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P276. Deconvolving Cell Type Proportions in Human Postmortem Brain Tissue From Bulk RNA-Seq Data

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Background: The brain contains a wide variety of structures, that in turn are made up of many different cell types with individual complex functions. Bulk RNA-seq from tissue homogenates obscures this complexity. Deconvolution seeks to estimate the composition of cell types in the sequenced tissue to examine biological differences between samples, or minimize confounding technical variables, such as differences in dissection, in downstream analysis.

Methods: Reference snRNA-seq Dataset

For the reference single nucleus RNA-seq dataset we utilized the data set from Tran et al. (https://doi.org/10.1101/2020.10.07.329839) which includes 70,615 high-quality nuclei across five human brain regions: nucleus accumbens (NAc), amygdala (AMY), subgenual anterior cingulate cortex (sACC), hippocampus (HPC), and dorsolateral prefrontal cortex (DLPFC). The ten cell types considered in the deconvolution of the tissue were Astrocytes (Astro), Endothelial (Endo), Macrophage (Macro), Microglia (Micro), Mural cells, Oligodendrocytes (Oligo), Oligodendrocyte Progenitor Cells (OPC), T cells, Excitatory Neurons (Excit), and Inhibitory Neurons (Inhib).

Cell Type Marker Gene Selection

To give the algorithms the best change of accuracy, we selected for highly specific genes to each cell type, ideally only expressed in the target cell type. To achieve this, we developed a marker selection strategy: calculating the ratio of the mean expression of each gene in the target cell type over the highest mean expression of that gene in a non-target cell type. The top 25 genes for each cell type were selected.

Deconvolution Algorithm Selection

We considered two different deconvolution methods: MuSiC (https://doi.org/10.1038/s41467-018-08023-x) and Bisque (https://doi.org/10.1038/s41467-020-15816-6), which showed strong performance (https://doi.org/10.1038/s41467-020-19015-1). MuSiC claims to be good for differentiating between closely related cell types, and Bisque showed the highest performance in their benchmark analysis on DLPFC data. Deconvolution was performed with MuSiC version 0.2.0 and the ReferenceBasedDecomposition function from BisqueRNA version 1.0.4, using the use.overlap = FALSE option.

Bulk RNAseq Datasets

We performed deconvolution on eight, in-house, post-mortem, human brain, bulk RNAseq datasets; spanning 5,787 samples, in seven brain regions (DLPFC, sACC, AMY, HPC, Caudate, Dentate Gyrus, and Nucleus Acumbens), and four diagnoses (MDD, Bipolar, SCZD, and control), as well as the GTEx v8 brain dataset. We will focus here on the deconvolution of the MDDseq data set containing 1,091 samples, with 704 (459 MDD, 245 Bipolar) cases and 387 control samples, from the sACC and AMY (Synapse syn22276064).

Results: Marker finding

The set of marker genes we identified is highly specific, confirmed by visualizing violin plots of gene expression over cell type, heat maps of pseudo-bulked cell type from each donor, and t-tests comparing against all other cell types (FDR < 5%). The resulting set of marker genes overlaps eight known marker genes (https://doi.org/10.1038/s41586-019-1195-2) and identifies new data driven marker genes (96%).

Method selection

We observed that MuSiC is highly sensitive to the set of marker genes, while Bisque is robust. Bisque also returns cell fraction estimates that were concordant with expected regional variability (https://doi.org/10.1101/2021.01.21.426000).

MDDseq results

The Bisque estimated cell type proportions showed a difference in means across diagnoses (MDD, Bipolar, control) for either brain region (AMY, sACC) in 22 out of 60 pairwise t-tests (testing ten cell types, in two brain regions, between three diagnoses; p.bonf < 0.05). Between samples from male and female donors 6 out of 20 t-tests are significant (testing for ten cell types, in two brain regions; p.bonf < 0.05). However, the effect size of the differences is minimal. Correlation analysis of the cell type proportions vs. quality surrogate variables (qSV) (https://doi.org/10.1073/pnas.1617384114) revealed qSVs may already adjust for variation in cell type composition. Each cell type has a significant correlation (p.bonf < 0.05) to at least four of the top ten qSVs.

Conclusions: We found that the mean ratio marker selection performed better than previous gene marker selection strategies. Based on the nine datasets and the expected cell type composition variability across human brain regions, we found Bisque to be the most accurate of the available deconvolution methods. The results reported for the MDDseq dataset represent a pattern of results seen in the other bulk RNA-seq human brain datasets.

Keywords: RNA-seq, Deconvolution, Single-Nucleus RNA-seq, Human Post-Mortem Brain, Bisque

Disclosure: Nothing to disclose.

P277. Setting the Stage for Chemogenetic Manipulations of Whole Brain Resting State Functional Connectivity in Nonhuman Primates

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Background: Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are modified G-Protein Coupled Receptors that can be targeted to select neuronal populations. Once expressed, pharmacological activation of DREADDs with a chemogenetic ligand allows for transient, remote, and reversible manipulation of cells expressing the receptors. Clozapine-N-Oxide (CNO) is commonly used to activate DREADDs, however, CNO demonstrates poor brain permeability, requiring large doses to be administered and is metabolized into clozapine. Recent studies have shown that low dose clozapine (CLZ) and novel ligand Deschloroclozapine (DCZ) have a higher affinity for DREADDS with minimal off-target binding. The utilization of DREADDs in nonhuman primates (NHP), though limited in comparison to studies using rodents, allows for the causal perturbation of targeted cells in a model species with brain structures more similar to those found in humans. Functional Magnetic Resonance Imaging (fMRI) is a non-invasive neuroimaging approach that measures fluctuation in hemodynamic signals locally and across the whole brain. fMRI has identified altered functional connectivity in many neurological and psychiatric disorders.

The aim of this study was to lay the groundwork for combining DREADDs manipulation with resting state fMRI in NHP. To this end, we first determined the infection and transport patterns of common AAV serotypes in macaques (see Cushnie et al., 2020).

Methods: We evaluated the effects of chemogenetic ligands on resting state functional connectivity prior to DREADDs transduction in NHP. Macaques underwent a series of resting state fMRI and structural MRI scans at 10.5 Tesla at UMN's CMRR. Acutely before the start of each scan, Macaca fascicularis ($n = 2$, female), received a systemic injection of chemogenetic ligand (DCZ 0.1mg/kg, DCZ 0.2mg/kg, CLZ 0.1mg/kg, CLZ 0.2mg/kg) or vehicle. NHPs were anesthetized with ketamine (10 mg/kg intramuscularly), and subsequently they were intubated using 1% isoflurane and monitored using standard physiological monitoring. For functional imaging, we used a simultaneous multi-slice gradient echo planar imaging approach at 0.75 mm isotropic with whole brain coverage (MB = 2, GRAPPA = 3). We collected resting-state data for 6 runs, ~2 hours/session.

Functional connectivity based on gradient and spin echo (T2* and T2) imaging was analyzed using a custom pipeline of a combination of FSL, AFNI and the CONN toolbox; neuroimaging software. Images were corrected for motion distortion using mcflirt (images are registered to the first image). Images were corrected for echo planar imaging distortions resulting from long readout trains using FSL topup. Slice scan time correction was applied to adjust for offsets in image time acquisition using a custom written C++ routine. Lastly, images were corrected for physiological artifacts through a multi-step denoising pipeline. Global white matter, CSF, and venous blood time courses were estimated using singular value decomposition and combined with respiration and heart rate. All images were analyzed in their original reference space.

