < 0.05$). Additionally, there was a trend toward a higher level of fragments between 167-177 bp sizes ($p = 0.06$) in the major depression group, suggesting that cellular apoptosis is a possible mechanism of release of ccf-nDNA in this sample.

Conclusions: Our findings indicate that ccf-nDNA levels are elevated in older individuals with major depression. The observed trends in ccf-nDNA fragment size distribution warrant further investigation with a larger sample size to determine the diagnostic utility of ccf-nDNA. In future studies, we aim to perform a more detailed examination of ccf-nDNA fragmentation patterns to gain deeper insights into the mechanisms underlying ccfDNA release in depression.

Keywords: Circulating cell-free DNA, Late Life Depression, Liquid Biopsy, next generation sequencing

Disclosure: Nothing to disclose.

P259. Essential Oils From Medicinal Plants Block Bifenthrin-Induced Depressive-Like Behavior

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Background: Agricultural and domestic control of pests with bifenthrin (BF) has increased human exposure and the risk of pesticide-induced depressive disorder among other harmful effects of this synthetic pyrethroid. The impact of the physical environment on mental health demands treatment approaches targeting causal mechanisms. With the previous mechanistic insights into the antidepressant-like properties of the essential oils of *Pimenta pseudocaryophyllus* (Gomes) LR Landrum (Myrtaceae; OEPP), *Spiranthera odoratissima* A. St. Hillaire (Rutaceae; OESO), *Hydrocotyle umbellata* L. (Araliaceae; OEHU), *Lafoensia pacari* A. St (Lythraceae; OELP), *Murraya koenigii* L. Spreng. (Rutaceae; OEMK), this study assessed the attenuation of depressant-like properties of BF after OEPP, OESO, OEHU, OELP, OEMK, and isolates (oleanolic acid and β -Caryophyllene) treatments.

Methods: Daily oral dose of BF 2.0mg/kg or vehicle 10 mL/kg for 14 days in association with oral vehicle 10 mL/kg, OEPP, OESO, OEHU, OELP, OEMK (all essential oils at the dose of 250 mg/kg), oleanolic acid (OA 10 mg/kg), β -Caryophyllene (BC 10 mg/kg), or fluoxetine (FLX 10 mg/kg) was explored to establish depressant-like behavior or its attenuation in Swiss mice [5-week-old with weight = 28 ± 4 g; $n = 10$ (# male = 5, # female = 5)] randomly distributed into the treatment groups prior to the forced swimming and tail suspension tests (FST and TST, respectively) exposure on the day-14. Corticosterone and cytokines were subsequently quantified. Experimental procedures approved by the Ethics Committee of the Federal University of Goiás (protocol number 052/21) adhered to the NIH Guidelines for the Care and Use of Laboratory Animals. Data were subjected to ANOVA followed by Dunnett's s or Bonferroni's post hoc tests and expressed as mean $\pm$ SEM ($p < 0.05$ was considered statistically significant).

Results: In this preliminary screening, BF increased the immobility time in FST and TST models while increasing the swimming and movement time. These depressant-like behavioral readouts were attenuated consistently in animals treated with OELP and OEMK [FST: $F(9, 90) = 10.7$, and TST: $F(9, 90) = 5.8$, respectively, $p > 0.05$]. The blockade of BF-induced changes in cytokines and corticosterone levels matches well with the previous report and complements *in vivo* findings.

Conclusions: With the dearth of pharmacological interventions in mitigating pesticide-induced depression, these results could further the study on the management of BF effects, bolster research efforts on identifying promising active ingredients in these essential oils, and enrich the scope of environmental neuroscience.

Keywords: Essential Oils, Pesticides, Preclinical assessments, Depression

Disclosure: Nothing to disclose.

P260. Accelerated TBS for Treatment-Resistant Depression: Results From a Randomized Controlled Trial and its Open-Label Phase

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Background: Highly-intensive accelerated theta-burst stimulation TBS (aTBS) treatments present high response rates for treating depression. However, aTBS interventions with relatively lower costs and easier to deliver are currently lacking. Our objective was to determine whether a novel, 78-min aTBS protocol was effective and safe for treatment-resistant depression.

Methods: The TTT (TBS Three Times daily) was a randomized, double-blinded and sham-controlled trial (RCT) in which 100

patients received active or sham aTBS sessions. The open-label phase offered additional aTBS. The study was conducted from July 2022 to June 2024 in a tertiary hospital in São Paulo, Brazil. Included participants were aged 18 to 59 years in a treatment-resistant (i.e., failure of at least two first-line treatments) depressive episode, and a Hamilton Depression Rating Scale, 17-item version (HDRS-17), score above 16. Exclusion criteria were other psychiatric disorders, except for anxiety; and severe clinical conditions. Participants received 45 active or sham stimulation sessions over 15 weekdays. On each day, three 6-min intermittent TBS (triplets of 50 Hz at 5Hz over 2 seconds, followed by a 8-second interval, totalling 1200 pulses per session), spaced by a 30-min interval, were applied over the left dorsolateral prefrontal cortex using the BeamF3 non-neuronavigated method. Non-responders (< 50% improvement from baseline) received 1-6 additional aTBS days of stimulation over 6 months. Analyses were carried out in R, version 4.4.0. For all continuous outcomes, we used 2-level linear mixed-effects regression models (LMM), assuming a linear relationship over time. LMMs included a discrete factor for treatment group, a continuous time factor over 6 time points (baseline and weeks 1-5) and their interaction as fixed effects as well as random effects for the patient-individual intercept and slope. Binary outcomes were modeled using logistic regression with group as the independent variable. LMMs were estimated using restricted maximum likelihood estimation (REML) and significance of model factors was determined using Satterthwaite approximation to degrees of freedom. Effect sizes were reported as Cohen's d (emmeans::eff_size function (Lenth)) for continuous outcomes and odds ratios (OR) for binary outcomes. Blinding integrity was evaluated using Pearson's χ^2 -squared test with Yates' continuity correction (correct guess of group by patient and rater [no, yes]) and independent t-tests (confidence in guess).

Results: Of 2847 prescreened and 431 individually screened volunteers, 100 participants were enrolled and 89 completed the study. Their mean (SD) age was 41.69 (8.80) years, and 84 (84%) were women (Table 1). Dropouts were balanced between groups, being related to non-adherence to the study protocol. Patients receiving active treatment showed superior improvement than sham at study endpoint ($\beta\Delta=4.11$, $t(87.74) = 3.66$, $p < .001$, $d = 0.65$ CI95% 0.29 to 1.00). The mean improvement rates of active and sham aTBS were respectively 54.7% (21.49) and 31.87% (27.04) in the HDRS scale. At the study endpoint, we observed significantly higher response (active: $n = 26$ [52%]; sham: $n = 11$ [22%], OR = 4.11, CI95% 1.66 to 10.13) and remission (active: $n = 17$ [34%], sham: $n = 8$ [16%], OR = 2.73, CI95% 1.03 to 7.24) rates in the aTBS group than in the sham group (Table 2). In terms of cumulative rates (i.e., having reached cutoff at any point during trial, as performed in the SNT trial (Cole et al. 2022)), we observed significantly higher response in the aTBS group than in the sham group (active: $n = 30$ [60%]; sham: $n = 20$ [40%], OR = 2.45, CI95% 1.1 to 5.47), while not in remission were shy of significance (active: $n = 19$ [38%]; sham: $n = 16$ [32%], OR = 1.42, CI95% 0.62 to 3.22). The frequency of all adverse events was similar between groups, except for higher rates of scalp pain (17.4% vs. 4.4%) and increased distractibility (17.8% vs. 4.6%) in the active group at weeks 1 and 3, respectively. Group membership guessing was at the chance level for both groups, and also for both raters and participants. They were also only moderately confident (60-65%) in their guesses. A total of 29 patients showed significant improvement when receiving active aTBS during the open-label, cross-over phase ($= -7.25$, CI95% -8.65 to -5.85), which was significantly larger than their response to sham treatment during the RCT ($= 4.35$, CI95% 2.06 to 6.60). Of the 29 individuals that took part in the crossover, 15 responded. At the end of the RCT phase, the 19 partial responders to active aTBS enrolled in the follow-up phase. Considering all patients (those who responded in the RCT phase, in the open-label cross-over phase and in the extended-treatment phase), the overall response rate was 64% in 6 months.

Conclusions: Compared to other accelerated protocols, our novel aTBS intervention demonstrated a similar improvement for treatment-resistant depression, while being likely less costly and easier to deliver. Clinicaltrials.gov - NCT05388539.

Keywords: Depression, theta burst transcranial magnetic stimulation, Human Clinical trial, repetitive transcranial magnetic stimulation (rTMS)

Disclosure: MagVenture: Grant (Self). SoterixMedical: Grant (Self). FlowNeuroscience: Advisory Board (Self). FlowNeuroscience: Grant (Self)

P261. Preliminary Evidence of Synaptic Deficits in Older Adults With Mood Disorders and Associations With Symptom Severity and Suicide Risk

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Background: Adults aged 65 and older have the highest risk of dying due to suicide than any other age group. Therefore, studying depression, anxiety, and suicide risk in older adults is crucial. Converging evidence suggests synaptic deficits contribute to symptoms associated with stress-related mood disorders. Using positron emission tomography (PET) targeting the synaptic vesicle glycoprotein 2A (SV2A), we previously provided the first in vivo evidence demonstrating lower synaptic density associated with greater depression symptoms in young/middle adulthood. Here, we extend this work into older adults and examine relationships with mood and anxiety symptom severity, as well as suicidal ideation and suicide risk.

Methods: Middle to later adulthood individuals ($n = 18$, 6 male, 56.8 ± 11.6 years) with chronic ($n = 15$) or past ($n = 3$) mood disorders and healthy control (HC) individuals ($n = 22$, 13 male, 57.0 ± 11.8 years) participated in PET imaging with [18F]SynVesT-1. The PET parameter, Volume of Distribution (VT), was calculated using a metabolite-corrected venous plasma input function and served as the measure of synaptic density in primary corticolimbic brain regions of interest (ROIs). The Montgomery-Åsberg Depression Rating Scale (MADRS) and the State-Trait Anxiety Inventory (STAI) were used to evaluate depression and anxiety symptoms, respectively. In addition, we performed a principal component analysis on responses to individual related items on the MADRS (item 10), the Hamilton Depression Scale (HAM-D item 3), and Beck's Depression Inventory (BDI item 9) to generate a composite "Suicide Factor Score" to assess suicidal ideation and suicide risk.

Results: A multivariate analysis revealed significantly lower corticolimbic synaptic density, as measured by [18F]SynVesT-1 and PET, in older adults with mood disorders relative to HC ($F_{3,36} = 3.64$, $p = 0.022$). The most pronounced differences were in dorsolateral prefrontal cortex (dlPFC) and orbitofrontal cortex (OFC), where synaptic density was 22% lower in older adults with mood disorders relative to HC, followed by 20% lower hippocampal synaptic density (all ROIs p 's < 0.01). Among individuals with chronic or past mood disorders, lower corticolimbic synaptic density was associated with greater severity of depressive symptoms ($r = -0.50$, $p = 0.037$). Specifically, lower corticolimbic synaptic density was associated with greater severity of psychic anxiety symptoms (e.g., inner tension; $r = -0.60$, $p = 0.008$), as well as both state ($r = -0.52$, $p = 0.026$) and trait ($r = -0.61$, $p = 0.007$) anxiety symptoms.

A principal component analysis performed on MADRS, HAM-D, and BDI suicide items yielded a one-factor solution that explained 64.9% of the total variance. Individual item responses were multiplied by their respective factor loading values and then

summed to generate a composite Suicide Factor Score, with higher values indicating greater severity suicidal ideation. Lower synaptic density in the dlPFC ($r = -0.53$, $p = 0.037$) and OFC ($r = -0.51$, $p = 0.046$) were associated with higher scores on this measure.

Conclusions: Here, we replicate and extend our previous findings to provide evidence of synaptic deficits in older adults with mood disorders relative to age-matched older adults with no psychiatric history. Further, we demonstrate an inverse relationship between SV2A PET measures of synaptic density and severity of depressive and anxiety symptoms, as well as a putative marker of suicide risk. These findings support the need for future research aimed at identifying psycho-social and biological mechanisms driving synaptic deficits in later life depression and suicide risk and the possible benefit of interventions tailored to the needs of older adults which target synaptic function.

Keywords: Synaptic Vesicle Glycoprotein 2A (SV2A), Positron Emission Tomography (PET) Imaging, Later-life Depression, Suicide Risk

Disclosure: Nothing to disclose.

P262. Proteomic Analysis of ADRD Risk Among Middle-Aged Individuals With Major Depression

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Background: Major depression (MD) has been long associated with an increased risk of Alzheimer's disease and related dementia (ADRD). However, the mechanisms underlying such association are not well understood. In this analysis, we investigated the biological mechanisms linking MD and ADRD using proteomic data from the UK Biobank proteomic and genomic data.

Methods: We included 3,615 individuals with a history of MD at baseline in this analysis, with total follow-up time of approximately 13 years. Proteomic data included 2,920 plasma proteins (OLINK Explore HT 3072 panel). Statistical analyses were carried out using penalized Cox proportional hazard regression and random forest models.

Results: Penalized Cox proportional hazard regression identified a panel of 6 proteins that were significantly associated with the risk of ADRD in MD individuals (GFAP, NFL, VGF, HPGDS, GET3, PSG1, adjusted p -value < 0.05). Random forest analysis showed that this protein panel had a high predictive power to future diagnosis of ADRD among MD individuals (C-statistics = 0.79).

Conclusions: We identified a small protein panel that can accurately identify individuals with MD at risk of developing ADRD. This panel involves proteins related to neurodegeneration and astroglial activation, neuroplasticity, immune and vascular function. Therefore, our results provide novel evidence of biological mechanisms underlying the association between MD and ADRD.

Keywords: Alzheimer disease, Depression, Proteomics

Disclosure: Nothing to disclose.

P263. Radiomics in Psychiatry: Classifying Major Depression Using Multi-Scale Feature Extraction of Serotonin-1A Receptor PET Data

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Background: Higher serotonin-1A (5-HT1A) receptor binding has been well documented in major depression (MDD) relative to

healthy volunteers (HVs) using positron emission tomography (PET) and the outcome measure that approximates receptor density with the fewest assumptions (BPF). However, a clinically translatable biomarker has yet to emerge. Radiomics, which is in its infancy in psychiatry, extends beyond conventional neuroimaging techniques (e.g., PET mean BPF in a region or functional magnetic resonance imaging (MRI) connectivity) to quantify multi-scale textural and shape information through mathematical extraction of interrelationships between voxels. Radiomics has yielded FDA-approved clinical tools in radiology/oncology to inform radiation treatment planning (Microsoft Radiomics App). The purpose of this investigation is to harness radiomics to extend established findings of 5-HT1A elevation in the raphe nuclei of individuals with MDD in an effort to develop biomarkers to inform clinical decision-making. Classifying individuals into those with MDD and those who are healthy (HVs) is explored here. Future analyses are planned in this sample, including examining effects of antidepressant treatment, predicting antidepressant treatment outcome and relating scan findings to specific domains of depression symptomatology.

Methods: Previously published dynamic [11C]WAY100635 PET scans were leveraged for secondary analysis. Participants were currently depressed, medication-free MDD ($n = 46$) and HVs ($n = 30$) (58% female; age 18-70 years). After standard preprocessing, including motion correction and coregistration, voxel-level binding potential (BPF) was quantified using a metabolite-corrected arterial input function and Basis Pursuit. PyRadiomics, an open-source platform that extracts a large panel of radiomic features, was run on voxel-level 5-HT1A BPF values in the whole brainstem, segmented with FSL FIRST, given brainstem raphe nuclei are the site of 5-HT1A autoreceptor expression. Feature classes extracted included first order, 2D and 3D shape-based, and gray level co-occurrence, run length, size zone, dependence, and neighboring gray tone difference matrices. The remaining analyses were run in R v4.3.1. Applying established radiomics workflows, affinity propagation (AP) clustering was used for data reduction, operating on the pairwise similarities between radiomics features across participants. AP clustering offers the advantages of data-driven selection of the number of clusters and selection of exemplar features that best represent each cluster. The set of exemplar features were retained to construct machine learning models. Ten repetitions of re-randomized five-fold cross-validation was used to estimate mean area under the curve (AUC) of the receiver operating curve for common models (linear discriminant analysis, recursive partitioning, k-nearest neighbor, support vector machines, and random forest). Of these five models, the model that maximized AUC is reported. For comparison of radiomics to canonical PET region-level analyses, a linear regression was also run for each exemplar feature. Both machine learning models and linear regressions were repeated using raphe nuclei mean 5-HT1A BPF values for comparison. For multiple comparisons correction of the radiomics linear regressions, the effective number of tests based on the exemplar feature similarities were used. Standardized betas (β) are reported as effect sizes; age and sex were covariates in all models.

Results: Data reduction of the 107 total radiomics features yielded 16 distinct clusters, each with an exemplar feature best representing the cluster. These exemplar radiomic features offered significant improvements when used within a machine learning model classifying MDD from HV participants, over canonical raphe nuclei mean BPF ($t = 4.5$, $p < 0.001$). The radiomics model (random forest) had on average across repetitions 0.75 ± 0.13 AUC, $52.7 \pm 20.9\%$ sensitivity, $79.3 \pm 14.8\%$ specificity, while the raphe mean BPF model had 0.64 ± 0.12 AUC, $47.0 \pm 22.5\%$ sensitivity, $72.2 \pm 14.4\%$ specificity. In canonical linear regressions applied to radiomics features, 8 of the 16 exemplars significantly distinguished participants with MDD from HVs ($p < 0.05$), with one exemplar surviving multiple comparisons correction ($\beta = -0.69$,

standard error (SE) = 0.23, $p = 0.003$). This feature was from the gray level size zone class, which quantifies features of zones within each PET scan that have similar 5-HT1A BPF levels. The feature that distinguished participants with MDD from HVs after correction quantifies the variance in zone size volumes. In comparison, as expected, raphe nuclei mean 5-HT1A BPF was higher in participants with MDD relative to HVs ($\beta = 0.63$, SE = 0.23, $p = 0.007$).

Conclusions: This is a first extension of radiomics to dynamic, fully-quantified PET data in psychiatry to extract meaningful information beyond standard voxel- or region-level binding potentials. Feasibility for improved classification of MDD is demonstrated based on 5-HT1A data, expanding the possibility for biomarker development in this arena. Planned work will pool additional [11C]WAY100635 scans as an independent testing dataset and leverage post-antidepressant PET scans from a subset of these participants to inform treatment effect and outcome predictions. The current radiomics pipeline will also be optimized, e.g., by expanding outside the brainstem and integrating previously multi-modal MRI data.

Keywords: Major depression, serotonin 1a receptor, Positron Emission Tomography (PET) Imaging, radiomics, machine learning classification

Disclosure: Nothing to disclose.

P264. Central and Peripheral ATP Bioenergetics in Young Adults With Depression

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Background: Major depressive disorder (MDD) frequently emerges early in life and is associated with suffering, disability and suicide. The symptoms of low energy and fatigue represent a core dimension of MDD that is particularly associated with disability because it interferes with all aspects of living in the world. Understanding and addressing this problem in the earlier stages of depression will be critical for restoring health and averting long-term negative outcomes. However, the biological mechanisms underlying fatigue in depression are poorly understood, thwarting progress in treatment development. The current work seeks to identify the molecular roots for energy failure in the brain. A recent advance in technology allows for the in vivo investigation of bioenergetic mechanisms in the brain. Functional 31P MRS-MT imaging measurement at ultrahigh field (7 Tesla) can be used to measure steady-state concentration of various phosphorus metabolites, intracellular pH, as well as enzyme activities and adenosine triphosphate (ATP) production rates, both under resting conditions and task conditions. Here, we sought to pair this novel approach for measuring brain energetics with a parallel approach for measuring ATP in blood cells, allowing for a simultaneous investigation of bioenergetics of the brain and body in an early stage of depression.