Results: We examined whole-brain changes in resting-state functional properties after systemic injections of vehicle or chemogenetic ligand. Specifically, we assessed changes in functional connectivity and intrinsic timescales. Resting-state functional connectivity is characterized by temporal correlations between spontaneous blood oxygenation level-dependent (BOLD) signals. Intrinsic timescales, a fundamental local property, are temporal fluctuations in the neural signal that are task and variable independent. Thought to reflect the amount of temporal integration of a region, intrinsic timescales are closely related to the brain's functional hierarchy. fMRI has identified altered functional connectivity and intrinsic timescale patterns in many neurological and psychiatric disorders.

The relationship between functional connectivity and drug is highly correlated between subjects, suggesting a relatively consistent change in resting-state functional connectivity in response to drug conditions (for both DCZ and CLZ). Functional connectivity between subjects is least correlated during vehicle conditions (most likely due to individual innate differences) and the highest correlation is observed following administration of CLZ doses. Compared to CLZ, DCZ has a lesser effect on functional connectivity and may be best suited for future studies. These results were also consistent for intrinsic timescale analysis.

Conclusions: This proof of principle study revealed local and whole-brain functional connectivity patterns prior to DREADDs expression, setting the stage for more detailed network interrogation using chemogenetic manipulations and resting-state fMRI in NHPs.

Keywords: Chemogenetics, Functional MRI (fMRI), DREADDs, Brain Anatomy

Disclosure: Nothing to disclose.

P278. Glucocorticoid-Priming of Inflammatory Response to LPS Challenge in Microglia

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Background: Glucocorticoids are renowned for their immunosuppressing effects. The immunomodulatory effects of glucocorticoids have been shown to extend to microglia, altering both their morphology and function. However, previous reports have largely focused on the acute effects of glucocorticoids on microglial processes. Glucocorticoids tend to produce an "inverted-U" dose-response with varying effects depending on the time-scale of treatment (acute versus chronic). Here, we examined the long-term effects of acute glucocorticoids on microglial responses to immune challenge.

Methods: We used mouse BV2 microglial cells as an in vitro model to examine the effects of glucocorticoids on the immune-activating effects of lipopolysaccharide (LPS). To examine LPS-induced alterations in chromatin and transcription within microglia, we administered the synthetic glucocorticoid dexamethasone (10nM), cross-linked chromatin after 90 min for glucocorticoid receptor (GR) chromatin immunoprecipitation (ChIP), and collected RNA for RT-qPCR after 180 min. We also co-treated cells with both LPS (1.0 µg/ml) and dexamethasone (10 nM) for 24 hr, and collected media for ELISA to quantify inflammatory cytokines and RNA for RT-qPCR. Additionally, we compare these results with microglia isolated from two in vivo manipulations of the glucocorticoid system, acute dexamethasone (10mg/kg, IP, 4 hr) and chronic social defeat stress ([CSDS], 10 days) in male C57BL/6J mice. Following each manipulation microglia were isolated using magnetic cell sorting for the microglia/brain macrophage marker CD11b and then RNA was isolated for RT-qPCR. Group differences were examined using unpaired student's t-tests.

Results: Dexamethasone treatment upregulated expression of mRNA encoding the clock gene period 1 (Per1) ($p < 0.05$) and the kappa-opioid receptor (KOR; Oprk1) ($p = 0.05$), a receptor previously implicated in responsiveness to stress, in microglial BV2

cells (n's = 5 plates per group). Consistent with previous findings, we found that co-administration of dexamethasone and LPS attenuated release of Il-1ß from microglia compared to release following treatment with LPS alone ($p < 0.05$, n's = 3 plates per group). In our in vivo manipulations, acute dexamethasone significantly downregulated Il-1ß ($p < 0.01$) and upregulated Oprk1 ($p < 0.01$) in microglia, whereas following a 10-day CSDS paradigm there was an upregulation of Il-1ß ($p < 0.01$) and downregulation of Oprk1 ($p < 0.05$) (n's = 6-8 per group).

Conclusions: These experiments demonstrate the potential for glucocorticoid-regulated gene expression to have lasting effects on microglia activation. We found initial evidence that acute glucocorticoids can have a differential effect on proinflammatory cytokines with acute dexamethasone decreasing Il-1ß expression, whereas CSDS enhanced Il-1ß expression in microglia. Interestingly, we found that dexamethasone elevated levels of Oprk1 mRNA, consistent with previous reports indicating that KORs are expressed in microglia and regulated by stress. Expression of KORs on microglia, where they would presumably act to inhibit inflammatory processes, is important considering that KOR antagonists are currently being evaluated as treatments for depressive illness, which has previously been associated with inflammatory processes. Future work will examine the mechanisms by which these responses are regulated, including measuring GR binding to response elements upstream of both cytokines and Oprk1 as well as the mRNA expression following co-treatment of dexamethasone with LPS to determine if there are alterations in GR binding at GR response elements. Additionally, we will overexpress Oprk1 in BV2 cells to examine if lasting effects of glucocorticoid-treatment on microglial inflammatory responses are mediated by glucocorticoid-induced Oprk1 expression.

Keywords: Glucocorticoid, Microglia, Kappa Opioid Receptor

Disclosure: Nothing to disclose.

P280. Dose Dependent Effects of Transcranial Photobiomodulation on Depression Severity in Major Depressive Disorder

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Background: Transcranial photobiomodulation (t-PBM) with near-infrared (NIR) light penetrates into the cerebral cortex and is absorbed by the mitochondrial enzyme cytochrome c oxidase (CCO), stimulating the mitochondrial respiratory chain. t-PBM also significantly increases cerebral blood flow (CBF) and oxygenation. Small studies have reported that t-PBM may be an effective treatment in major depressive disorder (MDD). However, relationships between t-PBM dose (irradiance and/or total energy) and clinical or biological effects are unclear. In this experimental medicine study, we evaluated the dose-dependent, clinical effects of t-PBM in MDD subjects.

Methods: We enrolled subjects meeting DSM-5 criteria for MDD, not treatment-resistant (0-2 failed antidepressants in the current episode), either unmedicated or on stable doses of antidepressants, with no other significant medical or psychiatric comorbidities. All subjects underwent 4 t-PBM sessions, 1 week apart, administered in random order, with either sham or 3 combinations of t-PBM parameters: 1) Pulse wave (PW), average irradiance 300mW/cm2, 42Hz, 33% duty cycle, 4.3 KJ total energy; 2) Continuous Wave (CW), 300 mW/cm2 irradiance, 2.4 KJ total energy; 3) "Low dose": CW, 50 mW/cm2 irradiance; 1.4 kJ total energy. Other t-PBM parameters were kept unchanged (808 nm; 12.0 cm2 × 2 treatment area; delivered to the prefrontal cortex, bilaterally, corresponding to the F3 and F4 electrode location). All subjects were rated by study investigators for their depression severity with the Montgomery-Asperg Depression Rating Scale (MADRS) at baseline and one week after each t-PBM session. Each t-PBM, dose-specific, antidepressant effect was assessed by computing the change in the MADRS total score from baseline to one week after the index t-PBM dose. The mean changes in MADRS total scores obtained after each t-PBM dose were compared to the effect of sham (paired t-test, with significance at 0.05).

Results: We analyzed data from the first 15 MDD subjects (11 White, 1 Black, 1 Asian, 2 Multiracial and 2 Latinos; age = 34.6 ± 11.3; 60% female) undergoing all 4 experimental sessions. We found that a single session of either PW (delta -5.7 ± 9.4, $p < 0.04$), CW (delta -6.9 ± 8.6, $p < 0.01$) or "low dose" CW (delta -5.9 ± 6.5, $p < 0.01$) was more effective than sham (delta -2.0 ± 8.4). CW appeared to be slightly better (>25% decrease in MADRAS total score) than PW (21%) or "low dose" CW (22%), however these differences were not statistically significant.

Conclusions: These findings suggest that a single session of t-PBM might induce significant antidepressant effects within one week of treatment. This is important as t-PBM could become a candidate treatment to expedite antidepressant response in patients with MDD. The data also suggest that specific parameters of t-PBM (CW rather than PW, higher irradiance and higher total energy) might be more effective. More clarity on the latter point will likely arise when the complete sample ($n = 30$) is analyzed.

Keywords: Transcranial Photobiomodulation, Near-Infrared Light, Neuromodulation, Neurostimulation, Depression

Disclosure: Niraxx Light Therapeutics Inc, Stock / Equity, Board Member, Advisory Board, Founder (Self)

P281. Associations Between Altitude, Suicidal Ideation, and Depressive Symptoms Among University Students in the Healthy Minds Study

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Background: There is increasing evidence that suicide rates in the U.S. and other countries are associated with altitude of residence, with higher suicide rates in high-altitude regions like the Rocky Mountain West. The psychopathological mediators of this association remain poorly characterized, with mixed findings regarding associations between altitude and depression. To attempt to replicate findings linking altitude and suicide and to shed light on possible psychopathological underpinnings of that association, we examined correlations between altitude of residence, suicidal ideation, suicide attempts, depressive symptoms, and previous diagnoses of depression in a large retrospective dataset of university students in the Healthy Minds Study.

Methods: We obtained data for 260,967 students from the Healthy Minds Study, which a large multiyear anonymous cross-sectional database of university students, including multiple sociodemographic measures, psychiatric symptom scales such as the PHQ-9, and self-reported lifetime history of suicidal ideation and attempts. Altitude, latitude, and longitude for each campus were determined using Google Earth. Moran's I was calculated for each outcome variable to determine whether it was spatially autocorrelated. Correlations between altitude and self-reported diagnoses, suicidal ideation, suicide attempt, PHQ-9 score, and PHQ-9 item 9 (suicidal ideation) were estimated using a mixed model with repeated measures a spatial Gaussian covariance structure, with individual sociodemographic variables treated as fixed effects. Multiple imputations were utilized to address missing data.

Results: 53% of participants were women, 65.8% Caucasian, 94.5% enrolled in a four-year program, and the average age was 22.5 (+ 5.2) years. The average altitude was 255.7 (+ 358.9) *m*. The mean PHQ-9 total score was 16.0 (+ 5.6). Moran's I indicated each outcome variable was spatially autocorrelated. In geographically-weighted mixed models, and after correction for multiple hypothesis testing, altitude was significantly correlated with any diagnosis of depression ($p = 0.0014$), a diagnosis of bipolar depression ($p = 0.0022$), but not a diagnosis of major depressive disorder. However, the interaction between gender and altitude was strongly significant, and major depressive disorder was significantly positively correlated with altitude for women but not for men. Similar results obtained for bipolar depression. PHQ-9 total score was significantly and positively correlated with altitude ($p < .0001$). PHQ-9 item 9 was also significantly correlated with altitude ($p = 0.007$) and the interaction terms of altitude with a history of depression was significantly correlated with PHQ-9 item 9. Lifetime suicide attempt was significantly correlated with altitude ($p < .0001$), although lifetime suicidal ideation was not.

Conclusions: Our results are consistent with previously published population-level evidence of an association between altitude of residence and various forms of psychopathology, including suicide, but augment those findings by examining outcomes among a large nationally-distributed sample of young adults who have provided individual psychometric data. Our results also identify, for the first time, statistically significant associations between altitude, depressive symptoms as measured by the PHQ-9, and an historical diagnosis of depression. We observed particularly significant associations between altitude and a diagnosis of bipolar depression. Consistent with findings from previously published animal studies, the effect of altitude on depressive diagnosis was greater for women than men. Our results reinforced findings that increasing altitude is associated with increasing risk of suicide, as we observed that past-week suicidal ideation as measured by PHQ-9 item 9 was significantly correlated with altitude, and that a history of suicide attempt was associated with altitude. The study was limited by several factors. First, the altitude to which participants were exposed could only be inferred from the altitude of their campus. Second, although participants reported their year in school, the duration of their exposure to the local altitude was unknown, as it was unknown whether they had previously resided near the university or at a similar altitude. Similarly, assessment of suicidal behavior (suicide attempts) was limited to participants' lifetime self-reported history; it was unknown, however, whether reported suicide attempts occurred while the participant was enrolled at the university or when they were residing elsewhere. Finally, the magnitudes of the associations with altitude observed in our study were, although consistently significant, usually small. Nevertheless, these findings support previously identified links between suicide rates in the United States and altitude, imply that increases in depressive symptoms may mediate this association, especially in women and in persons with bipolar disorder, and argue for the importance of further investigating mechanisms by which altitude could contribute to psychopathology.

Keywords: Suicide Risk Factors, Major Depression Disorder, Altitude **Disclosure:** Nothing to disclose.

P282. Alterations in the Functioning of Striatal Subregions are Associated With Anhedonia as a Function of Striatal Dopamine Concentrations in Adolescents With Depression

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Background: Depression affects about 20% of adolescents and substantially increases the risk for suicidality, substance abuse, as well as academic and interpersonal difficulties. It is a heterogeneous condition with varying degrees of severity and course, but little is known about the etiological processes underlying clinical features and outcome. Anhedonia, including loss of interest, motivation, and pleasure, is a core symptom of depression that has been associated with poorer treatment response and worse overall outcomes. Adolescent depression and anhedonia have been associated with changes in reward-related behaviors and alterations in the dopaminergic reward system, including the striatum. However, it remains unclear whether specific patterns of alterations in the functioning of the striatum may be associated with anhedonia relative to other symptoms of depression. To address this gap, we examined, in a sample of adolescents with varying levels of depressive symptoms, whether resting state striatal regional homogeneity– an index of regional synchronization generally shown to be reduced in adult depression– was associated with symptoms of anhedonia and to what extent this relationship may be moderated by striatal dopamine concentrations.