Methods: We recruited young adults with ($N = 11$) and without ($N = 11$) MDD to participate in the study. All participants underwent a diagnostic assessment including the Mini International Neuropsychiatric Interview, the Montgomery-Åsberg Depression Rating Scale (MADRS), and the Fatigue Severity Scale. On a separate day, they completed a multimodal imaging scan at 7 Tesla that included (i) brain structural information with 1H MRI; (ii) blood-oxygen-level dependent (BOLD) contrast based functional magnetic resonance imaging (fMRI) data in response to a visual stimulation, presented as a 8Hz black-red reversal checkerboard;

and (iii) 3D 31P MRS-MT imaging data with and without gamma-ATP saturation under both resting and stimulation conditions. After each human brain study, a 3D 31P MRS imaging with identical spatial resolution was obtained on a known concentration ATP phantom for quantification of phosphorus metabolites content in the visual cortex. The fMRI data were processed and analyzed to identify significant clusters of activation associated with the visual task. Activation clusters were used as a region of interest (ROI) in the 31P MRS imaging data analysis. We then carried out 31P spectral fitting of the ROI under four conditions. ATP molar concentration as an internal reference in each brain was quantified first. Metabolic contents of other phosphates, rate constants and fluxes or rates of CK and ATPase reactions were then determined within the ROI for both resting and activated states. In addition, participants underwent a blood draw. ATP was measured in peripheral blood mononuclear cells (PBMCs) using an ATP bioluminescence assay. We also used the Agilent Seahorse XF T Cell Metabolic Profiling Kit, which simultaneously measures the flux of extracellular acidification and O₂ consumption under basal conditions and after serial addition of mitochondrial inhibitors and uncoupler (oligomycin A, BAM15 and rotenone/antimycin A).

Results: For 31P MRS, data from 6 participants in the control group, and 5 participants in the MDD group were complete and usable. In resting-state conditions, the MDD group showed significantly greater ATPase-flux in the occipital cortex than healthy controls. During rest, greater FSS scores (and, at a trend, MADRS scores) correlated with greater resting brain ATPase flux levels. FSS also correlated with greater resting brain inorganic phosphate [Pi] contents. During the visual task, the groups showed similar levels of ATPase flux, but the depressed group showed lower concentrations of phosphoethanolamine (PE) and intracellular pH. Higher MADRS scores correlated with lower brain pH during the task.

All participants had usable data for the analysis of ATP in PBMCs. The MDD group showed higher levels of ATP ($p < 0.05$) in comparison to the control group, and both MADRS and FSS scores correlated positively with blood ATP levels. However, the Agilent Seahorse assay demonstrated a lower rate of O₂ consumption in the depressed group following mitochondrial uncoupling, indicating a reduction in capacity to generate ATP under stress.

Conclusions: Here we demonstrate, for the first time, that depression, and particularly fatigue, is tied to abnormalities in ATP bioenergetics that can be measured both in the brain and the blood in young adults with depression. Unexpectedly, we found evidence of increased ATP production in both brain and blood during the resting state in young adults with depression, which correlated with depression symptoms and with fatigue, paired with evidence of reduced capacity for making ATP at the mitochondrial level in the blood. The novel findings suggest a compensatory mechanism that occurs early in the disease stage.

Keywords: Depression, adenosine triphosphate (ATP), Bioenergetics

Disclosure: Nothing to disclose.

P265. Feasibility of the MGH CareDoc Platform for Mobile App Psychotherapy and Digital Phenotyping of Older Adults

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Background: Although mobile application (App) delivered psychotherapy may increase accessibility, there are few resources specifically designed for older adults. Digital phenotyping using smartphone sensors may provide objective measures of behavior

change, but older adults have often not had equitable representation in research studies. We aimed to determine the feasibility of the new, customizable, MGH CareDoc mobile application platform for delivery of a randomized, controlled trial of mentalizing imagery therapy (MIT) and caregiver skills training (CST) for older adult family caregivers of persons living with dementia. We further aimed to capture data from passive smartphone sensors for use in digital phenotyping, and determine whether smartphone data could be used to identify those with normal circadian (daytime) versus disturbed circadian (daytime and nighttime) usage patterns.

Methods: 60 family caregivers of persons living with dementia (mean age 68.3 (8.5 SD)) were randomized to receive 8 weeks of MIT with CST, or CST alone, delivered by the MGH CareDoc platform. CareDoc collected App usage data, passive smartphone sensor data (screen on/off events, geolocation, and accelerometer), and ecological momentary assessments of stress. Caregivers received daily lessons including text learning modules, videos, and audio recordings on MIT, CST, or both, delivered by CareDoc. Feasibility of daily lessons was assessed as usage of an average of at least 3 learning modules per week for 8 weeks. Feasibility of smartphone sensor data collection was assessed by collection of screen on/off events, geolocation, and accelerometer data. Screen on/off events were inspected to determine feasibility of identifying circadian smartphone usage phenotypes, with normal circadian usage defined as usage between 5am and 11pm, and disturbed circadian usage defined as usage between 11pm and 5am. Usability was evaluated with the system usability scale (SUS).

Results: Feasibility of daily lessons was demonstrated by > 75% accessing an average of at least 3 learning modules per week for 8 weeks. Feasibility of smartphone sensor data collection was demonstrated by collection of screen on/off events, geolocation, and accelerometer data in more than 75% of participants. Two distinct phenotypes, characterized by normal circadian usage and disturbed circadian usage, were identified, each comprising about 33% of the population, with the rest falling into an indeterminate phenotype. Usability ratings averaged 88 ± 11 (SD), indicating excellent usability by older adults.

Conclusions: For older adult caregivers, delivery of mobile app psychotherapy and collection of smartphone sensor data was feasible over 8 weeks with the MGH CareDoc Platform, with evidence of persistent engagement with the mobile App over time. Two phenotypes of circadian phone usage were identified, which could potentially indicate those with normal and disturbed circadian rhythms. Usability was high, suggesting promise of the MGH CareDoc platform for delivering customizable psychotherapy interventions for older adults.

Keywords: Digital phenotyping, Mobile application, older adults

Disclosure: Nothing to disclose.

P266. Assessing the Feasibility of Online Body Size Estimation Using a Digital Avatar Tool and Video Capture

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Background: Body image disturbance is a core characteristic of eating disorders and body dysmorphic disorder and is linked to poor body size estimation (BSE) accuracy. Somatmap 3D is a digital avatar tool used to measure body image disturbance that can quantify body-part specific BSE accuracy in combination with actual measurements. Somatmap 3D has previously

demonstrated sensitivity in detecting differences in BSE accuracy in those with anorexia nervosa and those with body dysmorphic disorder compared to controls, as well as in fashion models. Normative models of BSE accuracy are important for research and clinical purposes, as they allow for determining, on an individual level, potential deviance referenced against those of similar demographics and body types. However, general population samples are not highly accurate (with a general tendency for underestimation), and BSE accuracy can additionally vary by factors including gender and BMI. To create such a normative model requires large general population samples, yet it is logistically difficult to measure actual body part sizes in person on a large scale. Machine learning tools have been developed to estimate body sizes from video capture of one's body, which can be obtained remotely. Using such tools in conjunction with Somatmap 3D, we conducted an online pilot study to determine the feasibility, fidelity, and BSE results from data collected online compared to in-person.

Methods: We used Prolific.com to recruit and distribute demographic and psychometric surveys via REDCAP and the Somatmap 3D weblink for the tasks. We included participants from the general population, ages 18-65, living in Canada, the United States or the United Kingdom; of all gender identities and racial/ethnic backgrounds; who provided informed consent; who stated a willingness to comply with all study procedures; and for whom < 5% of their previous tasks on Prolific were rejected for quality issues. Participants were excluded who, by self-report, were currently using psychiatric medications or who had a current substance use disorder. A completion rate of 100% of survey responses was required for the participants to move on to the Somatmap 3D tasks. For the Somatmap 3D task, participants were instructed to adjust the avatar's 24 different body parts' sizes (girth and length) to best represent their current body's size/shape. To obtain estimated measures of participants' actual body sizes, an industry partner created a custom algorithm that reads video capture from a smartphone application to estimate body part sizes for the same 24 body parts. This algorithm was trained and tested on a sample of 42 male and female adult volunteers. The model estimations, compared with the in-person hand-measurement protocol (<https://doi.org/10.17504/protocols.io.e6nvwdpr2lmk/v1>) for each independent body part (the "ground truth"), showed a median absolute value error of 3.11% (IQR 2.6 to 3.72%). We then qualitatively compared results from the Prolific-collected sample with BSE accuracy data from Somatmap 3D and in-person-obtained tape-measured measurements from the general population.

Results: 201 participants (94 males, 107 females, mean $\pm$ SD age: 35 ± 11) obtained the survey link from Prolific and visited REDCAP. Only two participants failed more than one of the four attention checks, and their data was deemed unusable. A total of 117 participants provided usable measurements from both Somatmap and the body size estimation app.

There were no statistical outliers for any of the self-estimated (from Somatmap 3D) or actual body part sizes (from the phone app). Further, the self-estimated and actual body size estimates were anatomically plausible compared with the Civilian American and European Surface Anthropometry Resource (CAESAR) Project North America data set. This is a database of physical dimensions obtained by laser scanning and tape measurements for men and women (N = 2391) of various weights and heights between the ages of 18 and 65. The actual body size estimations from the app were within the minimum and maximum ranges of the comparable body part measurements from the CAESAR dataset. No self-estimations from Somatmap were lower than the minimum and none exceeded 10% above the maximum in this dataset. Further, BSE from the online procedure (N = 117, 68 females, 49 males, mean $\pm$ SD age: 33 ± 10) was comparable to BSE from previously-collected in-person-measured data of individuals in the general

population ($N = 104$, 60 females, 44 males, mean $\pm$ SD age: 26 ± 7): absolute BSE error across body parts ranged from 6%-30% (median 11.6%; IQR 9.2 to 17.8%) for online and 6% to 31% (median 13.4%; IQR 10.2-18.2%) for in-person.

Conclusions: In sum, this pilot experiment shows the feasibility of online data collection of body size estimation. While usable data was not obtained from every online participant, this pilot helped establish and improve our quality control workflow. The results suggest both the fidelity of smartphone app-based actual body size estimation, in the form of low error compared with in-person obtained data, and comparable participant BSE obtained with the combination of the smartphone app and Somatomap 3D for online compared with in-person data. Large-scale online data collection can aid in establishing normative models of BSE, which can be used for clinical and research purposes, including helping characterize neural, behavioural, and emotional contributors to BSE accuracy.

Keywords: Body size estimation, Body dysmorphic disorder, Eating disorders, Digital avatar, Normative modelling

Disclosure: Nothing to disclose.

P267. Mouse Digital Phenotyping: From Food Preference to Circadian Rhythms to Videographic Analysis

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Background: Neuroscientific study of behavior is often limited to experimenter-defined tests and tasks including cognitive tests, spatial tasks, stress tests and other assays. This stands in contrast to reports by patients that baseline spontaneous activity is often strongly perturbed in stress-related disorders such as depression and anxiety. New tools are needed in rodent neuroscience to better quantify baseline home-cage activity to enable the neural correlates of stress and disease.

Methods: We developed the mouse Digital Homepage (DHC) using digital phenotyping principles in human populations but with an eye towards studying combined behavior and brain biology using the advantages of animal models. The system continuously records multiple variables at sub-second resolution including continuous sucrose water preference, fatty/regular food preference, wheel running, actigraphy and dozens of free behavior classes using video data. We developed a software suite to collect, process, analyze and present data for both daily user interface and downstream analysis of preprocessed data. We sought to test the ability of the DHC system to record over long durations of time, across multiple animals at once and to register behavioral change in response to environmental challenge.

Results: We found that the DHC could record for 8 weeks or more continuously across 16 animals simultaneously. The sub-second resolution of behavioral quantification was able to be maintained throughout. In most cases mouse behavior stabilized after 2 weeks of acclimatization in the DHC, supporting the importance of long-term recordings. We observed a strong circadian modulation in many behaviors, with the strongest modulation seen in sleep and wheel running. As an environmental challenge, we introduced a 12-hour shift in the timing of visible light and found that behaviors shifted in 2-7 days, depending on the behavior. We also observed strong inter-individual variation across a variety of measurements, suggestive that even in

genetically inbred lines, we variation that may have relevance to human behavior.

Conclusions: We have established the utility of this new DHC system to record multiple behaviors at high resolution over weeks. This system can be used to study a variety of phenomena from stress, drug response, metabolic alterations and circadian rhythmicity. We also plan for simultaneous DHC behavior recording with brain recording methods such as electrophysiology (already in development), fiber photometry, as well as brain control methods such as optogenetics. We believe this mouse digital phenotyping opens important new avenues into the study of alterations of spontaneous behavior and how those link to brain circuit activity.

Keywords: Mouse models, circadian rhythm, individual differences, Behavioral Phenotyping, Digital phenotyping

Disclosure: Nothing to disclose.

P268. Measurement of Schizophrenia Symptoms Through Speech Phenotyping From PANSS Recordings: A Cross-Study Validation

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Background: Speech is a clinically meaningful indicator of schizophrenia symptom severity. Speech phenotyping in individuals with psychiatric illness often relies on task paradigms embedded into proprietary platforms. Such platforms typically use closed-source code to phenotype speech. Here, we use audio recordings of Positive and Negative Syndrome Scale (PANSS) interviews as source data for patient speech. We then use open source code to calculate speech measures and evaluate them as meaningful indicators of schizophrenia symptom severity.

Our group has previously demonstrated the feasibility of this approach. Using ~180 hours of PANSS interview recordings from a Phase 2 clinical trial (NCT03697252), we demonstrated that such clinical interactions are a reliable source of audio for speech phenotyping (under review at Biological Psychiatry). Here, we report results from an expansion of this project using ~2,000 hours of PANSS interview recordings from three separate clinical trials, leading to what we believe is the largest study on speech-based digital phenotyping in psychiatry to date.

Methods: During three schizophrenia clinical trials sponsored by Karuna Therapeutics, a Bristol Myers Squibb company (NCT03697252, NCT04659161, and NCT04738123), PANSS interviews were audio recorded. Interviews are recorded as part of standard practice in drug development to ensure standardization in scale administration. These recordings were used for digital phenotyping of patient speech, focusing on the following behaviors: amount of speech, rate of speech, pause characteristics, emotional sentiment, and lexical richness. Audio processing was conducted using OpenWillis, an open-source Python library for digital phenotyping (github/bklynhlth/openwillis). The processing included (a) transcription of audio files into text, (b) diarization of patient and clinician speech, and (c) analysis of speech characteristics from the patient's transcript. The measures quantifying the listed behaviors above were compared against PANSS scores using a mixed effects model, with age, sex, race, and the speech measure as fixed effects and patient IDs and visits as the random effects.

Results: A total of 3,482 PANSS interviews were analyzed, totaling approximately 1,984 hours of audio data.

The score on the PANSS positive subscale was associated with a greater amount of speech as measured by the adjusted speech length in minutes (β 3.355; p -value 0.000), adjusted speech length

in words (β 0.027; p -value 0.000), turn length in minutes (β 13.121; p -value 0.000) and turn length in words (β 0.094; p -value 0.000), an increased rate of speech as measured in words per minute (β 0.015; p -value 0.000) and syllables per minute (β 0.009; p -value 0.000), a change in pause characteristics through reduced pause length mean (β -3.819; p -value 0.000) and variance (β -0.572; p -value 0.000) between words, and reduced lexical richness as measured by moving average type token ratio (MATTR; β -13.008; p -value 0.000).

By contrast, the score on the PANSS negative subscale was associated with a reduced amount of speech as measured by the adjusted speech length in minutes (β -2.905; p -value 0.000), adjusted speech length in words (β -0.025; p -value 0.000), turn length in minutes (β -8.838; p -value 0.000) and turn length in words (β -0.082; p -value 0.000), a decreased rate of speech as measured in words per minute (β -0.017; p -value 0.000) and syllables per minute (β -0.010; p -value 0.000), a change in pause characteristics through increased pause length mean (β 4.969; p -value 0.000) and variability (β 1.088; p -value 0.000) between words and increased pre-turn pauses (i.e., time elapsed between the clinician's question and the patient's response; β 0.784; p -value 0.000), and increased lexical richness as measured by MATTR (β 5.302; p -value 0.000).

Certain measures showed the same directional relationship with both positive and negative symptoms. For these, we compared the measures against the overall PANSS score, which was associated with decreased positive emotional sentiment (β -20.472; p -value 0.000) and increased negative emotional sentiment (β 48.358; p -value 0.000).

Conclusions: This work demonstrates that recordings of clinical interactions are a reliable data source for speech-based digital phenotyping. Relationships between speech measures and schizophrenia symptom severity observed were in line with existing literature. The size of the dataset led to a robust observation of these relationships. Use of recordings from multiple studies allowed for an independent validation of the results across the studies.

We plan to continue this work by looking at more recently reported higher order linguistic features that may help quantify aspects of schizophrenia difficult to measure such as disorganized thought and abstract thinking. Speech measures identified as meaningful in assessing schizophrenia symptom severity could be used as features in higher order models predictive of overall disease severity and in stratifying patient populations.

The analysis was done retrospectively on archival data collected as part of standard practice in drug development. This suggests an opportunity to expand this work further using datasets from additional studies otherwise not being utilized. This has implications beyond speech phenotyping in schizophrenia, allowing for similar work to be done in other psychiatric and neurological conditions where the illness may manifest in speech behavior.

Keywords: Digital phenotyping, Automated natural speech analysis, Schizophrenia (SCZ)

Disclosure: Brooklyn Health: Founder (Self). AiCure: Stock / Equity - Privately Held Company (Self)

P269. Racial Discrimination Effects on Subcortical Brain Functional Development

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Background: Experiences and perceptions of racial discrimination are a form of stress that often impacts disadvantaged racial or ethnic groups, contributing to disparities in health and behavioral outcomes. Identifying effects of racial discrimination on brain

development and mental health outcomes may aid in the reduction of health disparities in racial minority youth. For this study, we used data from the Adolescent Brain Cognitive Development (ABCD) Study to identify effects of racial discrimination on neurodevelopment based on subcortical-cortical resting-state fMRI.

Methods: We included 3,321 racial and/or ethnic minority youth with resting-state fMRI scans at baseline (8.9-11.1 years old) and the 2-year follow-up (10.6-12.8 years old), which were divided into a training sample (80%, $n = 2658$) and a testing sample (20%, $n = 663$). Perceived racial discrimination was downloaded as a variable (dim_y_ss_mean) representing the mean score of seven child-reported items. Values from the first and second follow-up visits were averaged to represent reported discrimination during the 2-year analysis timeframe. Resting-state functional connections of three bilateral structural regions-of-interest – amygdala, hippocampus, and nucleus accumbens – with 13 large-scale cortical networks were selected for analysis due to known associations of these subcortical regions with racial discrimination, stress, and psychiatric symptoms. To examine brain development, residualized change in functional connectivity over the 2-year analysis timeframe was calculated for each connection and each subject. Then, for each of the three subcortical regions separately, a linear regression model using elastic-net regularization in R software (glmnet()) examined the ability of developmental changes in the functional connections to predict perceived discrimination. Tuning parameter and variable selection took place within a 10-fold cross-validation of the training sample, and a final model was tested on the independent testing sample. Model significance was assessed with Spearman rank correlations between actual and predicted values. To reduce bias related to many potential confounding variables, observations for each model were weighted according to the calculated propensity to report discrimination based on calculations in WeightIt() in R software, which included demographic variables, psychiatric variables, pubertal stage, head motion, and functional connectivity values at baseline.