Methods: Consistent with the NIMH RDoC framework, adolescents between 12-17 years old ($n = 75$; 46 females) were recruited to participate in this study. They were oversampled for clinically high levels of depression, with 56 adolescents scoring ≥ 40 on the Children's Depression Rating Scale-Revised (CDRS-R) and 19 adolescents having no current or past psychiatric diagnosis, and no parent with a psychiatric diagnosis. Participants completed a clinical interview, self-report measures, and an fMRI protocol that included two 6 min. resting state sessions. Adolescents reported symptoms of anhedonia using the Snaith-Hamilton Pleasure Scale (SHAPS) and symptoms of depression using the Mood and Feelings Questionnaire (MFQ). Resting state fMRI data was preprocessed using fMRIprep 20.1.1. Regional homogeneity (ReHo), a measure of intrinsic, local connectivity at a voxel-level spatial scale, was calculated within the striatum. Mean standardized T2* was calculated within the striatum and is used as a proxy for (inverse) tissue iron concentration, which in turn acts as a proxy for dopamine concentration. To examine the relationships between ReHo, mean T2* intensity, and anhedonia symptoms, we used a voxel-wise moderated mediation approach controlling for age, sex, and symptoms of depression (excluding items on the MFQ assessing anhedonia). A clusterwise permutation approach was used to control the familywise error rate.

Results: After controlling for age, sex, and other depressive symptoms, we found a significant moderation effect indicating that striatal tissue iron concentration moderated the relationship between ReHo and anhedonia within the bilateral caudate and the left putamen. Simple slopes revealed that reduced ReHo was associated with higher levels of anhedonia in adolescents with higher levels of tissue iron concentration in the right caudate (peak $T = 4.17$), and with lower levels of anhedonia in adolescents with lower levels of tissue iron concentration in the same region (peak $T = 2.99$). Lower tissue iron concentration in the left putamen was associated with higher levels of anhedonia overall (peak $T = 2.79$).

Conclusions: Findings indicate that a specific pattern of alterations in striatal neurobiology is associated with anhedonia in adolescents with varying levels of depressive symptoms. Namely, intrinsic connectivity in subregions of the striatum is associated with anhedonia but the direction of this relationship is contingent upon levels of striatal dopamine concentrations. While there is a need to determine the underlying mechanisms, such findings point to the need to examine whether dopamine-targeted pharmacotherapy may be particularly effective for a subset of adolescents with depression characterized by anhedonia. They also signal the need to deepen our understanding of the development of the striatal dopaminergic system in adolescent depression. Future work will examine to what extent these patterns of striatal functioning predict trajectories of anhedonia symptoms over 2 years.

Keywords: Adolescent Depression, Striatum, Dopamine, Anhedonia, Resting State Intrinsic Connectivity**Disclosure:** Nothing to disclose.

P283. Preference for Extrasynaptic GABA-A Receptors Conveys a Wider Therapeutic Window Between Anxiolytic and Sedative-Like Effects in Rats for the Positive Allosteric Modulator, PRAX-114, Compared With Zuranolone

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Background: Neuroactive steroid (NAS) positive allosteric modulators of GABA-A receptors (GABAAR PAMs) are demonstrating promise as rapid acting antidepressants with potential to address the unmet need in major depressive disorder (MDD). The antidepressant effects of NAS are thought to depend on potentiation of extrasynaptic GABAAR because benzodiazepines (BZs), which only interact with γ-subunit-containing GABAAR (predominantly synaptic GABAAR), are anxiolytic but not antidepressant. As α1 subunit-containing synaptic GABAAR mediate the sedative effects of BZs, we hypothesized that an extrasynaptic GABAAR-preferring NAS would be better tolerated due to reduced activity at this subtype of synaptic GABAAR.

Here we compare PRAX-114, a novel extrasynaptic GABAAR-preferring PAM, to zuranolone, another NAS in clinical development for MDD. To determine whether extrasynaptic preference results in an improved therapeutic window, the effects of both compounds on EEG β-frequency were measured as a translational biomarker of GABAAR activation and related to brain concentrations associated with anxiolytic and sedative-like effects.

Methods: Positive allosteric modulation of GABA-induced current (IGABA) by PRAX-114 and zuranolone were measured using manual patch clamp in vitro electrophysiology in CHO cells expressing human extrasynaptic (α4β3δ) or synaptic (α1β2γ2) GABAAR ($n = 6$-11/concentration).

Effects of PRAX-114 (0.3-10 mg/kg) and zuranolone (0.3-3 mg/kg) on β-power were measured in a cohort ($n = 8$) of telemetered rats using a cross-over design. Anxiolytic effects of PRAX-114 (1-10 mg/kg) and zuranolone (0.1-3 mg/kg) were evaluated in the conditioned emotional response (CER) assay in rats ($n = 12$-13).

Effects of PRAX-114 (3-30 mg/kg) and zuranolone (1-3 mg/kg) on 5-choice serial reaction time (5-CSRT) responding were assessed in rats as a sensitive measure of changes in attention ($n = 12$). Finally, effects of PRAX-114 (10-60 mg/kg) and zuranolone (3-30 mg/kg) on rat spontaneous locomotor activity (sLMA) were assessed as a surrogate for sedative-like side effects ($n = 10$).

Brain concentrations of PRAX-114 and zuranolone were measured using mass spectroscopy in tissue collected from satellite rats. Terminal samples were collected from a subset of rats after sLMA.

Results: PRAX-114 potentiated IGABA at extrasynaptic (EC50 353nM; Emax 910%) and synaptic (EC50 2241nM; Emax 1789%) GABAAR, indicating a 6.3-fold preference for extrasynaptic receptors in terms of potency. Zuranolone potentiated extrasynaptic (EC50 106nM; Emax 993%) and synaptic (EC50 293nM; Emax 3237%) IGABA, but with only a 2.8-fold preference for extrasynaptic receptors. In terms of efficacy, PRAX-114 had 10.5-fold less PAM activity at synaptic vs. extrasynaptic GABAAR, at a concentration (262 nM) achieving PAM activity equivalent to the maximal GABA effect at extrasynaptic GABAAR. Equivalent in vitro profiling of zuranolone (60 nM) indicated a 2.6-fold preference for activity at extrasynaptic receptors.

PRAX-114 achieved dose-dependent increases in β-power ($F4,34 = 31.72$; $P < 0.0001$) in rats. There was a strong relationship between brain concentration and β-power fold increases 1 h post-dose, with a 1.6-fold increase achieved at an interpolated brain concentration of 1205 ng/g and dose of 4.5 mg/kg. Zuranolone also produced dose-dependent increases in β-power ($F3,21 = 53.00$; $P < 0.0001$), with a 1.6-fold increase achieved at a brain concentration of 352 ng/g and a dose of 0.7 mg/kg.

PRAX-114 ($F3,36 = 12.24$; $P < 0.0001$; ED50 3.2mg/kg) and zuranolone ($F3,33 = 13.85$; $P < 0.0001$; ED50 0.5mg/kg) both increased CER suppression index to ~0.5 indicating robust anxiolysis. The brain concentration of PRAX-114 at the CER ED50 (EC50) was 305 ng/g, a concentration interpolated to produce a 1.1-fold increase in β-power. For zuranolone, the CER EC50 was 66 ng/g; similarly interpolated to produce a 1.1-fold increase in β-power.