Results: Brain development over two years was significantly associated with reported discrimination for nucleus accumbens (cross-validation: $\rho = 0.09$, $p < 0.001$; independent testing: $\rho = 0.11$, $p = 0.004$) and hippocampus: (cross-validation: $\rho = 0.05$, $p = 0.01$; independent testing: $\rho = 0.09$, $p = 0.02$). Amygdala functional connections did not demonstrate significant associations with discrimination in this sample. The functional connections that most significantly contributed to the significant results were between nucleus accumbens and frontoparietal, retrosplenial temporal, and sensorimotor (hand) networks, as well as between hippocampus and cingulo-opercular and sensorimotor (mouth) networks. Each of these connections showed decreases related to perceived discrimination. Further analysis suggested discrimination-related brain changes were consistent with an accelerated brain maturation.

Conclusions: The results indicate potential neural mechanisms that may underlie behavioral effects of racial discrimination, which may depend on accelerated maturation of cortical-subcortical brain connections.

Keywords: racial discrimination, Resting State Functional Connectivity, Adolescent Brain and Cognitive Development Study, Brain development, machine learning

Disclosure: Nothing to disclose.

P270. Neural Response to Social Reward Buffers Against the Effects of Minority Victimization on Sleep and Suicidal Ideation in Youth

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Background: Adolescence is characterized by heightened sensitivity to social feedback and sleep disturbances that may exacerbate risk for suicide, particularly among minoritized youth (e.g., sexual/gender, and/or racial/ethnic minorities). Minority Stress Theory suggests that social threat events (e.g., victimization) exert psychological and physiological stress that contribute to sleep disturbances, predicting enhanced risk for suicidal ideation (SI). However, individual differences in responsivity to positive social experiences may buffer against the negative effects of victimization on sleep, with implications for mitigating SI. Neural response in the ventral striatum (VS) during social reward—a key brain region critical to reward processing—may be relevant for understanding resilience against social threat experiences. In a diverse sample of youth with varying SI severity, we examined sleep disturbance as a mediator linking victimization and SI, as well as whether VS response to social reward moderates the relationship between victimization and sleep disturbance.

Methods: Participants ($n = 98$; aged 14–22; 50% sexual and/or racial minority) were from a larger study examining mood symptoms based on familial history of affective disorders and/or sexual orientation identity, and matched on key demographic characteristics (sexual orientation, race/ethnicity, sex). They completed self-report measures of identity-based victimization (gender, sexual orientation, race/ethnicity, body size), sleep disturbance (PROMIS—Sleep Disturbance), and SI (Suicidal Ideation Questionnaire). An index of victimization was based on the total score across categories. 74 participants completed an fMRI task involving receipt of peer social feedback. Region-of-interest analyses examined bilateral VS activation during receipt of positive feedback (vs. baseline fixation). Between-group differences in victimization, sleep disturbance, and SI were examined using ANOVA. Mediation and moderation analyses were examined via linear regressions using the SPSS PROCESS macro. All analyses controlled for sexual/racial minority status and sex.

Results: SMY (vs. heterosexual youth) reported significantly more severe victimization ($p = .004$), sleep disturbance ($p = .011$), and SI ($p = .001$). Racial minorities reported lower SI severity than White youth ($p = .023$). No other significant group differences were found. Sleep disturbance mediated the association between victimization and SI: Total victimization was positively associated with sleep disturbances ($p = .009$), which in turn predicted more severe SI ($p = .009$), indirect effect: $B = .09$, $\text{BootSE} = .06$, 95% CI [.01; 0.22]. Moderation analyses revealed that the victimization and sleep disturbance link was significant at lower (-1 SD) but not higher ($+1$ SD) VS response to social reward ($p = .038$).

Conclusions: Results highlight sleep disturbance as a pathway to SI, particularly among victimized youth. However, VS response to social reward may protect against the effects of victimization on sleep, with implications for mitigating SI. Findings extend beyond sources of mental health disparities and suggest potential neurobiological mechanisms underlying individual differences in risk and resilience for suicide among minoritized youth. Given the cross-sectional nature of the current research, however, future longitudinal studies are needed. Nevertheless, our findings have implications for intervention, suggesting sleep health and social reward functioning as important treatment targets.

Keywords: suicide, Health Disparities, Social reward, Sleep disturbances, Adolescence

Disclosure: Nothing to disclose.

P271. A Single-Cell Atlas of the Rat Nucleus Accumbens Reveals Molecular Signatures of Oxycodone Addiction

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Background: The opioid crisis is one of the most devastating public health challenges, with addiction rates skyrocketing in recent years. Addiction phenotypes are the results of complex interactions between genetic and environmental factors with multiple genes and their regulatory networks differentially affected by individual susceptibility or drug exposure.

Methods: We measured single nucleus gene expression and chromatin accessibility in the nucleus accumbens (NAc) of 85 outbred heterogeneous stock (HS) that were subjected to extended access to prolonged abstinence from oxycodone intravenous self-administration (IVSA) and exhibited varying levels of addiction-like behavior. Using this data, we employed latent factor analysis and eQTL analysis.

Results: Latent factor analysis revealed a single latent factor that was significantly associated with oxycodone intake, which we refer to as OxyLF. OxyLF primarily consisted of genes expressed in D3 MSNs. Cell abundance analysis revealed that increased D3 MSN abundance was associated with oxycodone intake. We also discovered a single haplotype block upstream of the gene *Oser1*, which contained multiple SNPs that were significantly associated with the OxyLF values.

Conclusions: Our analysis demonstrates an association between oxycodone intake in rats and cell abundance and gene expression programs in D3-MSNs. The identification of the *Oser1* QTL also provides evidence for a potential genetic basis.

Keywords: single-cell genomics, Opioid addiction, Latent Factor Analysis, behavioral genetics, outbred rats

Disclosure: Nothing to disclose.

P272. Two Faces of Reward: Alcohol-Cue and Monetary Assessment of Reward Circuit Functioning Reveals Neural Predictors of Relapse in Alcohol Use Disorder

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Background: Because alcohol use disorder (AUD) is associated with abnormal functioning of the reward circuit of the brain, functional brain imaging may allow prediction of post-treatment relapse. Specifically, the extant literature documents the “hijacking” of the reward system, where alcohol cues preferentially activate reward regions of the brain at the expense of other environmental rewards. Accordingly, we used functional brain imaging to quantify reward circuit function in treatment-seeking veterans with AUD and followed them for 6-months to assess their recovery.

Methods: Veterans with a primary diagnosis of AUD ($n = 127$) were enrolled from residential treatment programs, with 85 individuals included in the final analyses. Participants completed self-report questionnaires, clinical psychodiagnostic assessments and two reward tasks – the monetary incentive delay (MID) task and an alcohol-cue reactivity paradigm – while undergoing fMRI. Follow-up assessments were conducted for six-months post-treatment to assess recovery status. Binary logistic regression models with backward conditional parameter estimates were used to evaluate clinical and neural measures to understand outcome predictors, with bootstrapped validation.

Results: By 6-months following standard residential treatment, 67% of veterans had relapsed. Clinical measures, including smoking status and days since last drink, provided 78% overall classification accuracy for predicting relapse. Neural markers of monetary rewards, including the nucleus accumbens, ventral tegmental area and medial prefrontal cortex activation differences, as well as hyperactivation in nucleus accumbens and

ventral tegmental area to alcohol cues was predictive of relapse and correctly classified 87% of treatment outcomes.

Conclusions: The current study is the first to examine two independent facets of reward circuit function related to treatment outcomes in the same treatment-seeking individuals with AUD. Brain biomarkers of reward circuit function can provide important information about who may be at highest relapse risk following treatment. Importantly, results can guide future novel therapeutics to decrease brain-based risk factors, such as non-invasive brain stimulation or mindfulness-based neurofeedback.

Keywords: alcohol use disorder, relapse and treatment outcome, reward circuitry

Disclosure: Nothing to disclose.

P273. Phenotypes for Alcohol Use Risk and Escalation of Alcohol Use in a Longitudinal Adolescent Cohort: Association With Mu Opioid Receptors

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Background: Alcohol is one of the most commonly used recreational drugs among adolescents, however further investigation is needed to distinguish markers of brain-related risk for alcohol use from those of its consequences on adolescent brain maturation. Considerable heterogeneity in findings is observed across analyses that test regional effects on a per-region basis, an approach that may overlook broader brain-wide patterns of variation. Examination of the spatial patterns of alcohol related effects could provide further insight into biological systems involved in the observed effects. The endogenous opioid system is implicated in alcohol dependence, and the mu opioid receptor is one of the primary targets of naltrexone, used in the treatment of alcohol dependence. This analysis uses multi-level modeling to characterize spatial patterns of between- and within-person effects related to alcohol use, and to examine their relationship to the distribution of the mu opioid receptor.

Methods: Participants ($n = 151$; 55% female) aged 12-17 were invited to undergo 3 MRI scans (Neuroventure study). Participants were drawn from a population-based cohort based on their high- or low-risk for substance misuse. In total, 136 (90%) participants had high-quality MRI (T1-weighted) and behavioural data available for two or more timepoints (381 scans). Alcohol use was disaggregated into between- (vulnerability) and time-varying within-person components using multi-level modeling. Analyses controlled for age, sex, cannabis use, and tobacco use. Sensitivity analyses of cortical thickness phenotypes utilized independently published data from studies examining early initiation of alcohol use (ABCD study, $n = 9804$) and polygenic risk score for problematic alcohol use (ABCD study, $n = 3013$). Data for regional availability of mu opioid receptors was obtained from an open dataset ($n = 204$) and parcellated using Neuromaps. Spatial associations were tested with non-parametric spatial permutation tests using the ENIGMA toolbox.

Results: In the Neuroventure sample, cortical thickness was lower in years when participants' alcohol use exceeded their own average level of alcohol use ($F_{1,25378.3} = 3.92$, $p = 0.047$). The effect of a within-person increase in alcohol use of once-per-week on thickness was comparable to 14% of the annual rate of age-related cortical thinning (mm/year). Overall, greater mean alcohol use was associated with a pattern of effects that varied across brain regions (region*alcohol interaction: $F_{33,25368.9} = 3.24$, $p < .0001$), characterized by a pattern of lower thickness in frontal, cingulate, insular and medial temporal cortices and greater cortical thickness in parietal and occipital cortices. This spatial pattern followed the distribution of MORs ($r = -0.53$, $p = .007$). Similarly, the distribution of MORs predicted cortical thickness

phenotypes associated with polygenic risk score for problematic alcohol use ($r = -0.41$, $p = .024$; ABCD baseline), early initiation of alcohol use in adolescents ($r = -0.52$, $p = .010$; Neuroventure sample) and in 9-10 year-olds ($r = -0.47$, $p = .018$; ABCD baseline). In contrast, the cortical thickness phenotype reflecting within-person increases in alcohol use was not significantly associated with the distribution of MORs ($r = -0.32$, $p = .084$).

Conclusions: This study provides evidence that within-person escalation of alcohol use during adolescence is associated with brain-wide reductions in cortical thickness. Analysis of between-person effects across independent cohorts provides evidence of a cortical thickness phenotype associated with risky alcohol use that precedes the onset of alcohol use, detectable as early as 9-10 years-old. This cortical thickness phenotype was associated with the distribution of MORs. Finally, greater between- and within-person alcohol use were associated with spatially distinct patterns of effects on cortical thickness, which may reflect distinct underlying biological origins.

Keywords: alcohol, adolescent, longitudinal, mri, cortex

Disclosure: Nothing to disclose.

P274. Nicotine E-Liquid Composition Regulates Nicotine Vapor-Seeking Behavior in Adolescent Male and Female Rats

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Background: Propylene glycol (PG) and vegetable glycerin (VG), two constituents highly present in most nicotine e-liquids, have been shown to impact nicotine's delivery and subjective effects in e-cigarette users. As with traditional cigarettes, evidence suggesting increased susceptibility to e-cigarette use in vulnerable populations exists. For example, it is well known that e-cigarette use is most prevalent in adolescents, with e-cigarettes now preferred over traditional cigarettes within this population. Similarly, it has also been shown that females prefer higher concentrations of nicotine in e-cigarettes and display greater symptoms of nicotine dependence relative to males. Thus, the goal of the present study was to examine the self-administration of nicotine vapor plumes composed of varying ratios of PG and VG, in male and female adolescent rats.

Methods: Adolescent male ($n = 20$) and female ($n = 21$) rats were passively exposed to vapor plumes containing 24 mg/mL nicotine and of one of three PG:VG ratios (50:50, 80:20, or 20:80) for 6 consecutive days. Following passive exposure, rats were allowed to self-administer nicotine vapor with their assigned PG:VG ratio using nosepoke responses on a fixed ratio 1 schedule with a 2-min timeout period after delivery, in 90-minute daily sessions. Rats self-administered plumes with varying concentrations of nicotine for a total of 24 days, in the following order: 24 mg/mL nicotine for 6 days, 6 mg/mL nicotine for 12 days, 12 mg/mL nicotine for 6 days. All rats then underwent 14 days of extinction training before being tested for the reinstatement of vapor-seeking behavior, following the presentation of a single vapor plume containing 24 mg/mL nicotine and their assigned PG:VG ratio. Total nicotine vapor plume deliveries and active port nosepokes were compared between PG:VG ratio groups and sexes for each nicotine concentration using mixed-model ANOVA, followed by Fisher's LSD or t-test post-hoc analyses. Blood plasma cotinine levels were also compared between PG:VG ratio groups and sexes on day 6 of passive vapor exposure and day 12 of nicotine vapor self-administration, using one-way ANOVA and Fisher's LSD post-hoc analyses.

Results: Adolescent rats self-administered nicotine vapor plumes at all concentration of nicotine used, regardless of the

PG:VG ratio. While no differences were seen between males and females in the self-administration of any of the nicotine vapor concentrations tested for any of the PG:VG ratios used, a significant effect of PG:VG ratio was observed across all rats and nicotine concentrations tested ($F(2,35) = 3.27$, $p < 0.05$). Specifically, results reveal that rats had significantly less nicotine vapor-seeking behavior when self-administering the PG80:VG20 ratio relative to the PG50:VG50 ratio ($p = 0.02$) and the PG20:VG80 ratio ($p = 0.04$). Analysis of extinction of nicotine vapor self-administration did not result in differences between sexes or PG:VG ratios. However, a test of drug-primed reinstatement occurring 3 weeks after extinction showed that females had significantly more nicotine vapor-seeking behavior than males when using the PG20:VG80 ratio ($p = 0.05$). Analysis of blood plasma cotinine levels did not result in any significant effects of sex or PG:VG ratio on either of the two days assessed.

Conclusions: The described study provides a preclinical model of nicotine vapor self-administration that results in robust and persistent nicotine vapor-seeking and delivery. Findings suggest that the reinforcing effects of nicotine vapor are regulated by the composition of the e-liquid, with formulations higher in PG resulting in reduced vapor-seeking behavior. These findings are in line with clinical studies reporting higher aversive sensations (e.g., unpleasant “throat hit”) when nicotine e-liquids containing higher levels of PG are used. Higher reinstatement of nicotine vapor-seeking behavior was also observed in females relative to males, a finding that aligns with clinical studies in e-cigarette users and preclinical studies using nicotine intravenous self-administration. The development of a valid and reliable pre-clinical model of e-cigarette use will be necessary to determine how the unique constituents of e-liquids contribute to nicotine abuse liability and whether they promote substance use disparities in vulnerable populations.

Keywords: Nicotine, E-Cigarette, E-Liquid, Adolescents, Rat

Disclosure: Nothing to disclose.

P275. Role of Cortical CRF Neurons in Stress-Induced Alcohol Consumption

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Background: Stress is a major contributing factor driving relapse in individuals with alcohol use disorder (AUD), which is characterized as a chronic and relapsing neuropsychiatric condition. Corticotrophin-releasing factor (CRF) is a stress neuropeptide that has long been the focus of alcohol research, largely in the context of extended amygdala and hypothalamic circuitry, however CRF is expressed fairly ubiquitously throughout the cortex and many subcortical regions. One such region that has been implicated in regulating alcohol consumption is the medial prefrontal cortex (mPFC).

Methods: Here we utilize several transgenic mouse lines to interrogate the role of CRF in the dorsomedial PFC (dmPFC), specifically the prelimbic (PL) cortex in regulating stress-enhanced alcohol consumption. First, we characterized the expression, distribution, morphology and molecular phenotype of CRF+ neurons in the PL in order to better understand how changes in CRF neuron activity in response to stress and alcohol might be influencing PL output activity. We then characterized the effects of chronic stress (forced swim stress, FSS) and alcohol (chronic intermittent ethanol, CIE) exposure on CRF neuron physiology in the PL using Crh-Cre::Ai14 reporter mice and slice

electrophysiology recordings with biocytin labeling. We next utilized Crh-Flox mice to conditionally knockdown Crh expression in the PL and examine the effects on stress-enhanced alcohol consumption using the CIE-FSS model that models chronic stress effects on drinking. And finally, we inhibited CRF+ neurons in the PL using chemogenetics in Crh-Cre mice to determine whether blocking CRF neural activation by stress was sufficient to preclude escalated alcohol drinking.

Results: Expression of CRF is found nearly evenly distributed throughout layers 2-5 of the PL and infralimbic (IL) cortices of the mPFC, with slightly (but not significantly) denser expression in PL compared to the IL ($p = 0.12$), and an increasing gradient from anterior to posterior PL. While, CRF has been reported to be co-expressed in both GABAergic and glutamatergic neurons in the PL, we found that approximately 92% of neurons were GABAergic. The GABAergic-CRF subtype in the PL can be further categorized into those that go into depolarization block, with approximately 33% of CRF+ neurons in naive mice entering depolarization block at higher current injection steps. CRF+ cells differ in morphology, which correlates to physiological phenotype. Interestingly, when exposed to the CIE-FSS paradigm, GABAergic-CRF neurons become hyperexcitable, showing decreases in rheobase ($p < 0.01$) and a significant leftward shift in the dose response curve for current-evoked action potential firing ($p < 0.05$). Unexpectedly, ~96% of GABAergic-CRF neurons in PL are sensitive to depolarization block at current steps higher than 100pA, which represents a significant shift in excitability in this population after stress and alcohol exposure. Further, we knocked down Crh in the PL through Cre-Lox excision in the Crh-Flox mice and, as expected, we saw > 55% knockdown in functional CRF protein product as confirmed by western blot. Using this knockdown approach, we show a complete blockade of the stress-enhanced escalation of alcohol consumption in Crh-knockdown mice exposed to CIE-FSS compared to Crh-intact CIE-FSS mice and unstressed controls ($p < 0.05$, and $p < 0.01$, respectively). Finally, chemogenetic inhibition of CRF+ neurons in the PL prior to alcohol access significantly reduced alcohol drinking ($p < 0.05$) regardless of exposure to stress.

Conclusions: Taken together, we have identified the dmPFC as a critical hub for CRF effects on stress and alcohol interactions. Chronic alcohol and stress recruit CRF in many regions throughout the brain, but GABAergic-CRF neurons in the PL mPFC are particularly sensitive to these effects becoming persistently hyperexcitable in response to chronic stress and alcohol exposure. Hyperexcitability and activity in local CRF circuitry in the PL are crucial for driving stress-enhanced alcohol consumption, as knockdown of Crh in the PL completely blocks this effect, however inhibition of these neurons prior to drinking was sufficient to reduce consumption regardless of stress history. While intact CRF signaling in the PL is crucial, future transcriptomic experiments will examine synaptic changes induced by excessive CRF signaling in response to chronic stress and alcohol to identify additional druggable targets for treatment of this sensitive population, as the CRF receptor system is known to be a dynamic target incapable of sustained reductions in drinking.