Dose-dependent decreases in the number of trials completed in the 5-CSRT task were observed for PRAX-114 ($F4, 55 = 18.98$; $P < 0.0001$) and zuranolone ($F4, 55 = 8.93$; $P < 0.0001$). Brain concentrations associated with a 50% reduction in trial number (EC50) were 2756 ng/g for PRAX-114 and 355 ng/g for zuranolone.

For PRAX-114, the ED50 (59 mg/kg) for reduced locomotion ($F3,36 = 3.89$; $P = 0.0166$) was associated with a brain EC50 of 14460 ng/g, 47-fold higher than the EC50 for anxiolysis. For zuranolone, the ED50 for reduced locomotion (9.9 mg/kg; $F3,36 = 20.72$; $P < 0.0001$) corresponded to a brain EC50 of 1598 ng/g, 24-fold higher than the EC50 for anxiolysis.

Conclusions: When compared side-by side, PRAX-114 had greater preference for extrasynaptic GABAAR than zuranolone (~2-fold and 4-fold greater in terms of potency and efficacy, respectively). The relationships between brain concentrations required to increase β-power, and those associated with anxiolytic effects, were similar for the 2 molecules suggesting that extrasynaptic preference does not limit anxiolytic activity. Interestingly, in the 5-CSRT task, the PRAX-114 EC50 was calculated to achieve a 2.3-fold increase in β-power, whereas the zuranolone EC50 achieved only a 1.6-fold increase in β-power. Consistent with this, the separation between brain concentrations associated with reductions in sLMA and anxiolytic effects was ~2-fold greater for PRAX-114 than zuranolone. The data presented support our hypothesis that greater preference for extrasynaptic GABAAR results in improved tolerability without compromising anxiolytic efficacy.

Keywords: Rapid Antidepressant, Novel Therapeutics, Efficacy and Tolerability, Neuroactive Steroid, Anxiolytics**Disclosure:** Praxis Precision Medicines, Inc.: Employee, Stock/Equity (Self).

Pfizer Inc: Stock / Equity (Self)

Biogen: Employee, (Spouse)

P284. The Role of Lactate Metabolism in Bipolar Disorder: Preliminary 7T MRSI Data

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Background: Bipolar disorder is a devastating psychiatric disease affecting 2-5% of people. Abnormalities in mania-related and depression-related behavioral energy are principal features of bipolar disorder suggesting energy metabolism dysfunction may play a role. Lactate is an energy source that is produced adaptively to compensate for low oxygen availability and is efficiently used by mitochondria and astrocytes, yet its role in neural function is unclear. Lactate was considered to be a biproduct of anaerobic metabolism however recent work has shown that its therapeutic use by infusion in traumatic brain injury (TBI) results in structural and cognitive improvement in humans and rodents, suggesting

lactate plays an important and poorly understood role in neural health and functioning. Precuneus is a metabolic hot spot, is related to both task positive (cognitive, emotional, and theory of mind) activity and task negative (i.e., consciousness, introspection, and memory) activity, and precuneus dysfunction has been shown to be related to bipolar disorder. Our comparison region, the dorsal anterior cingulate cortex (dACC) is a task positive region that is established in the literature as important for differentiating bipolar disorder from healthy during emotion processing and other tasks. Furthermore, failure to deactivate this region during rest has recently been shown to be related to elevated depression in youth at risk for bipolar disorder. We aim to aid in understanding mania-related and depression-related behavioral energy in bipolar disorder through an examination of lactate availability. We examine lactate availability in precuneus compared to the better characterized dACC during an emotion processing task and during rest. Given the findings of improvements in neural health related to TBI with lactate infusion, and inconsistent lactate findings related to bipolar disorder, we expect reduced lactate in bipolar participants relative to healthy participant and we explore relationships with current mania and depression scores.

Methods: Eight adults (3 male) (mean age = 22.11, SD = 3.61) with controlled pediatric onset bipolar disorder and 1 male healthy adult (age = 27.67 years) from the Course and Outcome of Bipolar Youth (COBY) study were recruited for this study. Magnetic Resonance Spectroscopic Imaging (MRSI) was acquired on a 7-Tesla scanner using a 16-channel transceiver array, MP2RAGE imaging, and a fast homonuclear edited spectroscopic imaging sequence to detect the 1.3ppm lactate (Lac) both during an emotional face processing task (4 X 3 minutes 12 seconds) and during resting state (4 X 3 minutes 12 seconds). Lac is expressed as ratio to the creatine (Cre) resonance. On scan day, clinical depression and mania measures were collected using the Center for Epidemiologic Studies Depression scale (CES-D) and Altman Mania Rating Scale (AMRS) respectively. We used one sample t-test for bipolar disorder participants using the healthy participant test value and used correlations to examine relationships with mania and depression scores.

Results: Coby participants showed reduced task related precuneus Lac/Cre availability: mean = .054 (.03), range: 0.017-0.086, $t(6) = -3.93$, $p = .008$, Cohen's d = .03 relative to healthy male Lac/Cre availability = .102 and reduced precuneus Lac/Cre availability during rest: mean = .040 (.02), range: 0.017-0.080, $t(6) = -9.55$, $p < .001$, Cohen's d = .02 relative to healthy male Lac/Cre availability = .115.

Coby participants showed reduced task related dACC Lac/Cre availability: mean = .020(.01), range: 0.013-0.038, $t(4) = -22.43$, $p < .001$, Cohen's d = .01, relative to healthy male Lac/Cre availability = .148 and reduced dACC Lac/Cre availability during rest: mean = .043 (.02), range: 0.016-0.070, $t(4) = -3.67$, $p = .021$, Cohen's d = .03, relative to healthy male Lac/Cre availability = .084.

Higher subthreshold mania at scan was related to higher dACC task related Lac/Cre availability (Spearman's rho = .949, p.014) all other ps > .423. Higher subthreshold depression was trend related to lower dACC rest related Lac/Cre availability (r = -.868, $p = .056$) all other ps > .377.

Conclusions: Preliminary results suggest that lactate availability in key neural regions may differentiate bipolar disorder from healthy and may be related to mania-related and depression-related behavioral energy. Lactate availability was lower in well-controlled pediatric onset bipolar disorder adults, relative to healthy adult, in both regions of interest, precuneus and dACC. Additionally, higher dACC lactate availability during emotion processing was related to higher subthreshold mania and lower dACC lactate availability during rest was trend related to higher subthreshold depression scores. Findings suggest that lactate availability in key neural regions may represent a novel feature of bipolar disorder diagnosis relative to healthy and lactate availability may be differentially related to key symptoms of bipolar disorder. Larger samples could clarify these preliminary results.

Keywords: Bipolar Disorder, MRSI, Lactate, Precuneus, dACC**Disclosure:** Nothing to disclose.

P285. Sex Differences in Sleep, Mood and Brain Functional Connectivity in Alcohol Use Disorder

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Background: Growing evidence suggests greater vulnerability of women than men to the adverse effects of alcohol on mood and sleep. High sensitivity to the aversive consequences of drinking on sleep and negative affect might accelerate the progression into AUD in women. However, the underlying neurobiological mechanisms are still poorly understood.

Methods: Here we examined sex difference in resting state functional connectivity (RSFC) in alcohol use disorder (AUD) using a whole-brain data driven approach and tested for relationships with self-reported sleep and mood. To explore whether sex effects vary by AUD severity, we studied two separate cohorts: non-treatment seeking $n = 70$ AUD participants (29 females) from the Human Connectome project (HCP), and recently detoxified $n = 102$ treatment seeking AUD participants (34 females) at the National Institute on Alcohol Abuse and Alcoholism (NIAAA), who were older and had greater AUD severity than HCP participants. For RSFC analyses, a significance level was set at FWE-corrected $p < .05$ using non-parametric Threshold Free Cluster Enhancement approach (TFCE).

Results: Women with AUD reported greater sleep problems than men with AUD (pFWE = .005 and .048), while sex differences in mood were only observed in severe AUD (pFWE < .001). Sex differences in RSFC were distinct in low and high severity AUD participants. While sleep problems in women with low AUD severity were associated with increased cerebellar RSFC with salience (SN) and sensorimotor networks (SMN) presumably resulting in hyperarousal; for women with severe AUD, sleep and mood problems were associated with reduced RSFC between SN and visual network, presumably reflecting internally directed attention.

Conclusions: The current study revealed sex differences in RSFC that relates to sleep and mood problems in women with AUD. AUD severity modulates sex differences in sleep, mood and RSFC, which might reflect neuroadaptive processes with AUD progression that needs to be tested with longitudinal data in the future.

Keywords: Alcohol Use Disorder, Sex Differences, Resting State Functional Connectivity, Sleep, Mood**Disclosure:** Nothing to disclose.

P286. Dose Dependent Effects of Transcranial Photobiomodulation on Brain Hemodynamics in Major Depression

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Background: Transcranial photobiomodulation (t-PBM) with near-infrared (NIR) light penetrates into the cerebral cortex and is absorbed by the mitochondrial enzyme cytochrome c oxidase (CCO), stimulating the mitochondrial respiratory chain. t-PBM also significantly increases cerebral blood flow (CBF) and oxygenation. Small studies have reported that t-PBM may be an effective treatment in major depressive disorder (MDD). However, relationships between t-PBM dose (irradiance and/or total energy) and clinical or biological effects are unclear. In this experimental medicine study, we evaluated the dose-dependent effects of t-PBM in MDD subjects.

Methods: We enrolled subjects meeting DSM-5 criteria for MDD, not treatment-resistant (0-2 failed antidepressants in the current episode), either unmedicated or on stable doses of antidepressants, with no other significant medical or psychiatric comorbidities. All subjects underwent 4 t-PBM sessions in the MRI scanner, 1 week apart, administered in random order, with 1) sham (no energy emitted); 2) High dose: Pulse wave (PW), average irradiance 300mW/cm2, peak irradiance 900 mW/cm2, 42Hz, 33% duty cycle, 4.3 KJ total energy; 3) Medium dose: Continuous Wave (CW), 300 mW/cm2 irradiance, 2.4 KJ total energy; 4) Low dose: CW, 50 mW/cm2 irradiance; 1.4 kJ total energy. Other t-PBM parameters were kept unchanged (808 nm; 12.0 cm2 × 2 treatment area; delivered to the anterior prefrontal cortex, bilaterally). Resting state multi-echo (3), multi-band (2) fMRI was recorded on a 3T Siemens Trio using a 12ch head coil (TR = 2500ms, TE1 = 12.8ms, TE2 = 32.33 ms, TE3 = 51.86 ms, 60 slices, slice thickness 2.5mm) before, during and after t-PBM, using measures of the change in blood-oxygenation-level dependent (BOLD) signal on fMRI as marker of target engagement (t-PBM effect on cerebral blood flow). The BOLD signal was preprocessed using standardized automated tools [AFNI]. In order to test whether t-PBM modulated the BOLD signal during and after tPBM, we performed a region-of-interest (ROI) analysis taking into account the illuminated region of the brain. We extracted the signal from the transverse frontopolar giry, bilateral (ROIs 6 and 81 from the Desikan atlas) and separated the resulting time series into the pre-, peri-, and post-stimulation segments. We then performed spectral analysis in order to measure the BOLD power during each segment, employing the Thomson multitaper technique to increase the signal-to-noise ratio of the ensuing power estimates. This produced three spectra for each echo and dose (before, during, and after stimulation). We then tested for significant differences in BOLD power spectrum both during and after stimulation in each t-PBM dose, compared to sham (Wilcoxon rank sum test, corrected for multiple comparisons by controlling the false discovery rate at 0.05).

Results: We analyzed data from the first 7 MDD subjects (age = 32.1 ± 13.1; 57% female) undergoing all 4 experimental sessions. We found a dose-dependent effect of t-PBM on the BOLD. Namely, low-intensity t-PBM produced a marked decrease in BOLD that was observed in all three echos ($p < 0.05$, $n = 7$). The reduction in BOLD was most pronounced near 0.03 Hz at echos 2 and 3. In contrast, CW 300 mW/cm2 t-PBM increased BOLD power, with a significant increase resolved near 0.1 Hz at all 3 echos ($p < 0.05$, $n = 7$). This suggests that higher irradiance CW t-PBM increased the power of the "fast" component of the BOLD signal during stimulation. However, no significant differences from sham were observed during PW 300 mW/cm2 stimulation. We were also not able to detect any significant BOLD changes after tPBM (at any echo or t-PBM dose).

Conclusions: We found a U-shaped, dose-dependent effect of t-PBM on the BOLD, with the medium dose leading to an increase in the hemodynamic effect. These findings suggest that specific parameters of t-PBM (total energy, irradiance, CW versus PW) modulate the effect of near-infrared light on cerebral blood flow. This is important, as t-PBM doses optimized for their hemodynamic effect might also offer superior clinical efficacy.

Keywords: Photobiomodulation, Hemodynamic Effect, BOLD fMRI Signal, Irradiance, Total Energy

Disclosure: Allergan, Biogen, Jazz, Sage: Consultant (Self)
Otsuka: Contracted Research (Self)

P287. Neural Processes of Inhibitory Control in American Indian Peoples are Associated With Reduced Mental Health Difficulties

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Background: American Indian (AI) populations suffer from substance use disorder and suicide at rates disproportionately high relative to the general population. However, our understanding of these increased rates is limited by a lack of contextualization of the risk factors unique to AI by the extant literature. Sociopolitical and historical contexts of these populations drive increased risk burden (e.g., low socioeconomic status, trauma exposure) among AI communities. Intriguingly, when researchers account for relevant risk factors AIs often display equivalent or lower rates of mental health concern than the general population. Furthermore, research indicates that AIs also experience high levels of positive mental health factors. Combined, these finding indicate that despite high levels of risk burden for psychopathology, AIs may exhibit protective factors against mental health difficulties. Although some public health literature suggests that cultural factors (i.e., spirituality, connectedness) may benefit well-being and be associated with lower mental health difficulties, this is an understudied area particularly with respect to potential neural mechanisms of these effects. Inhibitory control has a well-established neuroscientific profile and has been associated with lower levels of mental health difficulties. The goal of the current work is to investigate the impact of risk factors on prevalence of suicide risk (SR) in a sample of AI individuals and examine inhibitory control as a potential mechanism of mental health protective factors. It was hypothesized that when accounting for sociodemographic factors and trauma exposure AI would display equivalent rates of suicide risk and substance use disorders as non-Hispanic White (NHW) participants. Furthermore, among the AI sample it was expected that individuals with no SR would demonstrate greater activity in dorsolateral prefrontal cortex and inferior frontal gyrus as compared with individuals with SR when engaging inhibitory control.