Keywords: Alcohol dependence, chronic stress, Corticotropin-releasing factor (CRF)

Disclosure: Nothing to disclose.

P276. Neural Substrates of 17 β -Estradiol-Induced Potentiation of Cocaine-Primed Reinstatement in Ovariectomized Female Rats

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Background: There are gender differences in patterns of behaviours related to cocaine use disorder (CUD), with women being more sensitive to relapse in the face of stress, cocaine-associated stimuli, and to the interoceptive stimuli evoked by cocaine itself when compared with men. These gender differences involve ovarian hormones, with high levels of 17 β -estradiol (E2) being associated with higher rates of cravings compared with low levels of E2. Furthermore, in rat models, the magnitude of cocaine-primed reinstatement is greater in females than males, and this difference is shown to be driven by E2 in females. Determining the neural substrates of this effect, such as how and where E2 is influencing reward and reinforcement circuitry, may aid in the prevention of relapse to cocaine use.

Methods: For this preliminary data, fourteen female rats were ovariectomized and implanted with indwelling jugular catheters. After 2 weeks of recovery, rats were allowed to self-administer intravenous cocaine (0.5mg/kg/infusion) in compound with the illumination of a cue light in operant conditioning chambers in 2hr daily sessions. Cocaine infusions were administered after responses on the active lever on a fixed-ratio 1 schedule of reinforcement for 24 daily sessions. There was a 20-s timeout after each infusion earned. After acquisition, the reinforcer was then swapped with saline, and cocaine seeking was extinguished over 22 daily sessions. After the final day of extinction, rats were treated with either E2 (N = 7) or vehicle (N = 7) 24h and 1h before a 90-min reinstatement session primed by 10mg/kg cocaine administered intraperitoneally. Rats were perfused and the immediate early gene, cFos, was quantified as a marker of neuronal activation in the nucleus accumbens core (NAcC) using immunohistochemistry.

Results: When compared against the last extinction session, cocaine-primed reinstatement of active lever pressing was greater in rats pretreated with E2 than rats pretreated with vehicle [Treatment*Timepoint: $F(1,12) = 4.801$, $p = .049$, $\eta^2 = 0.286$]. This effect was driven by greater active lever pressing during the reinstatement session rather than during the extinction session in rats treated with E2 compared with rats treated with vehicle ($LS_D = 21.400$). There was a trend toward a greater number of cFos+ cells in the NAcC in animals treated with E2 relative to those treated with vehicle ($p = .071$).

Conclusions: These findings suggest that interactions between the actions of cocaine and estrogen receptors in the NAcC, a key hub of the reinforcement circuitry, could have clinical implications. These should be considered when developing pharmacological and behavioural interventions in women with cocaine use disorders and could help prevent turning a lapse into a relapse.

Keywords: cocaine reinstatement and taking, estrogen receptor, Drug Relapse

Disclosure: Nothing to disclose.

P277. Withdrawal From Chronic Alcohol Increases Panic-PTSD-Relevant Behaviors in Male and Female Mice

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Background: Alcohol use disorder (AUD) increases risk to develop comorbid psychiatric disorders like panic disorder and PTSD. Treatments for these disorders are limited and co-occurrence worsens treatment outcomes. This points to a need for novel treatments. AUD, panic and PTSD have overlapping genetics and symptomology suggesting they share underlying molecular mechanisms and neurocircuitry. Emerging evidence supports dysregulated acid-base homeostasis as a shared mechanism in AUD, panic and PTSD. 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 evokes acidosis, a potent homeostatic threat, which positively correlates with withdrawal severity. Thus, alcohol's effects may result from a drive to restore homeostasis. Mechanisms combating threats to homeostasis can cause lasting changes in the reactivity in these systems. This can increase risk for later psychiatric disorders (i.e. panic disorder, PTSD). Homeostatic stressor CO₂ inhalation (non-hypoxic) produces acid-base imbalance and elicits greater fear/anxiety, hyperventilation, and cardiovascular effects (even panic attacks) in panic disorder and PTSD patients, in previously alcohol-dependent individuals, and during alcohol withdrawal. Thus, dysregulated acid-sensing mechanisms likely contribute to AUD, panic and PTSD pathophysiology. They may also facilitate the increased risk to develop panic or PTSD in previously dependent individuals. Investigating the effect of alcohol use on CO₂ sensitivity may yield unique and impactful findings, increase our understanding of comorbidity and help prevent or treat these disorders. Therefore, we recently developed a model to investigate the role of chronic alcohol use and withdrawal on CO₂ sensitivity in mice. Additionally, men and women differ in their rates for these disorders with rates of AUD higher in men, while panic disorder and PTSD are more common in women. Thus, we sought to understand how male and female mice differ in their responses to CO₂ inhalation following withdrawal from chronic alcohol.

Methods: Adult male and female C57/BL6J mice received control (4-methylpyrazol (4-MP) 9mg/kg) or ethanol (2g/kg) + 4-MP injections for 9 days then underwent the CO₂ inhalation paradigm. On the last day of treatment (day 9), mice were habituated to the inhalation chamber for 7m. They received their last injection immediately after removal from the chamber and were returned to their homecages. The next day at 24h of withdrawal mice were exposed to breathing air or 5% CO₂ inhalation for 10m. After 90 m, brain tissue was collected for immunohistochemical studies investigating the effects on neuroimmune factors and regional neuronal activation. We quantified fear-relevant defensive behaviors (freezing and rearing) in response to CO₂ inhalation. Data were analyzed by 3-way ANOVA. $n = 10-12$ /group

Results: We found significant overall effects of sex ($p < 0.05$), ethanol treatment ($p < 0.01$) and air/co₂ inhalation ($p < 0.01$) on freezing in the CO₂ inhalation paradigm. Similar overall effects were seen on rearing. Overall, males had higher freezing, while females had higher rearing with the CO₂ inhalation paradigm. However, we found withdrawal from chronic ethanol increased CO₂-evoked defensive behaviors (increased freezing, reduced rearing) in both male and female C57BL/6J mice ($p < 0.05$). Posthoc tests revealed that within each sex, there were significant differences between the ethanol treated, CO₂-exposed mice and all other treatment groups (vehicle treated/air, vehicle/CO₂, and ethanol/air). There were no other significant differences between groups. Interestingly, ethanol treated male mice had increased variability in their CO₂ responding compared to female mice.

Conclusions: These data suggest chronic ethanol use and withdrawal increases panic and PTSD-relevant behaviors in male and female mice. Ongoing studies are investigating the molecular mechanisms and circuitry underlying this effect. Overall, these data support the use of this highly translational chronic alcohol use/withdrawal and CO₂ inhalation model to improve our understanding of shared mechanisms underlying comorbid AUD, panic and PTSD.

Keywords: alcohol withdrawal, panic disorder, PTSD, comorbidity, Acute Stress

Disclosure: Nothing to disclose.

P278. Ventral Tegmental Area Neurons Differentially Encode Fentanyl and Fentanyl Place Preference Depending on Sex and Downstream Projection Target

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Background: Neurons in the ventral tegmental area (VTA) undergo molecular and physiological changes in response to opioid use that are heavily implicated in drug relapse. Previous work indicates morphine exposure induces structural and functional adaptations in VTA dopamine neurons depending on their downstream projection target, however no work has examined how this translates to different circuit activity during behavior, or if this extends to the synthetic opioid fentanyl. Here, we use an intersectional viral strategy to monitor in vivo calcium dynamics and test the hypothesis that VTA neurons that project to nucleus accumbens (NAc) or medial prefrontal cortex (PFC) differentially encode aspects of fentanyl reward.

Methods: All experiments were conducted in young adult mice, using sex as a biological variable (defined gonadally, $n = 5-7$ per sex per projection). 8 week old mice received retrograde AAV-Cre in either the NAc or PFC, Cre-dependent GCaMP8s in the VTA, and a unilateral fiber optic cannula over the VTA. Mice underwent baseline behavior testing in a 3-chamber social interaction apparatus and an elevated zero maze, prior to entering into a 5 day, biased design fentanyl conditioned place preference experiment. During the pre-test day, mice were allowed free access to all 3 compartments. For conditioning days, mice received saline (10 mL/kg) in the morning administered in their preferred side, followed 4 hr later by fentanyl (0.2 mg/kg IP) in the afternoon in the non-preferred side. On the test day, mice were allowed free access to all 3 compartments. We recorded GCaMP8 with a wireless fiber photometry system (Telefipho) during all test and conditioning sessions, and aligned z-scored $\Delta F/F$ to TTL-timestamped behavioral events in MATLAB.

Results: After conditioning, all mice preferred the fentanyl paired side, with no difference in CPP score across sex or virus group. (Two-Way ANOVA, Sex: $F_{1,20} = 0.91$, Virus-Group $F_{1,20} = 0.06$, p 's > 0.4). We compared calcium transient amplitudes, durations, and frequency during conditioning across sex and projection neuron population. In females, VTA to NAc projections exhibited increased calcium transient amplitude during fentanyl conditioning relative to saline (Kolmogorov Smirnov $p < 0.0001$). In contrast, male VTA to NAc projections exhibited decreased amplitude (K-S $p < 0.0001$). Both sexes showed decreased transient duration, and increased transient frequency (Kolmogorov Smirnov all $p < 0.02$). For VTA to PFC projections, both sexes showed increased transient amplitude during fentanyl conditioning relative to saline (K-S p 's < 0.006). In contrast to the NAc projections, PFC projections had baseline sex differences in transient duration and frequency, but were insensitive to fentanyl. Entries to the fentanyl paired chamber were differentially encoded by the projection neuron populations in a sex-specific manner: Male VTA to NAc, but not VTA to PFC, exhibited increased $\Delta F/F$ during entry into the fentanyl paired compartment relative to saline (nested t-test, $t_{1,8} = 2.4$ $p = 0.04$). In contrast, female VTA to PFC, but not to NAc, had higher $\Delta F/F$ during entry into the fentanyl paired compartment relative to saline ($t_{1,12} = 2.2$, $p = 0.04$).

Conclusions: Our data demonstrate that neurons in the VTA do not respond uniformly to fentanyl and fentanyl-context associations, and that patterns of calcium activity are dependent on both downstream projection target (mPFC or NAc) and sex. Ongoing

experiments are asking if these patterns hold true when recording from exclusively dopamine neurons, and the extent to which dopamine receptor signaling in the downstream projection targets influences fentanyl place preference.

Keywords: Fentanyl, In vivo fiber photometry, conditioned place preference, Ventral Tegmental Area (VTA)

Disclosure: Nothing to disclose.

P279. The Effect of Minocycline on the Gut Microbiome in Individuals With Alcohol Use Disorder and Healthy Controls

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Background: In animal models of alcohol use disorder (AUD), the tetracycline antibiotic minocycline reduced alcohol consumption, preference, withdrawal symptoms, and alcohol-induced neurotoxicity. Further, minocycline improved anxiety- and depressive-like behaviors in rodents, and these effects were mediated and preceded by minocycline's effects on gut bacteria. Thus, minocycline's effects on the gut microbiome may be critical in its potential beneficial effects for AUD and other psychiatric disorders. However, the effects of minocycline on alcohol-related behaviors and the gut microbiome in humans have not been well studied. The purpose of the present study was to examine the effects of four weeks of treatment with minocycline, vs. placebo, on the composition of the gut microbiome in individuals with AUD and controls.

Methods: This study analyzed $n = 256$ fecal samples collected and sequenced from actively drinking, non-treatment seeking individuals aged 25-65 with DSM-5 AUD ($n = 30$; ≥ 14 or 7 drinks per week for men and women, respectively, and ≥ 5 heavy drinking days [HDD] per month [HDD = ≥ 5 drinks in a day for men, ≥ 4 drinks for women]) and healthy controls ($n = 26$; < 14 or 7 drinks per week for men and women, respectively and ≤ 2 heavy drinking days over the last 6 months) who were recruited from the general community to participate in a 4-week randomized, placebo-controlled minocycline trial (NCT04210713). Participants in both groups had to be generally healthy and medically cleared to participate in a medication trial; no participant with AUD had a clinically significant alcohol associated liver disease. Participants provided fecal samples at baseline (pre-medication) and 4 following weekly visits. Participants were provided a take home fecal sample collection kit consisting of a test tube with chaotropic solution (DNA/RNA Shield, Zymo Research) as well as collection and storage materials and instructions. Participants were instructed to collect a stool sample as close as possible to their next scheduled study visit and immediately store their sample in their freezer until that time. Upon arriving to the study baseline with their frozen sample, fecal samples were then stored in a -80°C freezer until sequenced. Medication adherence and stool sample collection adherence were both $> 95\%$. Hypervariable regions V3 and V4 of the bacterial 16S rRNA gene were amplified with primers 319F and 806R. High-quality amplicon sequences were obtained on an Illumina HiSeq 2500 modified to generate 300 bp paired-end reads. Amplicon sequence variants were generated by DADA2 and taxonomically classified using the RDP Naïve Bayesian Classifier trained with the SILVA v128 16S rRNA gene database. Comparisons of alpha diversity and taxa differences were performed using multivariable linear regressions. Principal Coordinate Analysis using Bray-Curtis dissimilarity was performed to assess beta diversity. Permutational multivariate analysis of variance (PERMANOVA) using adonis and mixed effect models in R were used to compare group differences at baseline and the

full model analyzing Medication, Group, Time, and their interactions; all models controlled for Age, Sex, BMI, and Diet. In addition, multivariate association with linear models (MaAsLin2) was used to compare relative abundance levels of individual taxa between groups.

Results: At baseline, the AUD group vs. control group had significantly different microbial composition (i.e., Beta Diversity; $R^2 = 0.02$, $F = 4.67$, $p < 0.05$), but groups did not differ in measures of alpha diversity. The abundance of several individual taxa differed between groups at baseline, including *Coprococcus* 3, *Phascolarctobacterium*, *Prevotella* 7 and 9, and *Lachnospiraceae* ND3007 group (FDR corrected $ps < 0.05$). Over four weeks of treatment, the group difference in microbial composition was maintained (Group main effect; $R^2 = 0.02$, $F = 4.67$, $p < 0.05$). Minocycline, vs. placebo, significantly altered microbial composition over time (Medication x Time; $R^2 = 0.01$, $F = 0.42$, $p = 0.002$), and this effect differed between AUD and control groups (Medication x Group x Time; $B = -0.01$, $p < 0.05$).

Conclusions: The composition of the gut microbiome differed between actively drinking individuals with AUD and healthy controls, and this effect may have been driven by differences in the abundance of taxa involved with short chain fatty acid production (*Coprococcus* and *Lachnospiraceae*), lipid metabolism (*Phascolarctobacterium*), and inflammatory processes (*Prevotella*). Further, four weeks of treatment with minocycline differentially altered the composition of the microbiome between individuals with AUD vs. controls. Next steps will be to determine how minocycline-induced changes in gut microbiome composition relates to primary outcome measures (i.e., alcohol consumption, cognitive performance, MRI, etc.) of the four-week trial.

Keywords: alcohol use disorder, Gut Microbiome, minocycline

Disclosure: Nothing to disclose.

P280. Smaller Hippocampal and Amygdala Volumes in Adolescents Prior to Initiation of Substance Use

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Background: Examination of youth prior to engagement in risk behaviors (e.g., substance use) may help establish neurobiological signatures that prospectively predict heightened susceptibility for initiation and continued use. This study prospectively investigated the initiation of substance use and hippocampal and amygdala volume, regions that undergo essential developmental changes during adolescence and that are known to be vulnerable to substance use.

Methods: Participants were 59 healthy, alcohol- and drug-naïve, 13-14-year-old adolescents. After completion of a baseline visit, participants were followed over three years, during which time they completed quarterly substance use assessments. Based on these assessments, youth were stratified into two groups: those who endorsed the use of alcohol, cannabis, or nicotine after baseline (initiators, $N = 21$) and age-matched adolescents who did not initiate the use of any substance (non-initiators, $N = 38$). Participants were matched on age, sex, and handedness. Magnetic resonance imaging data were acquired at 3.0 Telsa, and Freesurfer analysis was performed to measure structural subcortical brain volumes.

Results: A general linear model was used to examine the effect of initiation group on subcortical volumes, with sex and family history (FH) status of substance use included as covariates. Initiation of substance use was associated with smaller bilateral

hippocampal (left $p = .006$; right $p = .011$) and left amygdala ($p = 0.007$) volumes, as compared to the non-initiator group.

Conclusions: These findings suggest that in those who go on to initiate alcohol or other substance use, the differential pattern of subcortical volumes may reflect a potential neurobiological vulnerability to risk-taking. These findings are critical given that the hippocampus and amygdala play crucial roles in addiction due in part to their involvement in memory, emotion, and reward processing, which are key components of addictive behaviors. However, structural differences in other subcortical structures (e.g., caudate, putamen, pallidum, nucleus accumbens) could emerge as a later consequence of use. Ongoing longitudinal data analysis will further parse neurobiological vulnerabilities that reflect both antecedents and consequences of use on developing adolescent neurobiology.

Keywords: Adolescent, Substance Use Initiation, MRI, Hippocampus, Amygdala

Disclosure: Nothing to disclose.

P281. Disrupting Drug Memories to Diminish Drug Cue-Reactivity Using Reconsolidation and Methylphenidate in Addiction: A Double-Blind Randomized Clinical Trial in Individuals With Cocaine Use Disorder

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Background: Drug addiction is characterized by excessive salience attributed to drug at the expense of nondrug cues. Memories that have been previously associated with such salient drug cues may underpin intense physiological and psychological states (e.g., cue reactivity and craving) that may interfere with abstinence goals. Cue-exposure therapies, rooted in preclinical extinction paradigms that are associated with enhanced ventromedial prefrontal cortex (vmPFC) function in humans, have been widely used to diminish the salience of drug-associated cues in individuals with drug addiction with mixed results. Potentially by bypassing or diminishing vmPFC-mediated processes, reconsolidation renders the memory labile for disruption by altering it post-retrieval, previously shown in a behavioral study to decrease heroin craving. Here, in a double-blind, within-subject, randomized clinical trial using fMRI, we administered methylphenidate (MPH), a dopamine agonist that has also been shown to reduce drug cue-reactivity, to further perturb vmPFC function and enhance reconsolidation to improve extinction retention in cocaine addiction.

Methods: Eighteen individuals with cocaine use disorder (mean age: 40.6, 6 women) underwent Pavlovian conditioning in a novel task where two sets of distinct neutral visual cues (conditioned stimuli: CS+ and CS-) were partially reinforced with a 3-second clip of drug use (i.e., smoking a crack cocaine pipe) or a matched non-drug action (i.e., holding a pen near mouth) unconditioned stimuli, respectively. Four hours later, participants were orally administered 20 mg MPH or placebo. Then, an hour later (during peak MPH concentration) and in the MRI scanner, participants were presented two trials of some of the CS+ (i.e., reminded CS+) but not others (i.e., non-reminded CS+) to induce reconsolidation (i.e., retrieving the CS+ memory and updating it with a no explicit unconditioned stimulus association). Ten minutes later (as consistent with reconsolidation practices), participants underwent standard extinction during fMRI over the course of three runs. Participants returned the next day for estimates of drug biased behavior, whereby we administered a choice task in which drug >

pleasant choices index simulated drug seeking, as shown to correlate with concurrent and prospective (i.e., 6 months later) drug use. Participants returned a week later to complete the second, identical, study arm (i.e., same conditioning and extinction procedures and stimuli) with the remaining pill. The fMRI data were analyzed using standard procedures, with a cluster defining threshold of $Z > 3.1$, corrected to $p < .05$, small volume corrected to an atlas-based mask of the vmPFC (combined with a spherical mask of the amygdala for pertinent subcortical explorations).