Methods: Participants were drawn from the first 500 individuals recruited for the Tulsa-1000 (T1000), a naturalistic longitudinal study of 1000 people aged 18-55 ($n = 476$ with stop-signal task data). We employed propensity matching analysis to generate a matched sample of AI ($n = 76$) and NHW participants ($n = 76$) with respect to biological sex, age, education, income, reading ability, and trauma exposure. We then compared these groups on prevalence of suicide risk versus no suicide risk as indicated on clinician administered interview. Among the AI subsample, we then examined blood oxygen level dependent (BOLD) signal during a functional Magnetic Resonance Imaging (fMRI) scan during completion of the stop-signal task. Response inhibition was defined as the contrast of percent signal change during hard stop trials as compared to easy stop trials at a priori regions of interest (ROIs) drawn from original the SST development paper (dlPFC; IFG) and defined by the Brainnetome cytoarchitectural atlas. Whole

brain analyses were also conducted by adding SR into the linear mixed effect model used to extract BOLD signal and using conservative voxel-wise correction ($p < 0.005$) and auto-correlation function values for cluster size correction.

Results: Following the propensity matching procedure groups did not differ on the matching variables (p's 0.56-0.98). Comparing the propensity matched groups, the AI sample displayed lower occurrence of SR (29%) relative to the NHW participants (53%), $\chi 2 = 8.77$, $p = 0.003$, OR = 0.37). With respect to neuroimaging results for the a priori ROI analyses among AI individuals those without SR demonstrated increased activation in the left dlPFC ($t(54.2) = 2.19$, $p = 0.03$) and IFG ($t(51.97) = 2.26$, $p = 0.03$) in the hard versus easy contrast of the SST compared to those with no SR. Whole-brain linear mixed effects models maintained the effects related to SR indicating a significant cluster of increased activation in the dlPFC comprising 83 voxels with a center of mass at (-27.0, -45, 21).

Conclusions: Results indicate that (1) accounting for risk factors such as sociodemographic variables and trauma exposure the AI sample demonstrated lower levels of SR than NHW and (2) Among AI participants those with no SR showed higher levels of activity in executive control regions during the SST. These findings are a timely first step in examining SR is the AI population as AI demonstrated the largest increase in deaths by suicide of any racial group in the past decade. Results add to a burgeoning literature indicating the presence of protective factors among these communities and also that high prevalence rates of mental health difficulties may be largely driven by disproportionally high-risk burden within AI communities. The current study also posits inhibitory control as a potential neural mechanism underlying protective effect among AIs against suicide risk. These findings are limited in that they do not consider potential physical and mental health comorbidities. Furthermore, the parent data set for these secondary analyses did not include assessment of culturally specific risk and resilience variables for AI. Future research is needed to examine the specific factors related to mental health risk and protection among AIs. The current study provides an important first step towards culturally informed clinical neuroscience among AI populations.

Keywords: Inhibitory Control, Suicidality, Native Americans

Disclosure: Nothing to disclose.

P288. Clinical Outcome of Adolescents and Young Adults at Familial Risk for Bipolar Disorder

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Background: Bipolar disorder (BD) is highly familial. Studying offspring of parents with BD provides the opportunity to identify risk markers for illness development. Functional decline often predates BD illness and may be an early target for intervention. The Aretaeus Study was a 2-year, longitudinal observational study to assess psychosocial and biological predictors of emergent mood symptoms (depression, mania or hypomania) in youth offspring of a parent with BD. We hypothesized that functional impairment, prior to onset of any BD-spectrum diagnosis or clinical initiation of antipsychotics or mood stabilizers, would be predictive of clinical worsening or transition to BD-spectrum diagnosis.

Methods: Participants aged 15- 25 years with a biological parent with BD recruited from clinical and non-clinical settings were assessed every 3 months for up to 24 months. Volunteers were excluded if they had current or prior mood stabilizer or antipsychotic drug exposure, or a diagnosis of BD-spectrum disorders or psychosis. Global Assessment of Functioning (GAF) was assessed at all timepoints by trained clinicians using the modified GAF scale (Hall, 1995). Participants were stratified at screening by GAF as functionally impaired (GAF ≤ 70) or not impaired (GAF > 70). Clinical diagnostic and symptom rating scales assessed mania and depression symptoms using the Longitudinal Interval Follow-Up Evaluation (LIFE) Psychiatric Status Rating (PSR) score for DSM-IV-TR psychiatric disorders, the Bipolar Prodrome Symptom Scale – Full Prospective (BPSS-FP), and the General Behavioral Inventory. Participant-reported outcomes of depression, mania, sleep, anxiety, and social functioning were measured at regular intervals via a smart-phone app. Treatment was administered as deemed necessary by the participant's treating clinician without restriction.

Results: Participants ($N = 223$) were recruited at 17 US-based sites; 84 (37.7%) were in the Low GAF group and 149 (62.3%) were in the High GAF group. A majority (70%) completed the study, though significantly fewer Low vs. High functioning participants were study completers (59.5% vs. 76.3%; $p = 0.008$). The Low GAF group was younger (17.9 years vs. 19.3 years; $p < 0.001$) included slightly more females (66.7% vs. 59.0%; $p = 0.253$), but had similar race / ethnicity composition compared with the high GAF group (82% White and 90% Not Hispanic/Latino vs. 79% White and 86% Not Hispanic/Latino).

Low (vs. High) GAF participants had more frequent prior antidepressant (32% vs. 17%) and anxiolytic use (6% vs. 1%) at baseline. Proportion of weeks in the 6 months prior to baseline with clinically relevant symptoms of a Major Depressive Episode on the LIFE (PSR score >3) was greater for the Low vs. High GAF group (28%+38 vs. 6%+18, $p < 0.001$). The proportion of weeks during the 6 months prior to baseline with Mania/Hypomania symptoms was comparable between groups (Low GAF = 0.4% +3.3 vs. High GAF 0.8%+5.8, $p = 0.405$). Low (vs. High) GAF participants had more severe baseline BPSS-FP Index scores for both Mania ($9.3 + 9.0$ vs.$3.9 + 5.4$) and Depression ($20.7 + 14.9$ vs. $9.0 + 12.7$). Participant reported outcomes of depression and anxiety, but not mania, were worse at baseline in the Low vs High GAF group; depression – Quick Inventory of Depressive Symptoms-16 ($8.4 + 5.1$ vs. $4.3 + 3.4$), anxiety - General Anxiety Disorder-7 ($6.4 + 5.3$ vs. $2.5 + 3.2$), and manic symptoms - Altman Self-Rating Mania ($2.4 + 2.6$ vs. $1.6 + 2.2$).