Results: Our preliminary results indicate an increase in vmPFC function over the course of extinction (run3 > run1) during reminded vs. non-reminded trials in pill A as compared to pill B (to be unblinded upon trial completion at $n = 23$), $Z = 4.30$, $p = .003$, 24 voxels. Higher activity in this region further correlated (voxel-wise) with more drug-biased choices the next day ($Z = 4.06$, $p = .028$, 8 voxels), suggesting that those who show the highest bias towards drug (vs. alternative pleasant) cues benefit most from the (potentially) MPH-enhanced reconsolidation effects on the vmPFC.

Conclusions: Promoting the “forgetting” of disruptive memories in drug addiction may reduce drug-biased salience attribution and enhance self-control—two hallmark symptoms of drug addiction, to be further inspected upon trial completion and unblinding in time for the annual meeting. This entirely novel approach contributes to developing the foundation for effective, neuroscience-informed synergistic cognitive-behavioral-pharmacological strategies to target the persistent and cyclical pathology of drug addiction en route to tackling the ongoing addiction epidemic.

Keywords: methylphenidate, Extinction, Memory, fMRI, drug addiction

Disclosure: Nothing to disclose.

P282. The Effects of Alcohol-Induced Hepatic Steroid Hormone Dysregulation on Liver-Brain Axis Function

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Background: Alcohol consumption significantly contributes to numerous negative health outcomes and can be enhanced when the bioavailability of the steroid hormone estrogen is high, such as during the proestrus phase of the estrous/menstrual cycle in females. Intriguingly, chronic alcohol use may, in turn, be able to increase estrogen synthesis in non-ovarian tissues; humans with late-stage alcohol-related liver diseases robustly express aromatase, the P450 enzyme responsible for metabolizing androgens to estrogens, in the liver and display elevated levels of circulating estrogen. As estrogen signals in the liver to prevent steatosis and inflammation to maintain healthy liver function, alcohol consumption may homeostatically upregulate liver aromatase to increase local estrogen synthesis that can combat the burden of alcohol. However, this liver-derived estrogen may be able to enter the bloodstream (and thus brain) to participate in driving further alcohol drinking.

Methods: Male and female C57BL/6J mice underwent voluntary binge alcohol drinking (DID paradigm) or alcohol feeding (Lieber DeCarli diet) models of alcohol exposure and were sacrificed after the last alcohol exposure for measurement of aromatase protein in peripheral (gonads, liver, spleen, iWAT, sBAT, adrenals, and liver) and central tissues (BNST, PFC, and AMYG) via Western blot and estrogen in liver, blood, and brain via liquid chromatography tandem mass spectrometry. During EtOH DID, mice were systemically treated with the aromatase activity inhibitor letrozole or vehicle 10 min before alcohol access across cycles.

Results: Chronic alcohol consumption recruited aromatase expression specifically in the liver in both sexes, but earlier in males (3 wks) than females (7 wks), and persisted across increased alcohol drinking; there was a trend for increased E2 in the liver, blood, and brain of chronic alcohol drinkers. At these same time points, alcohol drinking became sensitive to acute letrozole treatment, and estrogen-sensitive measures of neuronal function in the BNST (where E2 can signal to drive alcohol drinking) were increased in males, suggesting increased E2 tone.

Conclusions: Estrogen synthesis may be recruited early in alcohol drinking as a homeostatic mechanism to stave off alcohol-induced liver pathology. This may establish a feedforward loop between alcohol, liver aromatase upregulation/estrogen synthesis, and neuroactive estrogen early in drinking that promotes the maintenance of heavy alcohol intake. Ongoing studies are evaluating these results in a nonhuman primate translational model of alcohol drinking and assessing other hormone levels in the liver and the signaling of estrogen in the brain that participate in these effects.

Keywords: estrogen synthesis, liver brain axis, Alcoholic Liver Disease, Alcohol consumption, extended amygdala

Disclosure: Nothing to disclose.

P283. Dopamine and Opioid Systems Jointly Influence Respiratory Behavior in OUD Patients Undergoing Treatment: PET Study With a Methylphenidate Challenge

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Background: Opioids and dopamine could synergistically modulate respiration[1-3]. Opioid overdose can lead to opioid-induced respiratory depression (OIRD)[4]. Therapies like methadone treatment therapy could impact respiration on heroid addicts[5]. Interestingly opioid-stimulant co-use is a growing problem in Opioid Use Disorder (OUD), and such co-use neuro-pharmacological interactions are recently being investigated[6]. Using dopamine receptor agonists, previous studies showed that, particularly D1 agonists, increase CO2 reactivity in the brainstem[7]. Thus, stimulants like Methylphenidate (MP) can modify the CO2 detection and the amount of inhaled air from the lungs. Our study is the first investigating chronic use of opioids on the respiratory system and its interaction with acute stimulant use, OUD use duration and OUD pharmacological treatment.

Methods: Participants: OUD participants on medication (Methadone (N = 21), Suboxone (N = 10)) and No Medication (NoMeds, N = 11), and controls (N = 32). PET Study: was conducted 3 hours before fMRI (when the respiration measures were taken); 2 sessions: Acute oral MP (60 mg), Acute oral Placebo (PL). D1 and D2 receptor densities were calculated via SRTM BPnd with C11-NNC and C11-RAC tracers. Respiration: For baseline (PL) and stimulant session (MP) recordings were taken. BIOPAC TSD221-MRI MR-safe fully-pneumatic respiration transducer measuring abdominal respiration connected to the TSD160A differential pressure transducer (with operational pressure of 2.5 cmH2O supported by the DA100C amplifier, where voltage output is 327.5 μ V/cmH2O.). Mean respiratory rate (number of respiratory peaks per minute), and respiratory volume (Respiratory amplitude divided by duration from the pre-peak minimum to the post-peak minimum). For each measure outliers with values 2*IQR above the 75% or below the 25% percentiles were excluded. BPnd and respiratory measures were age adjusted since age has been shown to influence BPnd, and in our study on respiration. For respiratory dependent variables we constructed linear models and ANOVAs

including REGION (Putamen, Caudate, NAcc) BPnds, and GROUP (Methadone, Suboxone, NoMeds, Control) were constructed for the tracers NNC and RAC (PL) separately (Separately in other model for OUD only, we also used opioid use duration; OUSE as an independent variable). We also explored MP related changes by comparing RAC and respiration contrasting PL and MP.

Results: Respiratory rate: There was a main effect of GROUP ($F(3,216) = 19.68, p < .001$), where Methadone group showed lower respiration rates than all other groups in the baseline PL measure. While MP intake slightly normalized it, this effect did not reach significance. OUSE was significantly influenced respiration rate ($F(1,60) = 31.5, p < .001$) in OUD; longer use indicated higher respiration rate. There was an interaction between D1 BPnd and Group on respiration rate ($F(3,147) = 5.73, p < .001$) where BPnd effect was significant for NoMeds ($F(1,15) = 4.79, p < .05$). RVT: There was a main effect of GROUP ($F(3,138) = 11.48, p < .001$), where Methadone group showed lower RVT than all other groups in the baseline PL measure. There was an interaction between D1 BPnd and Group ($F(3,144) = 4.36, p < .01$) where effect of BPnd effect on RVT was significant for NoMeds ($F(1,15) = 11.05, p < .005$). OUSE was significantly affected RVT ($F(1,51) = 45.42, p < .001$) in OUD; longer use indicated lower respiratory volume.

Conclusions: We have shown that OUD treatment type and OUD use duration influence respiratory rate and depth, together with dopamine receptor density, particularly D1. MP could mildly regulate and normalize respiration. Our results could explain why simultaneous use of stimulants and opioids in OUD might be a way to overcome respiratory difficulty and CO₂ related chemoreceptor function in the brainstem.

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Keywords: OUD, stimulants, respiration, PET

Disclosure: Nothing to disclose.

P284. Oral Metformin as a Treatment for Cocaine Use Disorder: Defining Biodistribution and Dynamic Glutamate Activity Driving Reduced Cocaine Cue-Induced Reinstatement

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Background: Cocaine-associated deaths in the U.S. continue to rise annually, many occurring as a result of relapse in use after a period of abstinence. There is no current FDA-approved treatment for cocaine use disorder (CUD) contributing to high rates of treatment attrition, relapse, and overdose. We have shown that metformin, a Type II Diabetes medication, can reduce cocaine cue-induced reinstatement (RST) when centrally injected into the nucleus accumbens core (NAcC), although striatal biodistribution, glutamate activity, and associated behavioral effects of oral metformin treatment for CUD remain unclear. We hypothesize oral metformin treatment will modulate the cocaine withdrawal-induced glutamate deficit during extinction, and ultimately reduce sensitized release of glutamate driving RST of cocaine. We additionally hypothesize that oral metformin will show stronger effects in female rats as compared to male rats, with expected dose- and cumulative time-dependent increases based on known sex differences on neuropharmacological responses and previous studies showing similar observations with homogenized brain samples. These studies elucidate translational potential and inform repurposing of metformin as a treatment for CUD, specifically influencing neuropharmacological changes that may induce therapeutic changes in motivated behavior.

Methods: EXPERIMENT 1: Metformin (1 mg/ml, 1.75 mg/ml, 2.5 mg/ml) was dissolved in drinking water and provided ad libitum in home cages for both male and female Sprague Dawley rats for different time periods (4 days, 7 days, 15 days, 30 days). Animal weight, water intake, and food intake were monitored daily followed by tissue collection (Dorsal Striatum, Ventral Striatum, Cortex, Cerebellum) for analysis of metformin biodistribution with liquid chromatography-mass spectroscopy (LC-MS). EXPERIMENT 2: Male and female Sprague Dawley received iGluSnFR3, a glutamate sensor, and optic fibers in the NAcC followed by intravenous jugular catheter for training on self-administration (SA) of cocaine. Metformin (2 mg/ml) was dissolved in the drinking water with ad libitum access throughout a 10-day extinction (EXT) period, followed by RST testing. A separate group of animals were trained in sucrose self-administration and tested in the same paradigm to assess naturalistic reward. In vivo fiber photometry recordings were done to measure changes of glutamate activity on the last day of SA; first, 5th, and 10th day of extinction; and during RST. Operant behavior events (lever press, tone/light cue, drug delivery) are synced with fiber photometry records using TTL pulses and behavior video recordings. Rat weight, water intake, and food intake were monitored daily. Raw photometry signal was z-scored to the isosbestic fluorescence and averaged across trials in each session.

Results: EXPERIMENT 1: Increasing doses of metformin results in a decrease of water intake across both female ($n = 48$, 4 rats per dose per time period; $p < 0.0001$ between treatment groups) and male rats ($n = 48$, 4 rats per dose per time period; $p < 0.0001$ between treatment groups), with those receiving 2.5 mg/ml oral metformin drinking the least throughout the 30 day period. The high dose also resulted in a decrease of food intake among male rats ($p < 0.0001$ between treatment groups). Preliminary LC-MS results show expected increases of metformin found in male rat cortex and cerebellum with increasing dose of metformin and increased time periods of cumulative treatment. Dorsal and ventral striatum LC-MS testing is currently ongoing, although is expected to yield similar results and higher concentrations among female tissue samples. EXPERIMENT 2: Preliminary RST data shows a reduction of active lever pressing during exposure to cues previously associated with cocaine after oral metformin treatment in female rats only ($n = 6$). Expression and measurement of iGluSnFR3 in vivo in the NA cC was validated during sucrose SA ($n = 3$), with decrease in signal during lever press and sustained increase of glutamate seen when sucrose was ingested. Although still in ongoing study, preliminary results show sustained increase

of glutamate during self-administration of cocaine ($n = 3$), with a marked decrease of glutamate fluorescence during RST in rats treated with oral metformin in EXT. Current experimental designs are focused on assessing sex differences in glutamate fluorescence underlying metformin treatment.

Conclusions: Oral metformin treatment during extinction following self-administration of cocaine has yielded promising therapeutic value in female rats with a reduction in cue-induced reinstatement. Due to sex differences in cocaine-seeking behavior after oral metformin treatment, we expect more efficient distribution and therefore higher concentrations of metformin among dorsal and ventral striatum of female rats. Preliminary fiber photometry measurements have confirmed dynamic changes of glutamate activity throughout cocaine SA, EXT, and RST suggesting neuroadaptations in exocytosis and synaptic uptake. Continuation of these studies will inform sex differences in physiological reactions to metformin and glutamatergic influence on cocaine-seeking behavior. Most importantly, our goal is to highlight promising evidence supporting the repurposing of metformin as a treatment to prevent relapse events in CUD.

Keywords: cocaine self-administration and reinstatement, metformin, extrasynaptic glutamate

Disclosure: Nothing to disclose.

P285. Association of Craving on Methamphetamine Use Outcomes With Naltrexone Plus Bupropion Versus Placebo: Findings From the ADAPT-2 Study

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Background: The Accelerated Development of Addictive Treatment for Methamphetamine Disorder (ADAPT-2) trial demonstrated efficacy of extended-release injectable naltrexone and oral bupropion in individuals with methamphetamine use disorder. This report evaluated whether self-reported craving for methamphetamine were associated with subsequent use of methamphetamine.

Methods: Participants of ADAPT-2 trial with data available to evaluate transition either from positive to negative urine drug screen (UDS) or from negative to positive UDS were included ($N = 357$). Urine samples were collected twice a week to test for the presence of methamphetamine. A visual analog scale for was used to measure self-reported craving for methamphetamine in the past week. Generalized repeated-measures mixed effects models were used to estimate the probability of transition from positive to negative UDS and vice versa.

Results: There was a significant interaction noted for VAS by treatment group by time ($p = 0.018$), where higher craving were consistently associated with lower likelihood of transition from positive to negative UDS at the next visit in the naltrexone-bupropion group. In the placebo group, higher craving was also associated with lower likelihood of transition from positive to negative UDS at the next visit, but this occurred earlier in the course of treatment. However, no such effect was present by week 6 of treatment. Among those with a negative UDS, higher craving was associated with higher likelihood of transition to a positive UDS ($p = 0.002$) in both treatment groups.

Conclusions: Higher craving was associated with poorer treatment outcomes, especially in the NTX-BUP treatment group. Systematically measuring craving may assist with informing clinical decision-making and guide the development of novel therapeutics.

Keywords: Methamphetamine use disorder, Naltrexone, bupropion, craving

Disclosure: Neurocrine, Navitor/Supernus, Janssen Research and Development: Contracted Research (Self). Psychiatric Clinics of North America: Honoraria (Self). Janssen Scientific Affairs, Boehringer Ingelheim: Consultant (Self). Worldwide Clinical Trials, Vicore Pharma, IQVIA, North American Center for Continuing Medical Education, Medscape/WebMD, Clinical Care Options, Physicians' Education Resource, H.C. Wainwright and Co.: Honoraria (Self).

P286. Identifying Reliable and Valid Personalized Targeting Biomarkers for Opioid Use Disorder Through Fronto-Parieto-Amygdala Network Interactions

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Background: Emerging research in opioid use disorders (OUD) increasingly focuses on noninvasive brain stimulation techniques, such as transcranial magnetic (TMS) or electrical (tES) stimulation, to engage and synchronize key regions within dysfunctional brain networks. Pathological drug use typically manifests as enhanced activity in fronto-limbic circuits, associated with aberrant reward processing and impulsive actions, and diminished activity in executive control circuits within the frontoparietal network. Effective treatment strategies may need to attenuate the impulsive reward circuit's response to cues or enhance the executive control circuit's response. This study aims to identify reliable biomarkers for targeting TMS/tES across frontal and parietal hubs linked to limbic areas, enhancing our ability to modulate the frontoparietal network's interaction with the amygdala. We hypothesize that modulating these networks can influence drug-related behaviors and craving.

Methods: Functional MRI (fMRI) data on drug cue reactivity (FDCR) were collected from 74 male participants with OUD, aged 34.55 ± 7.07 years, at baseline and repeated at least two weeks later without any specific medication or intervention. Cue-induced cravings were quantified before and immediately after fMRI drug cue exposure task using VAS scores. Reactivity to drug versus neutral cues was assessed across all brain sub-regions using the Brainnetome atlas for parcellation. The right medial amygdala, exhibiting the highest functional reactivity among subcortical regions, was chosen as the seed region for subsequent seed-to-whole brain psychophysiological interaction (PPI) analysis. For each participant, the locations and strengths of the most significant positive PPI connections linked to the subcortical seed in all significant clusters were identified. Additionally, correlations between region-of-interest (ROI)-to-ROI PPI connectivity and changes in craving scores were evaluated.

Results: Following drug cue exposure, craving scores significantly increased in both sessions ($P < 0.001$). Right medial amygdala, showing the highest FDCR (mean $\pm$ SD at test: 0.21 ± 0.47 , re-test: 0.12 ± 0.46), was selected as the seed region. PPI analysis from the right medial amygdala to whole brain identified significant clusters (P FDR-corrected < 0.05) within frontoparietal network indicating increased connectivity during drug cue exposure in both test and re-test sessions. These clusters were located in the right and left prefrontal and right and left parietal cortices (test: frontal: [6, 24, -22] 4341 voxels, right parietal [42 -58 -22] 3161 voxels, and left parietal [-34, -72, 48] voxels, re-test: right frontal [28, 42, 28] 2049 voxels, left prefrontal [-24, 26, 0] 1612 voxels, right parietal [44, -52, -22] 5897 voxels, and left parietal [-22, -84, -12] 4370 voxels). Significant ROI-to-ROI PPI increases were found from right amygdala to main nodes in the

frontoparietal network, with notable inter-individual variability in both locations (maximum positive PPI over frontal/parietal regions in both sessions was located within a cube with a volume greater than 33 cm³ in MNI space) and strength (ranging from -1.2 to 7.1). Additionally, in the re-test database, a significant negative correlation was observed between craving scores post-drug cue exposure and strength of frontoparietal PPI connections ($R = -0.27$, $P = 0.03$), as well as between frontoparietal and right fronto-amygdala PPI connections ($R = -0.34$, $P = 0.01$).

Conclusions: Our study demonstrates a consistent increase in connectivity between the amygdala and the frontoparietal network during drug cue exposure, observed across both test and re-test sessions. This increased functional coupling from limbic areas to regions managing executive functions underscores the robust interaction between drug cue-related value processing and the amygdala's arousal response. The negative correlation between craving scores and the strength of frontoparietal PPI connections further supports this interaction's role in craving modulation. The reproducibility of neural interactions across sessions highlights the reliability of our findings, despite inter-individual variability. These results suggest that multi-target neuromodulation interventions, such as focal dual-site in-phase frontoparietal synchronization using tES with alternating currents (tACS) or fMRI-guided TMS, could be tailored to enhance frontoparietal network activity while inhibiting its connections to the limbic network and the amygdala. This approach may be crucial for developing effective brain stimulation treatments for OUD, offering a promising avenue for future research and clinical applications.

Keywords: Functional MRI (fMRI), non-invasive brain stimulation, Targeted interventions, Test-Retest Reliability

Disclosure: Individior: Honoraria (Self)

P287. Examining the Modulatory Role for Galanin Signaling in Opioid Reward

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Background: BACKGROUND: Over three million Americans suffer from opioid use disorder (OUD), and opioid overdoses account for more than 75% of all drug-related overdose deaths in the United States. Not only are the effects of OUD devastating for individuals and families affected, but this epidemic also poses a significant economic burden on society. The cost of OUD in the United States was estimated at nearly \$1.5 trillion in 2020, and these costs are only expected to increase as a consequence of the pandemic among other factors. Despite this national public health crisis, there are only three FDA-approved medications for the treatment of OUD, and each of these have side effects including respiratory depression, diversion for illicit use, and a necessary detoxification period. Therefore, it is critical that we identify novel pharmacotherapies for OUD. One approach to developing new treatments involves targeting endogenous neurobiological circuits that modulate opioid addiction-related behaviors. Opioid reward is mediated, in part, by suppressing GABA transmission in the ventral tegmental area (VTA) via μ opioid receptor (MOR) activation, thus disinhibiting local dopamine (DA) neurons. Importantly, galanin is an endogenous neuropeptide that regulates opioid responses, most notably opposing opioid reward. Prior studies have demonstrated that mice lacking galanin globally exhibit increased morphine-induced locomotor activity and conditioned place preference (CPP), whereas ICV infusion of galanin has the opposite effect and suppresses morphine CPP. Finally, galanin receptor 1 (GalR1) can form heteromers with MOR,

and galanin signaling via MOR-GalR1 heteromers suppresses MOR-induced increases in extracellular DA. These findings suggest that galanin transmission in the VTA may represent a therapeutic target for OUD, but the neurocircuitry and molecular mechanisms underlying the ability of galanin to oppose opioid reward have not been identified. The current experiments were designed to determine 1) the endogenous source of galanin in the VTA, 2) the impact of galanin signaling in the VTA on morphine CPP, and 3) the co-expression of GalR1 and MOR in critical nodes of the reward pathway such as the VTA, rostromedial tegmental nucleus (RMTg), and the nucleus accumbens (NAc).

Methods: METHODS: To determine the endogenous source of galanin to the VTA, adult, male and female C57BL/6 ($n = 4$ per sex) mice were infused with a retro-GFP virus into the VTA, and brain-wide in situ hybridization was used to identify cells containing both GFP and galanin mRNA. To examine the role for VTA galanin in morphine CPP, male and female mice ($n = 4$ male/ 4 female per group) were implanted with indwelling bilateral cannulae in the VTA and received galanin (400 ng/side) or vehicle 20 min prior to morphine during CPP acquisition. Behavioral data from these experiments were analyzed via 2-way ANOVA. To verify that GalR1 and MOR are co-expressed and thus capable of forming heteromers in brain regions critical to reward seeking, we used in situ hybridization to identify mRNA expression of GalR1 and MOR in GABAergic (GAD1 +) and non-GABAergic (GAD1-) cells in the VTA, RMTg, and NAc ($n = 3-4$ per region).

Results: RESULTS: In experiment 1, the lateral hypothalamus was identified as a candidate source for galanin to the VTA. Additionally, we found that intra-VTA galanin infusion attenuated morphine CPP ($p = 0.02$). Ongoing analyses will quantify the co-expression of GalR1 and MOR mRNA in the VTA, RMTg, and NAc.

Conclusions: CONCLUSIONS: Together, these findings suggest that galanin signaling in the reward pathway represents a viable mechanism for modulating opioid reward and shed light on the mechanism by which galanin modulates morphine CPP. Furthermore, these behavioral studies lay the foundation for investigating galanin signaling within the VTA as novel therapeutic target for OUD.

Keywords: galanin, μ opioid receptor, conditioned place preference, opioid use disorder

Disclosure: Nothing to disclose.

P288. Effects of Chronic Voluntary and Passive Ethanol Exposure on Hyperalgesia and Ventrolateral Periaqueductal Gray (vlPAG) Neuronal Properties in Mice

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Background: Chronic ethanol use is associated with hyperalgesia, an effect that is especially apparent during abstinence. Ethanol withdrawal hyperalgesia has been demonstrated using several rodent drinking models, however, this phenomenon has not been reported in models of human binge drinking, such as the drinking in the dark (DID) paradigm in mice. Ethanol withdrawal reduces the activity and strength of inhibition from central amygdala GABAergic inputs onto ventrolateral periaqueductal gray (vlPAG) neurons, a critical node in descending pain modulation pathways. This project aims to investigate whether volitional ethanol consumption compared to passive ethanol exposure promotes similar changes in nociception and neuronal properties of vlPAG neurons.

Methods: Adult male and female mice were chronically exposed to voluntary ethanol consumption using DID paradigm

for 4 weeks (16 sessions, during which time water bottles were switched out for 20% ethanol; in Control groups, the water bottle was switched out for another water bottle). A separate cohort of mice was exposed to a passive chronic ethanol exposure (CIE) using vapor chambers for 4 weeks (16 sessions; during which time mice were exposed to vaporized ethanol; in Control groups, mice were exposed to air). To assess whether ethanol drinking in both models lead to hyperalgesia, nociceptive testing, von Frey and hot plate, occurred during the first day of abstinence after each week of DID or CIE and after 7 days of abstinence from the last ethanol exposure. To assess changes in the neuronal properties of vPAG neurons, separate cohorts of mice were subjected to the same DID or CIE paradigm and whole-cell patch-clamp slice electrophysiology recordings were performed in the vPAG after 7 days of ethanol abstinence.

Results: In both models, CIE and DID, mice reached levels of ethanol that resulted in blood ethanol concentration levels above NIAAA binge intoxication levels (80 mg/dL). For the DID paradigm, only alcohol-drinking male mice developed mechanical and thermal hypersensitivity after 21 days of abstinence compared to controls. DID followed by 7 days of alcohol abstinence reduces the frequency of spontaneous excitatory post-synaptic currents (sEPSCs) in the vPAG neurons in male and female adult mice. For the CIE paradigm, male and female mice developed mechanical and thermal hypersensitivity after 2 weeks of CIE and those effects persist even after 21 days of abstinence. We will further evaluate neuronal changes in the vPAG neurons after 7 days of abstinence from CIE.

Conclusions: DID model produced hyperalgesia in male mice. It is unclear whether the lack of effect in female mice was related to the specific experimental protocol used here. CIE model produced hyperalgesia in both male and female mice, and it occurred sooner than the mice exposed to DID. A history of DID drinking reduced frequency of sEPSCs in vPAG neuron and ongoing experiments will reveal whether similar effects are observed after CIE. These findings show that the DID model of binge alcohol drinking is useful for examination of neurobiological mechanisms underlying alcohol-associated hyperalgesia. However, CIE paradigm generates a faster and more robust model of alcohol-induced withdrawal hyperalgesia.

Keywords: Pain, Alcohol, hyperalgesia

Disclosure: Nothing to disclose.

P289. Retrieval of an Ethanol-Conditioned Taste Aversion Promotes GABAergic Plasticity in the Insular Cortex

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Background: Blunted sensitivity to ethanol's aversive effects can increase motivation to consume ethanol; yet, the neurobiological circuits responsible for encoding these aversive properties are not fully understood. Plasticity in cells projecting from the insular cortex (IC) to the basolateral amygdala (BLA) is critical for taste aversion learning and retrieval, suggesting this circuit's potential involvement in modulating the aversive properties of ethanol. Here, we tested the hypothesis that GABAergic activity onto IC-BLA projections would be facilitated following the retrieval of an ethanol-conditioned taste aversion (CTA).

Methods: Male and female C57/BL6 mice ($n = 52$) were injected into the BLA with a rg-AAV encoding tDTomato to allow for visualization of IC-BLA projecting cells. After a 3-week recovery period, mice were given access to a 0.15% saccharin solution for 30 min/day for 3 days and intake was recorded. On day 2, mice

received an i.p. injection of either vehicle (0.9% sterile saline) or 2 g/kg ethanol immediately after saccharin access. Whole-cell patch clamp recordings of miniature inhibitory postsynaptic currents (mIPSCs) were obtained from IC-BLA projecting cells 1 hr post ethanol-CTA retrieval on day 3, and local inhibitory synaptic input maps to IC-BLA projecting cells were created using a Laser Applied Stimulation and Uncaging (LASU) approach. PV-Cre mice ($n = 36$) were bilaterally infused into the insula with AAV8-hSyn-DIO-mCherry or AAV8-hSyn-DIO-hM4D(Gi)-mCherry, followed after a 3-week recovery period by the same 3-day conditioned taste aversion protocol described above. On day 3, mice received a pre-retrieval i.p. injection of 0.1 mg/kg Deschloroclozapine (DCZ). Behavioral data were analyzed using two-way ANOVAs using sex (male vs female) and treatment (saline vs ethanol) as dependent variables. Two-way ANOVAs using sex (male vs female) and treatment (saline vs ethanol) as dependent variables were used to compare between-group differences in electrophysiological measures. Two-way ANOVAs using treatment (saline vs ethanol) and distance to soma as dependent variables were used for synaptic input mapping experiments to compare summed cumulative synaptic inputs, and unpaired t-tests were used to compare total number of synaptic input sites in both layer 2/3 and layer 5/6.

Results: Frequency of mIPSCs was increased following retrieval of an ethanol-CTA in IC-BLA projection neurons (layer 2/3: $F(1,29) = 8.951$, $p = 0.006$; layer 5/6: $F(1,24) = 11.71$, $p = 0.002$). This increase in GABAergic plasticity occurred in both a circuit-specific and learning-dependent manner. Additionally, local inhibitory inputs onto layer 2/3 IC-BLA projection neurons were greater in number and strength following ethanol-CTA ($t(14) = 2.34$, $p = 0.03$). Finally, DREADD-mediated inhibition of IC parvalbumin-expressing cells blunted the retrieval of ethanol-CTA in male, but not female, mice.

Conclusions: Collectively, this work implicates a circuit-specific and learning-dependent increase in GABAergic tone following retrieval of an ethanol-CTA, thereby advancing our understanding of how the aversive effects of ethanol are encoded in the brain.

Keywords: ethanol, conditioned taste aversion, slice electrophysiology, Anterior insula, basolateral amygdala

Disclosure: Nothing to disclose.

P290. Neural Mechanisms of Social Facilitation of Cocaine Reward and Social Buffering of Cocaine-Induced Aversion

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Background: A growing body of research shows that social interactions influence motivation for drugs of abuse, drug reinforcement, and relapse. Despite this, investigations into how social interactions shape drug reward and aversion are few. Human and animal studies demonstrate that cocaine (COC) administration produces initial reward which then gives way to dysphoria. Indeed, rats develop a conditioned place preference (CPP) to the immediate effects of COC (0-5 min after administration) and a conditioned place aversion (CPA) to the delayed effects of COC (15 min after). Thus, it is likely that the rewarding and aversive effects of COC contribute to drug taking via positive and negative reinforcement mechanisms, respectively.

Methods: To assess how social interaction affects COC conditioned reward and aversion we used a place conditioning procedure in which male and female rats learn to associate a unique environment with either the immediate or delayed effects of one of three doses of COC (0.1mg/kg, 0.25mg/kg, and 1.0mg/kg, IV; $n = 6-12$ per group). Further, during each conditioning session, rats were either alone in the environment (as is typical in

these procedures) or they were paired with a same-sex cage mate that never received drug. All animals underwent a post-conditioning test to assess social facilitation of cocaine reward or social buffering of cocaine-induced aversion. Follow-up experiments used systemic administration of an oxytocin antagonist (atosiban) or activation of an inhibitory DREADD targeting oxytocin cells of the paraventricular nucleus of the hypothalamus (PVN) to assess the role of oxytocin in observed social effects on cocaine reward and aversion.

Results: We replicated previous studies showing that 1.0mg/kg COC produces robust CPP and CPA in male rats ($p < .05$). In males conditioned alone, 0.25mg/kg COC was unable to produce a CPP, however, in rats conditioned with a conspecific this same dose produced a robust CPP, demonstrating social facilitation of cocaine reward. Further, while 1.0mg/kg COC produced a CPA in males conditioned alone, rats conditioned with a conspecific failed to develop a CPA, evidencing social buffering of cocaine aversion. Here we also show that a similar dose of cocaine (1.0mg/kg) produced optimal CPP in female rats, however, no COC dose tested reliably produced CPA in female rats indicating greater variability in COC-induced aversion in female rats. Social facilitation of COC reward was not evident in female rats. In follow-up studies, pretreatment with an oxytocin receptor antagonist significantly reduced social facilitation of COC reward in male rats while not affecting social buffering of COC-induced aversion. However, atosiban's anxiolytic effects may have obscured its ability to block social buffering. Use of an inhibitory DREADD targeting oxytocin cells of the PVN revealed that inhibition of oxytocin signaling did indeed attenuate social buffering of COC-induced aversion.

Conclusions: Thus, female rats developed CPP for a similar dose of IV COC as male rats, however female COC-induced CPA was much more variable. Social interaction facilitated COC reward in males, but not females, indicating a sex difference in social facilitation of COC reward. Further, presence of a conspecific blocked COC-induced aversion in male rats. Pharmacological and DREADD interventions support a role for oxytocin in both social facilitation of COC reward and social buffering of COC-induced aversion.

Keywords: cocaine, Social buffering, social interaction, oxytocin

Disclosure: Nothing to disclose.

P291. Personalized Brain Stimulation Targets for Opioid Addiction Remission

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Background: Brain stimulation techniques, including transcranial magnetic stimulation and low-intensity focused ultrasound, have emerged as promising therapies for opioid use disorder (OUD). However, identifying optimal brain locations to target remains a significant barrier to success, because individuals with OUD display tremendous heterogeneity in symptomatology and brain function. Here, we attempted to identify personalized targets for brain stimulation therapy based on functional connectivity to a recently identified 'addiction remission' network, where individuals with lesions in regions exhibiting a particular connectivity pattern showed complete addiction remission (Joutsa et al., 2022 Nat. Med.). This network is predominantly characterized by strong positive connectivity with the salience network and strong negative connectivity with the default mode network. Because of recent evidence that OUD display reduced levels of brain network segregation, we hypothesized that, compared to controls,

OUD would show: 1) weaker regional peaks of maximal connectivity (i.e., stimulation targets) to the 'addiction remission' network; 2) less laterality of stimulation targets across the group, indicating less functional specialization, and 3) fewer individuals with maximal/minimal connectivity targets residing within the salience/default mode networks (that is, greater spatial dispersion of targets across individuals).

Methods: Thirty-seven individuals with OUD in recovery (22 male, 15 female) and 39 age-matched controls (25 male, 14 female) underwent resting-state functional magnetic resonance imaging. We performed seed-based functional connectivity between every voxel and the 'addiction remission' map. For each individual, the regions with the highest and lowest average correlation in a 6-mm sphere radius were considered the maximal 'inhibition' and 'stimulation' targets, respectively (i.e., two targets per subject). Then, for each target, we extracted the connectivity strength and the location (both the hemisphere and functional network that the target belonged to, according to the 'Yeo 7 network' functional parcellation). We performed two-sample t-tests for group differences in target connectivity strength, and chi-squared tests for group differences in target locations (i.e., left vs. right hemisphere, and salience/default mode networks vs others).

Results: Compared to controls, OUD showed significantly weaker maximal ($t = 4.08$, $p = 1 \times 10^{-4}$) and minimal ($t = 4.644$, $p = 1 \times 10^{-5}$) connectivity targets with the addiction remission network. There were significant group differences in lateralization for the stimulation targets (controls: 62% left hemisphere targets; OUD: 27% left hemisphere targets; $\chi^2 = 9.147$, $p = .002$). There were significantly more controls than OUD whose stimulation targets fell within vs. outside the default mode network (controls: 85% within default mode; OUD: 65% within default mode; $\chi^2 = 3.950$, $p = .047$).

Conclusions: The weaker maximal/minimal connectivity to the addiction remission network and the greater spatial variability of stimulation targets (i.e., individuals whose targets fell outside the default mode network) in the OUD compared to control groups is consistent with loss of functional segregation and/or increased variability in brain connectivity patterns due to OUD pathophysiology. However, while we hypothesized less laterality in the OUD group, we instead found that optimal stimulation targets were more likely to be in the right hemisphere in OUD, whereas targets were more likely in the left hemisphere in controls. Further studies are needed to evaluate the significance of these hemispheric differences. Overall, this work highlights the potential for precision medicine-based targets for OUD intervention.

Keywords: brain stimulation, Functional MRI (fMRI), opioid use disorder, personalized medicine, Default mode network (DMN)

Disclosure: Nothing to disclose.

P292. Sex-Specific Effects of Pain on Fentanyl Use and Dopamine Neuron Dynamics

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Background: Increasing rates of opioid overdose deaths in the US are a major public health concern that disproportionately impacts men compared to women. Interestingly, the most reported motivation for opioid misuse is pain relief. Still, it remains unclear how pain impacts opioid misuse liability and whether pain alters opioid reward in a sex-specific manner.

Methods: Here, we characterize sex-specific effects of inflammatory pain (produced by hind paw injections of Complete Freund's Adjuvant; CFA) on fentanyl self-administration in rats and elucidate underlying mechanisms within the mesolimbic

dopamine reward circuitry under the regulation of estrogen receptor signaling using wireless in vivo fiber photometry to evaluate ventral tegmental area (VTA) dopamine neuron calcium dynamics time-locked to fentanyl self-administration. We evaluate how fentanyl-evoked dopamine neuron responses evolve over several weeks of self-administration, under conditions of pain, and under the influence of variable ovarian hormone states.

Results: We reveal that pain time-dependently enhances fentanyl self-administration selectively in males, and this effect is associated with greater fentanyl-evoked calcium transient activity from VTA dopamine neurons. In females with pain, ovariectomy produces a male-like phenotype such that fentanyl use and corresponding VTA dopamine neuron activity are increased. While the protective effects of ovarian hormones are not solely driven by estradiol, per se, high estradiol states - naturally occurring in gonadally intact females or artificially produced in gonadally intact males - suppress fentanyl self-administration and evoked VTA dopamine activity through estrogen receptor beta signaling in the VTA.

Conclusions: In males, CFA inflammatory pain time-dependently enhances fentanyl-evoked VTA dopamine calcium transient responses during fentanyl self-administration, leading to higher rates of instrumental fentanyl use over time. In females, CFA does not alter fentanyl-evoked VTA dopamine calcium transient responses during fentanyl self-administration which corresponds to stable levels of fentanyl use. These effects can be recapitulated in males with exogenous application of estradiol which acts through estrogen receptor beta in the VTA to suppress fentanyl-evoked VTA dopamine activity. Similarly, females show enhanced fentanyl-evoked VTA dopamine activity and higher rates of fentanyl use after ovariectomy. Together these findings demonstrate that VTA estrogen receptor beta signaling has an inhibitory influence over opioid-evoked dopamine neuron activity that contributes to maladaptive opioid use under conditions of pain.

Keywords: opioids, Dopamine, sex differences, Pain, estradiol

Disclosure: Nothing to disclose.

P293. Examining (R)-Ketamine Reinforcement and its Dependency on Opioid Receptors

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Background: (R)-ketamine is an enantiomer of (R,S)-ketamine and is in clinical development as a medication for depression. (R)-ketamine has shown promising results in preclinical studies on treating depression-like behavior, showing greater and longer lasting antidepressant efficacy, and less psychotomimetic effects than (S)-ketamine.

(R)-ketamine is commonly regarded as a noncompetitive N-methyl-D-aspartate receptor (NMDAR) antagonist, but it also interacts with other receptors. (R)-ketamine has distinct pharmacokinetics and pharmacodynamics, and it has about fourfold lower affinities at NMDARs and μ opioid receptors (MORs) than (S)-ketamine.

Recently, our lab found that while rats will learn to self-administer (S)-ketamine at 0.5 mg/kg/infusion dose, they will not learn to self-administer (R)-ketamine at this dose. We also showed that this dose of (S)-ketamine was sufficient to increase brain activity in the nucleus accumbens (NAc). Further, we observed that repeated exposure of 20 mg/kg/day of (S)-ketamine had effects on MOR density and function. Here we delve into (R)-ketamine's effects using equimolar and equipotent doses to compare to our previous (S)-ketamine studies.

Methods: Rats ($n = 12$) underwent (R)-ketamine intravenous self-administration (IVSA) training (2 mg/kg/infusion) and their IVSA dose-response curve was determined with and without the presence of the opioid receptor antagonist naltrexone (3 mg/kg, SC). Rats were then exposed to additional IVSA sessions prior to extinction training.

Rats were exposed to 8 daily IV saline ($n = 6$) or (R)-ketamine (20 mg/kg/h ($n = 6$), or 60 mg/kg/h ($n = 6$), 60 min) infusions. On day 9, the rats were euthanized and MOR density was assessed using [3H]DAMGO. Adjacent brain sections were exposed to DAMGO- (10 μ M) or (R)-ketamine-stimulated (10 μ M) [35S]GTPyS autoradiography.

Functional ultrasound imaging (fUSI), a neuroimaging modality, was used to measure brain-wide changes in cerebral blood volume (CBV). Rats ($n = 9-10$) were pretreated with saline or naltrexone (3 mg/kg, SC) followed by a single IV injection of saline, 0.5 mg/kg, or 2 mg/kg (R)-ketamine 15 min later while imaging the mPFC and NAC.

Results: Rats acquired (R)-ketamine IVSA at a unit dose 4 times that of S-ketamine (2 mg/kg/infusion) and showed an inverted U-shaped dose-response function with highest responding to a 1 mg/kg/infusion unit dose. Naltrexone (3 mg/kg, SC) significantly blunted this dose response at the unit doses of 1 mg/kg ($P = 0.0375$) and 0.5 mg/kg ($P = 0.0012$). MK-801 (0.1 mg/kg, SC) significantly decreased (R)-ketamine infusions at the peak dose of 1 mg/kg/infusion ($P < 0.001$). Additionally, rats extinguished on the first day dropping from 57 active lever presses on the previous session to 8.

Unlike (S)-ketamine, repeated exposure to 20 mg/kg/hr IV (R)-ketamine did not affect MOR, KOR, or NMDAR receptor levels. Furthermore, this repeated exposure did not affect (R)-ketamine-stimulated [35S]GTPyS recruitment, but it did decrease DAMGO-stimulated [35S]GTPyS recruitment in the mPFC ($P = 0.0076$).

Unlike with (S)-ketamine, we found no change in peak change of CBV in either the shell or the core of the nucleus accumbens (NAc) after a single IV infusion of either 0.5 (equimolar dose to (S)-ketamine) or 2 mg/kg (equipotent dose to (S)-ketamine) (R)-ketamine. Additionally, we found that in the cingulate area (Cg1) and the secondary motor cortex (M2), 0.5 mg/kg (R)-ketamine did not cause a change in activity. However, the 2 mg/kg dose increased CBV significantly above baseline in Cg1 ($P = 0.165$) and above both baseline ($P < 0.001$) and the 0.5 mg/kg ($P = 0.035$) dose in M2. Interestingly, naltrexone pretreatment did not affect the peak of the activation in Cg1 or M2 at the 2 mg/kg dose; however, it elongated the time that the activation was above baseline and significantly increased the area under the curve in M2 ($P = 0.019$).

Conclusions: Our findings indicate that (R)-ketamine has reinforcing effects at sufficiently high doses and these effects may be due to an its effect on the opioid system. Moreover, (R)-ketamine reinforcement coincides with divergent regional changes in brain activity as compared to (S)-ketamine.

Keywords: ketamine, self administration, μ opioid receptor

Disclosure: Nothing to disclose.

P294. Dysregulations in Sleep Patterns Induced by Fentanyl Self-Administration and Acute Withdrawal are Altered by Xylazine Co-Use

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Background: Chronic use of fentanyl, a μ opioid receptor (MOR) agonist, is associated with severe withdrawal including disruptions in sleep quality. Importantly, individuals with opioid use disorder experience alleviation of withdrawal severity when given the insomnia medication suvorexant, indicating that sleep

dysregulation is a critical aspect of withdrawal. Xylazine, an adrenergic 2a receptor (A2aR) agonist, has been increasingly detected as an adulterant in the illicit fentanyl supply and clinical experiences indicate that xylazine may increase withdrawal from other substances including fentanyl. We have found that withdrawal from fentanyl+xylazine self-administration (SA) is exacerbated in female rats compared to fentanyl alone, an effect not observed in males. To date, no studies have evaluated the consequences of xylazine on sleep quality when it is co-used with fentanyl and thus we utilized piezoelectric sleep mats to non-invasively monitor sleep cycles throughout SA of and withdrawal from fentanyl with or without xylazine in the infusion.

Methods: 6 female young adult Long Evans rats underwent jugular vein catheter surgery followed by 10 2-hr sessions of saline, fentanyl (0.32 µg/kg/infusion), or fentanyl+xylazine (0.15 or 0.30 mg/kg/infusion) SA followed by acute (48 hrs) withdrawal. Throughout experimentation, rats were housed in sound attenuating chambers which contained home cages that rested on piezoelectric sensors (Adapt-a-Base; Signal Solutions, LLC), allowing for 22 hrs of continuous sleep monitoring. SA criteria were set at a minimum of 10 infusions and a 2:1 active:inactive lever discrimination for 3 consecutive days. Two forms of data were taken from the Signal Solutions SleepStats software: percentage of time spent asleep by hour, when the mats were monitoring the rodent, and percentage of time spent asleep by phase of the light/dark cycle. Rats were housed in a reverse 12h:12h light:dark cycle, with SA sessions occurring during the dark phase. Factors analyzed included phase of experiment, day of experiment, and experimental group. SA and sleep mat data were analyzed via appropriate ANOVAs with Tukey's post-hoc analyses.

Results: All rats met SA criteria within 10 SA sessions, and as we previously found, xylazine significantly suppressed fentanyl consumption during SA ($F_{2,6} = 71.38$, $p < 0.05$). Although preliminary, fentanyl SA induced an increase in total sleep duration as compared to saline SA across all days of SA ($F_{1,18} = 15.78$, $p < 0.05$). Xylazine co-use did not result in significantly different sleep duration compared to saline at either xylazine dose (p 's > 0.05). Acute withdrawal from fentanyl SA resulted in less sleep compared to saline after 48 hrs, which was attenuated by xylazine co-use (trending; $F_{3,5} = 11.37$, $p = 0.08$). Analysis of percent sleep time in the light versus dark cycle is ongoing.

Conclusions: Akin to prior clinical reports, fentanyl use resulted in significantly increased sleep duration after chronic use, and acute withdrawal from fentanyl resulted in a reduction in total sleep duration. Surprisingly, co-use of fentanyl with xylazine appeared to attenuate these fentanyl-induced effects. Mechanistically, it is possible that xylazine is inhibiting MORs when fentanyl and xylazine are co-used given that A2aRs and MORs can inhibit each other's function. Thus, it is possible that xylazine blocks fentanyl's effects on sleep duration through these mechanisms although additional studies are needed. Together, our results indicate that sleep dysregulations may be an important component of the fentanyl+xylazine withdrawal syndrome.

Keywords: Fentanyl Self-Administration, Xylazine, Sleep disturbances, Fentanyl withdrawal

Disclosure: Nothing to disclose.

P295. Neuronal Circuits Encoding Methamphetamine Seeking Behavior

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Background: The dopamine mesolimbic system modulates the reinforcing and rewarding effects of methamphetamine. However,

chronic methamphetamine users develop deficiencies in dopamine-related brain functions that become more severe with increased exposure and consumption of the drug. In addition, postmortem studies in heavy users reported indications of neurodegeneration and brain damage. How and whether these findings contribute to the reinforcing properties of methamphetamine and its persistent use is not completely known. We have previously reported that mice chronically exposed to methamphetamine self-administration showed signs of an increased oxidative environment of the ventral mesolimbic dopamine pathway. These include a reduction of the antioxidant molecule glutathione in the ventral striatum and an increase in mitochondrial oxygen consumption rates in the ventral striatum and the ventral tegmental area. An increase in oxidative stress could predict neurodegeneration and cellular damage. Here, we will present a preliminary study on the dopamine circuitry changes and neuronal assemblies activated by methamphetamine-seeking behavior in mice chronically exposed to methamphetamine self-administration.

Methods: We crossbreed FosTRAP mice with an inducible Cre-dependent TdTomato (TDT) reporter strain. This mouse model allows the genetic tagging of neuronal populations expressing c-Fos within an activated brain circuit. We implanted FosTRAP/TDT adult mice with a jugular catheter and trained them daily to nose poke for methamphetamine infusions (0.1 mg/kg/infusion). After training, mice were placed in a fixed ratio 3 (FR3) schedule to complete up to 12 weeks of methamphetamine self-administration exposure. We used classical tyrosine hydroxylase (TH) immunolabeling on brain slices to locate neuronal ensembles in dopamine mesolimbic regions activated during methamphetamine-seeking behavior.

Results: Our preliminary results indicated that mice exposed to methamphetamine self-administration for several weeks recruited additional brain circuits outside the mesolimbic dopamine pathway during methamphetamine seeking. Neuronal assemblies were mostly found in cortical regions but also in the cerebellum and hypothalamus. Then, to investigate if prolonged methamphetamine exposure leads to neurodegeneration, our current research efforts have focused on developing a methodology to visualize changes in dopaminergic circuitry in the whole mouse brain. For this, we combined TH immunolabeling and tissue-clearing procedures to collect images using confocal and light-sheet microscopy. In our initial full brain mapping, we found that TH+ labeling was decreased in the ventral tegmental area, the substantia nigra, and the striatum, which indicates a decrease in dopamine synthesis capabilities. We are working on annotating and registering neuronal assemblies by brain region to determine activation patterns during seeking behavior and co-labeling with TH-positive circuitry.

Conclusions: Our study offers initial insights into the brain circuits that appear to be involved in drug-seeking behavior following chronic methamphetamine self-administration. It will provide a new understanding of how highly addictive drugs gradually alter brain function, leading to drug misuse.

Keywords: Dopamine, Substance abuse disorders, Methamphetamine Self-Administration, Brain Circuits, c-Fos-expressing ensembles

Disclosure: Nothing to disclose.

P296. Females Experience Greater Clinical Benefit from TMS for AUD: A Translational Analysis of Sex-Differences in Preclinical and Clinical Treatment Outcomes

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Background: Women have experienced a disproportionate increase in AUD prevalence in the last decade. Transcranial magnetic stimulation (TMS) is a promising treatment tool for Alcohol Use Disorder (AUD). While several groups have shown robust sex-differences following TMS for other psychiatric disorders, it remains unclear if there are sex differences in treatment outcome for AUD. Here, we evaluate sex differences in TMS efficacy following two sham-controlled, experiments in preclinical and clinical models of AUD. Study 1: preclinical analysis of change in alcohol consumption following 14 sessions of prefrontal cortex TMS delivered to male and female mice. Study 2: clinical analysis of 3-month alcohol abstinence following 10 sessions of frontal pole TMS in AUD patients ($n = 50$, 20F).

Methods: Study 1: 36 C57BL/6J mice (18F) received 14 sessions of continuous theta burst stimulation (cTBS, $n = 12$, 6F), intermittent theta burst stimulation (iTBS, $n = 12$, 6F) or sham ($n = 12$, 6F) stimulation delivered to the prefrontal cortex (2 sessions/day, low intensity 16mT stimulation). Prior to, and after TMS, alcohol consumption was evaluated using a two-bottle choice, Drinking in the Dark paradigm. Brain tissue was assayed for BDNF gene expression via qPCR. Study 2: 50 (20F) individuals were randomized to receive active or sham frontal pole TMS (cTBS, 10 sessions, 1 session/day). Follow-up visits were conducted for 3 months. fMRI alcohol-cue induced functional connectivity data were measured from the stimulation site to the anterior cingulate, striatum and insula prior to TMS and at each follow up (Conn toolbox v20.b). Alcohol abstinence was analyzed using standard odds ratios.

Results: Study 1: Relative to same-sex sham controls, cTBS reduced alcohol consumption to a greater degree in female compared to male mice (female: $d = -0.45$; male: $d = -0.19$). Conversely, iTBS increased alcohol consumption to a greater degree in females compared male mice (female: $d = 1.5$; male: $d = 0.05$). Female mice also experienced a greater increase in prefrontal BDNF expression following iTBS, compared to male mice (female: $d = 0.84$, male: $d = 0.23$). cTBS reduced BDNF expression to a similar degree in both female and male mice (female: $d = -0.42$; male: $d = -0.53$). Study 2: Two and three months after active TMS, females were 5.25 and 8.4 times more likely to remain abstinent to alcohol, relative to males who received active TMS (2Mos. OR = 5.25, $p = 0.1$; 3Mos. OR = 8.4, $p = 0.08$). Additionally, women experienced a greater reduction in frontal-pole functional connectivity to the striatum, insula and cingulate cortices following active TMS treatment, relative to men ($F_{1,547} = 5.28$, $p = 0.02$). This reduction was most pronounced one month after treatment.

Conclusions: In a preclinical model, female animals experienced greater TMS-induced change in alcohol consumption and cortical BDNF, relative to male animals. In a human clinical trial, females were substantially more likely to remain abstinent to alcohol, relative to males receiving active TMS. Females also experienced a greater reduction in fMRI brain response to alcohol cues. Taken together, these translational studies suggest that females may be more receptive to brain and behavior changes following TMS than males in the context of AUD. While preliminary, these results warrant future, prospectively powered evaluations of sex differences in TMS treatment outcome for alcohol and substance use disorders.

Keywords: transcranial magnetic stimulation (TMS), Alcohol Use Disorder - Treatment, sex differences, Translational Neuroscience, fMRI

Disclosure: Nothing to disclose.

P297. Neural Correlates of Nicotine and Monetary Reward Processing Using an MRI-Compatible Vaping Device: A Preliminary Model-Based fMRI Study

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Background: Reward and risk processing are fundamental for decision-making and can be significantly altered by drug addiction. While it is crucial to understand drug-specific neural processes underlying decision-making, little is known about these processes in the context of actual drug use in humans. Here, by employing an MRI-compatible vaping device, we aim to fill this gap by (1) comparing fMRI responses to nicotine and monetary rewards, and (2) investigating decision-making patterns involving actual nicotine use and their neural underpinnings in smokers undergoing acute withdrawal.

Methods: We conducted two fMRI experiments using an MRI-compatible vaping device. Participants included healthy controls (HC) and regular smokers (smoking more than five cigarettes daily for over a year). Smokers abstained from nicotine, alcohol, and caffeine for 12 hours prior to the experiment. In Study 1, we compared fMRI responses to nicotine and monetary rewards using the reward and risk prediction error (PE) task (Preusschoff et al., 2008). The HC group ($n = 26$) received monetary reward and the smoker group received either monetary ($n = 6$) or nicotine ($n = 8$) reward during the task. In Study 2, we investigated the neural underpinnings of decision-making involving actual nicotine use where participants completed a context-dependent reinforcement learning task (Bavard et al., 2022). The HC group ($n = 33$) received the monetary rewards, and the smoker group ($n = 18$) completed two visits, performing the RL task with either monetary or nicotine rewards. An MRI-compatible vaping device delivered nicotine rewards to smokers inside the MRI. We used computational modeling and model-based fMRI for behavioral and fMRI analysis to identify the neural correlates of reward/risk PE and decision-making.

Results: Preliminary results from Study 1 suggest that smokers exhibit reduced reward PE signals in the striatum and reduced risk PE signals in the posterior parietal cortex compared to HCs. In Study 2, smokers showed greater fMRI responses to nicotine rewards compared to monetary rewards in regions including the striatum, the insula, and supplementary motor area.

Conclusions: Preliminary findings indicate that smokers may have distinct neurocognitive mechanisms for processing nicotine and monetary rewards. This research enhances our understanding of the neural processing of primary drug rewards, providing valuable insights into the neurocognitive aspects of addiction.

Keywords: Nicotine addiction, fMRI, Reward, Risk, Reinforcement learning

Disclosure: Nothing to disclose.

P298. Altered White Matter Development in Adolescents With Polysubstance Use

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Background: Polysubstance use (PSU), defined as the concurrent consumption of multiple psychoactive substances, is a growing public health concern, particularly among adolescents. This pattern of substance use can lead to more severe health outcomes, including an increased risk of developing substance use disorders. Despite the prevalence and severe implications of PSU, there is a paucity of research examining its neural underpinnings in the adolescent population. Diffusion Tensor Imaging (DTI), a non-invasive imaging modality that measures the diffusion of water molecules in brain tissue, offers a unique opportunity to explore the structural integrity of white matter tracts. Normative samples show a clear development trajectory of white matter, with increases in fractional anisotropy (FA) measures and decreases in mean diffusivity (MD) throughout childhood and

adolescence. Disruptions in these processes may profoundly affect cognitive and behavioral outcomes, including the onset of PSU. This analysis used longitudinal data from the ongoing Adolescent Brain Cognitive Development (ABCD) Study to discover whether altered white matter maturation is a risk factor for PSU, using a fully prospective design that began before SU onset.

Methods: Participants were part of the ABCD study, a 10-year, longitudinal study that enrolled 11,875 children at age 9 or 10 across 21 U.S. sites ABCD. We used data from 5032 children (data release 5.1) with baseline through Year 4 DTI measures and explored relationships between DTI measures and PSU. DTI measures collected at 3 timepoints (Baseline, age 9-10; Year 2, age 11-12; and Year 4, age 13-14) were fit using linear mixed-effects models with predictors for (a) linear and quadratic trends for age and (b) substance use (no use (NU), use of a single substance (SSU), or PSU) at Year 3 (age 12-13), controlling for age, sex, ICV, diffusion MRI quality control variables, and time. Models included random effects of scanner manufacturer, site, participant ID, and family ID. Statistical analysis was performed using R (version 4.4.0).

Results: At Year 3, 82 (1.6%) participants reported PSU, 348 (6.9%) participants reported SSU, and 4613 (91.5%) reported NU. All white matter tracts showed highly significant main effects of age in all participants across ages 9-15, indicating increases in FA and decreases in MD, as expected (all p values > 0.001 , β (absolute value) = 0.09 to 0.21). We found significant interactions between PSU and age on white matter development in several white matter tracts, revealing altered trajectories among PSU, but not SSU or NU youth. For FA measures in the right fornix, left corticospinal/pyramidal, cingulate cingulum, and anterior thalamic radiation regions, while NU and SSU adolescents exhibited gradual steady increases typical of normative development ($\beta = .11$ to 0.21, all $p < .001$), PSU showed significant interactions with age that significantly reduced those age-related increases, generally around ages 11-14 ($\beta = -.11$ to $-.13$, $p = .021$ to 0.039). No other white matter tracts exhibited significant irregularities in those with PSU.

Conclusions: Overall, PSU showed significant irregularities in white matter development compared to adolescents using none or one substance, particularly around ages 11 to 14, in white matter tracts that play a role in cognitive and emotional processing. Generally, gradual increases in FA with age were reduced in PSU. This abnormal white matter development during adolescence could be a risk factor for the development of PSU or other risky behaviors.

Keywords: DTI, brain development, polysubstance use, substance use disorders

Disclosure: Nothing to disclose.

P299. Epigenome-Wide Methylation Patterns Associated With Self-Rating of Effects of Alcohol and Alcohol Use Disorder

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Background: Patterns of excessive alcohol use and misuse have been associated with a range of acute and chronic consequences. There are a range of genetic and environmental factors that can contribute to the heterogeneity in alcohol use and alcohol use disorder (AUD), and understanding the underpinnings of these sources of variability can greatly help improving the diagnosis and prognosis of AUD. One of these determinants is the level of response to alcohol (LR), which can be measured using the Self-Report of the Effects of Alcohol (SRE), a retrospective

questionnaire that evaluates the number of drinks required to feel the effects of alcohol. The need for a higher number of drinks to experience these effects has been shown to be genetically influenced and predicts higher risks for heavy drinking and alcohol-related problems and outcomes. Recent studies have also identified a critical role for epigenetic changes in modulating the effects of genetic and environmental variables on gene expression in several psychopathological conditions, including AUD. Thus, the objective of this study was to identify epigenetic changes, specifically DNA methylation patterns, that are associated with SRE and AUD.

Methods: Participants were enrolled in the NIAAA Natural History Protocol conducted at the NIH Clinical Center and included individuals with AUD ($n = 383$) and without AUD ($n = 351$). Blood samples were collected from all participants, and genome-wide DNA methylation data were obtained using the Illumina Methylation EPIC BeadChip (850K). The LR to alcohol was measured using the SRE questionnaire, which evaluates the number of standard drinks required to experience four different effects of alcohol. To identify differentially methylated positions (DMPs) associated with LR to alcohol, SRE total (lifetime) scores were divided into low and high groups using the median split method. DMPs were then analyzed to identify hypo- and hyper-methylated regions between AUD and non-AUD groups, as well as between low-SRE and high-SRE groups, after adjusting for age, sex, BMI, race, principal components of control probes, smoking status, pack-years, and cell compositions. Additionally, we identified overlapping and unique CpG-associated genes for both AUD and SRE.

Results: Results of the comparison of DNA methylation between AUD and non-AUD groups identified 18,539 differentially methylated CpG sites, including 12,633 hypermethylated and 5,906 hypomethylated sites across exon, 3' UTR, 5' UTR, and gene body regions. Among the alcohol response groups, the low-SRE group showed 14,297 differentially methylated CpG sites compared to the high-SRE group. This included 4,097 hypermethylated and 10,200 hypomethylated sites across the exon, 3' UTR, 5' UTR, and gene body regions. We identified 5,519 genes with significantly altered CpG sites that overlapped between AUD and SRE groups, while 3,923 differentially methylated genes were unique to AUD and 2,436 to SRE groups. Some of the significantly altered CpG-associated genes that were common to AUD and SRE groups included HNRNPA1, LMF1, LRRC20, and PLEKHG4B. This was consistent with findings from previous epigenetic studies in AUD.

Conclusions: In conclusion, this study has identified several novel and overlapping CpG sites/genes associated with AUD and level of response to alcohol. These findings provide supportive evidence for the association of previously identified AUD-related genes with level of response to alcohol and suggest potential common gene pathways underlying the relationship between alcohol response and AUD. Future work will include examining these common gene pathways as well as pathways distinct to level of alcohol response in individuals across the spectrum of alcohol use and misuse.

Keywords: Epigenetic biomarkers, Alcohol response, Alcohol use disorder

Disclosure: Nothing to disclose.

P300. Development of a Safe, Widely Accessible Neuroimaging Tool to Measure Opioid Addiction Neurobiology

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Background: Carfentanil, an extremely potent synthetic opioid, that is significantly contributing to the opioid crisis due to its high potential for overdose. Paradoxically, a variant of carfentanil labeled with carbon-11 ([¹¹C]FCFN) has been a valuable tool in brain research for nearly four decades, specifically for studying the mu-opioid receptor (MOR) system and related addiction biology with PET imaging. However, the use of [¹¹C]FCFN in clinical research is substantially limited by its extreme potency which carries safety risks and the restricted availability of carbon-11, a radioactive isotope with a short half-life of 20.4 minutes.

Methods: Herein, we introduce an alternative, a fluorine-18 labeled fluorocarfentanil ([¹⁸F]FCFN), which can be manufactured at higher molar activity, administered at lower, safer dose and that will be easier to access due the widespread availability of the longer-lived ($t_{1/2} = 109.8$ min) fluorine-18. We arrived at this compound by synthesis of six fluorophenyl carfentanil derivatives, radiolabeling optimization and preclinical neuroimaging evaluation of a preferred subset.

Results: The ideal fluorination position was identified, and we show [¹⁸F]FCFN has ideal radiotracer properties: efficient brain uptake, and pharmacokinetics for PET imaging and specific binding in MOR-rich regions of interest (e.g. thalamus) with low non-specific binding. Selectivity was confirmed through in vitro and in vivo competition binding with the primary opioid receptor family members.

Conclusions: While further studies in non-human primates are needed to better understand its pharmacokinetics and safety, we anticipate [¹⁸F]FCFN will be quickly adopted for MOR imaging of the human brain.

Keywords: Positron Emission Tomography (PET) Imaging, CNS Radiotracer, neuroimaging, mu-opioid receptors, fluorine-18

Disclosure: Nothing to disclose.

P301. Exploration of the Behavioral Profile of Sequential Opioid-Stimulant Polysubstance Use Disorders in a Translational Rodent Model

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Background: Concurrent opioid and stimulant usage is increasingly responsible for overdose deaths, yet, it is unclear how the interaction of these compounds within the nervous system produces changes in drug-seeking and -taking behavior. The goal of this study was to compare polysubstance and single substance addiction behavioral patterns using a rodent self-administration model. We hypothesized that opioid-stimulant polysubstance use would have a unique, synergistic effect on addiction-related behaviors that is not present when either drug is used alone.

Methods: Adult rats ($n = 37$ males, 31 females) were implanted with indwelling jugular catheters and trained to lever press for fentanyl (0.0015 mg/kg/infusion), methamphetamine (0.1 mg/kg/infusion), or both drugs using an intermittent access protocol. We utilized a sequential model of polydrug use (i.e. separate use of multiple drugs modeled after human drug use patterns. Rats in the single drug conditions either self-administered fentanyl seven days a week (FENT) or methamphetamine five days a week (METH). Rats in the polydrug condition (POLY) completed daily fentanyl self-administration and five days a week of methamphetamine self-administration in separate sessions. After acquisition and escalation of drug intake over 3 weeks, we conducted either 1) an extinction and cue-induced reinstatement of drug-seeking test, or 2) a behavioral economics thresholding test. POLY rats completed these tests for one of the two substances.

Results: Total drug intake and lever pressing was similar in POLY and single drug groups. However, differences between groups emerged in the behavioral tests. When compared to METH rats, POLY rats showed higher levels of drug-seeking during extinction (two-way ANOVA; $F(1,23) = 6.634$, $p = 0.0169$), and higher levels of reinstatement of drug-seeking to a cue associated with methamphetamine use ($t(23) = 2.160$, $p = 0.042$). POLY and FENT rats did not differ in drug seeking during extinction or reinstatement of drug-seeking to fentanyl cues.

Preliminary results from the behavioral economics procedure revealed group- and drug-specific differences in hedonic setpoint (Q_0) and demand elasticity (α). The hedonic setpoint, or the initial intake of drug at minimal to no cost of consumption, for both drugs was altered in rats with a history of polysubstance use. When the price for drug consumption was low, POLY rats preferred a higher initial dose of fentanyl than FENT rats ($t(5) = 7.465$, $p = 0.0007$); yet POLY rats preferred a lower initial dose of methamphetamine than METH rats ($t(5) = 3.321$, $p = 0.021$). Demand elasticity, an inverse measure of motivation normalized to Q_0 , was lower for methamphetamine compared to fentanyl in animals with a single drug history ($t(5) = 3.064$, $p = 0.028$). Fentanyl demand elasticity decreased with polysubstance history ($t(5) = 2.841$, $p = 0.0362$), while there was a non-significant increase in methamphetamine demand elasticity with polysubstance history.

Conclusions: Our findings suggest that there are bidirectional and substance-specific trends in behavioral patterns in animals with a history of polysubstance use. These differences occurred even though the amount of fentanyl or methamphetamine self-administration between the polysubstance and single substance groups were comparable. These results emphasize the unique behavioral phenotype of opioid-stimulant polysubstance use and may indicate the recruitment of distinct neural circuits compared to those implicated in single substance addiction. Ongoing experiments will use whole brain light sheet microscopy to map neurocircuit activation patterns that may underlie these behavioral differences in poly- and single substance use. Understanding the neurobehavioral effects of polysubstance use is essential for the development of targeted, effective treatments for polysubstance addiction.

Keywords: polysubstance use, Rodent models, behavioral economics, Fentanyl Self-Administration, Methamphetamine Self-Administration

Disclosure: Nothing to disclose.

P302. Pharmacokinetic Assessment of High Affinity D4R-Selective Ligands as a Pharmacological Tool to Treat Substance Use Disorder

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Background: The dopamine D4 receptor (D4R), a G protein-coupled receptor, is predominantly expressed in the prefrontal cortex in which it plays an important role in cognition, attention, and decision making. Studies have indicated that D4R-selective ligands are promising candidate medications for neuropsychiatric conditions such as substance use disorders (SUD). D4R ligands have been shown to alter cognition and behavior in animal models of drug addiction. A better understanding of D4R-mediated signaling is essential to treating D4R-associated disorders, including SUD. Despite their clinical importance, there are currently no FDA-approved medications that selectively target

the D4R, nor are there any for cocaine use disorder treatment. The present study focuses on the design of next-generation D4R ligands featuring bioisosteric replacement of an amide scaffold with 1,2,3-triazoles, including in vitro and in vivo pharmacokinetic analyses.

Methods: The compounds were designed and synthesized by using a click chemistry reaction approach to bioisosterically replace the amide with triazoles. Compounds were purified and analytically characterized followed by CHN combustion elemental analysis. In vitro binding affinities were determined via [³H]N-methylspiperone radioligand binding using membranes prepared from HEK293 cells expressing dopamine D2-like receptors (D2R, D3R, D4R). The ligands were also studied in β -arrestin recruitment assays for their effects on D2R-like function; followed by in vitro and in vivo pharmacokinetic analyses with selected compounds.

Results: Compounds featuring a bioisosteric replacement of the amide linker with a triazole moiety were successfully synthesized and profiled using radioligand binding displacement assays and β -arrestin recruitment assays. Overall, triazole compounds had very similar efficacy and potency profiles compared to their amide analogs. We identified several compounds with nanomolar D4R binding affinity and excellent D2-like subtype selectivity (≥ 705 -fold versus D2R and D3R). The compounds displayed D4R partial agonist or antagonist activity in functional β -arrestin recruitment assays. In microsomal assays, triazole compounds had improved stability compared to their amide analogs, including a complete loss of non-Phase I metabolism. Based on these analyses, lead compound FMF-01-042 was selected for in vivo pharmacokinetic assessment in rats where preliminary results indicate displayed good brain penetration with AUC_{brain/plasma}~2.7.

Conclusions: The triazole compounds maintain their pharmacodynamic profiles but are more resistant to drug metabolism compared to their amide analogs. These ligands display high binding affinity and subtype selectivity for D4R with a range of efficacies. Given their improved metabolic stability, including FMJ-01-042 with its excellent brain penetration, this next generation of compounds is more suitable for behavioral testing. Development of these compounds may lead to a better understanding of the role of D4Rs in neuropsychiatric disorders such as SUD and provide insights to further targeted drug discovery.

Keywords: Substance abuse disorders, Dopamine D4 receptor, Triazole compounds, Partial agonist Ligands

Disclosure: Nothing to disclose.

P303. Association Between Pre-Menstrual Dysphoria and Alcohol Consumption in Naturally Cycling Females Who Drink Heavily and Smoke

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Background: Three out of four females of reproductive age experience premenstrual syndrome (PMS). PMS is characterized by a host of physical and emotional symptoms such as menstrual cramps, negative mood, weight gain and back pain. Pre-menstrual dysphoria disorder (PMDD) describes more severe experiences of PMS that significantly impairs quality of life. With a large population of female drinkers being of reproductive age and potentially experiencing PMS (with a smaller percentage experiencing PMDD), it is important to consider how premenstrual symptoms and premenstrual dysphoria impact alcohol use. This is especially relevant since females are more likely to drink in response to negative affect than males. To evaluate this effect, this study examines a sample of naturally cycling females who drink heavily and smoke cigarettes enrolled in a clinical trial for smoking cessation and drinking reduction. We tested the effects of

premenstrual dysphoria on three drinking outcomes measured over the course of a 30-day period prior to study enrollment: 1) any drinking (Y/N; Binary), 2) mean number of drinks consumed, and 3) heavy drinking (Y/N; binary). We hypothesized that premenstrual dysphoria would be associated with greater alcohol use such that naturally cycling females with greater premenstrual dysphoria would have a higher likelihood of drinking and heavy drinking and have a higher mean number of drinks consumed.

Methods: Naturally cycling female participants (N = 23) who were seeking treatment for smoking cessation and drinking reduction completed a battery of demographics, and alcohol and nicotine use assessments. Participants were interviewed using the Timeline Followback (TLFB) to retrospectively capture the past 30 days of alcohol use. The standard number of drinks reported in the TLFB were transformed into the binary drinking outcomes of any drinking or heavy drinking (≥ 4 drinks in a drinking occasion). All participants completed the Shortened Premenstrual Assessment Form (SPA-F), which evaluated the intensity of their premenstrual symptoms during their last cycle from seven days before their period to when menstruation began. Total SPA-F scores equal to or above 27 was considered the cut off for PMDD. We included a categorical variable (weekend versus weekday) to account for whether drinking took place on weekend days (Friday-Sunday) or weekday (Monday-Thursday). General and generalized linear mixed models tested the effects of premenstrual dysphoria, weekend drinking, and their interaction on the mean number of drinks consumed and the likelihood of any drinking and heavy drinking. For binary dependent variables (i.e., any drinking and any heavy drinking), we used a binary distribution and a logit link function. **Results:** In this sample of naturally cycling females who drink heavily and smoke, there was a significant interaction between premenstrual dysphoria (via SPA-F total score) and weekend versus weekday on likelihood of heavy drinking ($F_{1,665} = 4.46$, $p = 0.0351$, $CI = 0.005, 0.129$) controlling for AUD symptoms. Thirty percent of participants had a total SPA-F score that reached the clinical cutoff for PMDD. To help interpret this interaction, premenstrual syndrome symptom scores were entered in the model as a categorical variable based on clinical cutoffs (non-PMDD versus PMDD). In this updated model, there was a significant main effect of weekend ($F_{1,665} = 47.96$, $p < 0.0001$, $CI = 0.449, 1.573$), as well as a significant interaction of non-PMDD versus PMDD groups with weekend versus weekday on heavy drinking likelihood ($F_{1,665} = 10.7$, $\beta = 1.80$, $p = .0012$, $CI = 0.72, 2.89$) when controlling for AUD symptoms. Post hoc comparisons showed that the non-PMDD and PMDD groups both had a higher likelihood of heavy drinking on the weekend; however, there was no statistical difference between these groups on likelihood of heavy drinking on weekend or weekday (p 's > 0.05). There were no significant effects of premenstrual dysphoria, weekend drinking, or their interaction on likelihood of any drinking or mean number of drinks consumed controlling for AUD symptoms (p 's > 0.05). **Conclusions:** Results show that premenstrual dysphoria is not strongly associated with alcohol consumption among naturally cycling females who drink heavily and smoke. While the omnibus tests showed a significant interaction between premenstrual symptoms and weekday versus weekend on likelihood of heavy drinking, post hoc tests adjusting for multiple comparisons did not robustly demonstrate that premenstrual dysphoria moderates the relationship between weekend/weekday and likelihood of heavy drinking. A limitation of this study is the small sample (N = 7) of females that endorsed PMDD. A larger sample of females with severe premenstrual symptoms may show a more robust association between premenstrual dysphoria and alcohol consumption. Future work should investigate the relationship between menstrual cycle and alcohol consumption in females, specifically focusing on the luteal phase where PMS and PMDD occur. Further examination of the association between menstrual dysphoria and alcohol

consumption in females would allow future studies to personalize treatments for AUD to the menstrual cycles of female participants.

Keywords: Alcohol, premenstrual syndrome, premenstrual dysphoric disorder, alcohol drinking

Disclosure: Nothing to disclose.

P304. Probing the Molecular Correlates in the Nucleus Accumbens of Individual Differences in Volitional THC Consumption

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Background: The United States is in the midst of a significant paradigm shift around the perception and use of cannabis (*Cannabis Sativa*) with most Americans supporting legalization. Although many believe that cannabis use is safe and has low addiction liability, repeated large-scale reports indicate that 10-30% of users meet the criteria for cannabis use disorder (CUD). CUD is an addictive disorder is characterized by high levels of intake, poor control over use, and craving associated with significant negative impact on the individual. As cannabis becomes more widely available, it is essential to determine causal behavioral and neurobiological substrates that influence the development of CUD. CUD is highly comorbid with social anxiety disorder, and those who chronically use cannabis show deficits in impulse control and poor cost/benefit decision making. However, it is unclear from clinical data whether these phenotypes are predictive or are the result of cannabis use and the mechanisms contributing to these outcomes.

Methods: We examined whether affective and cognitive behaviors often associated with CUD predicted or responded to THC consumption using a translational model of volitional edible THC consumption. Prior to drug access, male and female Long-Evans rats ($n = 10-22/\text{group}$) were assessed for social approach or decision making using the three-chamber social interaction test and preclinical analogue of the Iowa Gambling Task, the rat gambling task (rGT), respectively. Following initial behavioral testing, animals were given daily access to either control or THC-infused gelatin for 1hr/day starting at a concentration of 0.133 mg/g which was subsequently doubled to 0.27 mg/g to assess titration of intake. Performance on the rGT was assessed in the morning prior to edibles sessions, and social interaction was re-tested after consumption levels were stable at the 0.27 mg/g concentration. Animals were sacrificed ~1.5hr following the final consumption session. To determine whether THC consumption was associated with alterations in the endocannabinoid system,

we assessed changes in endocannabinoid enzyme transcripts fatty acid amide hydrolase (Faah), monoacylglycerol lipase (Mgll), and cell activation cFos using qPCR in multiple mesocorticolimbic structures. We also used unbiased RNA sequencing of the nucleus accumbens core (NAcC) to deeply interrogate transcriptional changes associated with THC intake. For all studies, repeated measures ANOVA or mixed-effects models were used to assess behavioral and qPCR effects followed up by post-hoc tests of significant main effects with drug and sex as between-subjects factors. Corrections for sphericity, normality, and multiple comparisons were used when necessary. RNA-sequencing data was analyzed using the DESeq2 package in R.

Results: We observed stark sex and individual differences in THC consumption. Although male and female rats consumed similar amounts of control gelatin, males consumed significantly more THC at both concentrations. To determine whether there were individual differences in consumption regardless of sex, we used a k-means clustering approach to assess different levels of intake at the different concentrations. Three distinct groups emerged: low, moderate, and high consumers of THC. While low and moderate consumers exhibited titration of intake to preferred levels of consumption, high consumers doubled drug intake after the concentration increased. High THC consumption was predicted by both social avoidance and risky choice, mimicking results from individuals with CUD. From our qPCR screen we saw sex and drug differences in Faah, Mgll, and cFos in areas of the prefrontal cortex and nucleus accumbens, and VTA with the most pronounced changes in the NAcC (but not shell). RNAseq of the NAcC revealed that male and female THC animals exhibited distinct patterns of mRNA expression after consumption with divergent effects in expression of pre- and post-synaptic plasticity transcripts, recruitment of interneuron populations, and trans-synaptic signaling. In the individual differences analysis, there was distinct recruitment of histone demethylases and changes in endothelial cells in high consuming rats, suggesting epigenetic sensitivity to THC and potential impact on the blood-brain barrier.

Conclusions: Using a translational model of volitional THC consumption we were able to detect sex and individual differences of drug intake which echoed results from human users with CUD. THC consumption preferences were associated with changes in both the endocannabinoid system and plasticity in the nucleus accumbens core which may influence drug intake as well as decision making and social avoidance phenotypes. These results provide us with a robust model to assessment mechanisms underlying CUD-like behavior. Ongoing studies are investigating alterations in protein expression and levels of bioactive lipids such as the endocannabinoids to determine whether mRNA changes correspond to alterations in protein and lipid networks.

Keywords: cannabis use disorder, Decision Making, Social Behavior, molecular mechanisms, Translational research

Disclosure: Nothing to disclose.