Over the 2-year post-baseline period, the Low GAF group was twice as likely as the High GAF group to start a new psychotropic medication (36.9% vs. 17.3%), significantly higher for antidepressants (32.1% vs. 16.5%; $p = 0.007$) and a trend in difference for anxiolytics (6.0% vs. 1.4%; $p = 0.061$): the Low GAF group was significantly more likely to require a dose increase (16.7% vs. 5.0%; $p = 0.004$). Adverse events over 2 years were comparable in Low and High GAF groups (47 % vs. 49%), as were psychiatric AEs (13% vs. 10%). Serious psychiatric AEs were higher in Low versus High GAF group: there were 5 suicide attempts in the Low GAF group and none in the High GAF group.

Prospective 2-year data on the LIFE PSR score paralleled baseline differences: proportion of weeks with PSR scores >3 for Major Depressive Episode were significantly greater ($p = 0.008$ in the Low (29.3%+35) vs. High GAF group (10.7%+20). Both groups had a low proportion of weeks with PSR score >3 for mania/hypomania. There was a trend for worsening of depression symptoms in the High, but not the Low GAF group based on change from baseline for proportion of weeks with PSR scores >3 (4.7 vs. 1.3, $p = 0.232$).

Conclusions: Prospective longitudinal data of youth at familial risk for BD showed that a high level of functional impairment at baseline was associated with greater expression of symptoms of depression, anxiety, and manic symptoms. Baseline functional

impairment was associated with higher psychotropic medication use and more dose increases over two years of prospective observation. Participants with higher functioning worsened over two years in clinical symptoms, but never reached the level of symptoms of the group with low functioning at baseline, which continued to show high levels of depressive symptoms over 2 years. Functional impairment in youth at familial risk for BD appears to be strongly associated with long-term, clinically significant mood symptoms, especially depression, requiring intervention. Longer-term risk for BD requires further study.

Keywords: Bipolar Disorder, Familial Risk of Bipolar Disorder, Early Identification of Risk, Childhood Onset Bipolar Disorder, Clinical Heterogeneity

Disclosure: Janssen Research and Development, LLC: Employee (Self)

Johnson and Johnson: Stock / Equity (Self)

P289. Antidepressant Response of Electroconvulsive Therapy and Magnetic Seizure Therapy: Response Trajectories by Symptom Clusters

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Background: Electroconvulsive therapy (ECT) is a highly effective treatment for severe depression, particularly for cases with psychotic features, treatment-resistance, and acute suicidality. Magnetic seizure therapy (MST), in which seizures are induced using high-dose repetitive transcranial magnetic stimulation, can potentially offer similar efficacy as ECT, but with fewer adverse side effects. Although the efficacy rates between the two treatment modalities have been shown to be relatively similar, it is unclear whether they differentially act on depressive symptom dimensions and clusters.

Methods: This was a secondary analysis of a double-blind, randomized, controlled trial comparing the efficacy of ECT (ultrabrief pulse, right unilateral electrode placement) and MST (circular coil over vertex). The randomized clinical trial was conducted under an Investigational Device Exemption from the US FDA and was approved by the Institutional Review Boards of the New York State Psychiatric Institute, Duke University Medical Center, and UT Southwestern Medical Center. Participants were referred for treatment with ECT, between the ages of 21 and 81, and met DSM-IV-TR criteria for a major depressive episode in the context of major depressive disorder or bipolar depression based on the Structured Clinical Interview for Diagnosis, and had a baseline 24-item Hamilton Depression Rating Scale (HAMD) total score of 18 or greater. Exclusion criteria included a history of neurological disorders, head trauma, contraindications to transcranial magnetic stimulation, current unstable or serious medical illness, pregnancy, presence of implanted electronic devices, history of ECT in the prior six months or failure to previously respond to ECT, or a Mini Mental Status Exam total score less than 24. Data from an intent-to-treat sample of 72 participants were included in this analysis.

First, we conducted an exploratory factor analysis of HAMD subitems at baseline. All but one participant scored 0 on the item Insight; therefore, we removed this item from the factor analysis (thus the HAMD now comprised 23 items). The number of factors was determined using parallel analysis. The minimum residual approach was used with oblique, oblimin rotations to allow for between-factor correlations. Items with loadings > = 0.3 were included for each factor. The factor scores at each treatment, calculated as the sum of individual item scores at each treatment, were normalized to baseline factor scores.

Next, we performed latent class trajectory modeling to classify participants into classes based on fitting heterogeneous longitudinal polynomial response trajectories. We used the 24-item HAMD total score at each treatment for up to the eighth treatment, from a subset of 67 participants who had received a minimum of three ECT or MST treatments. We used a model with up to a cubic polynomial term, with quadratic random effects and a proportionality constraint to allow variance structures to vary across classes. To assess model adequacy, we calculated the average posterior probability of class membership (>0.7 in all classes is regarded as acceptable). We further assess model adequacy using odds of correct classification (>5 recommended) and mismatch (close of zero recommended). Finally, we explored whether baseline clinical, demographic characteristics, and treatment modalities were associated with response trajectories.

Results: For the baseline 23-item HAMD, the Kaiser–Meyer–Olkin sampling adequacy measure was 0.58, which was minimally adequate for factor analysis. The 23 items loaded onto 4 factors. Factor 1 consisted of hopelessness (loading score = 0.77), depressed mood (0.61), work and activities (0.56), helplessness (0.53), worthlessness (0.44), psychic anxiety (0.40), and suicide (0.34); Factor 2 consisted of late insomnia (0.76), general somatic symptoms (0.34), psychomotor retardation (0.34), and early insomnia (0.30); Factor 3 consisted of weight loss (0.60), gastro-intestinal symptoms (0.56), hypochondriasis (0.53), and somatic anxiety (0.43); Factor 4 consisted of middle insomnia (0.70), guilt (0.64), and depersonalization (0.34). Over the first 8 treatments of ECT or MST, Factors 1 and 4 showed substantial reductions relative to baseline (z-scores~-1.5 at treatment 8), whereas Factors 2 and 3 only slight reductions (z-score > -1). There was no difference between ECT and MST on the magnitude or speed of symptom factor score reduction.

In the 3-class model, the response trajectories showed distinct temporal patterns. The first class, which we labeled "nonresponse" ($N = 10$, 14.9%), showed minimum HAMD reduction over the eight treatments. The second class, labeled as "linear response" ($N = 46$, 68.7%), showed steady linear reduction in HAMD. The third class, labeled as "rapid response" ($N = 11$, 16.4%), showed maximal HAMD reduction within the first three treatments. Further exploratory analysis showed that the three classes did not differ in baseline depression severity (symptom factor scores, or total HAMD), age, sex, or treatment modality.

Conclusions: ECT and MST did not differ in their impact on symptom dimensions or the speed of symptom improvement. The most significant improvements observed were in the core mood symptoms of depression. We identified distinct classes of response trajectories. Future work is needed to identify predictors of response class using discrimination analysis with additional psychosocial and physiological covariates.

Keywords: Electroconvulsive Therapy, Magnetic Seizure Therapy, Factor Analysis, Latent Class Analysis

Disclosure: Nothing to disclose.

P290. Identification of BNST Gene Networks Modulating Susceptibility and Resilience to Chronic Stress Using Single-Nucleus RNA Sequencing

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Background: Mood and anxiety disorders can develop as a result of the interaction between genetic predispositions and environmental risk such as traumatic and/or chronic stress. As with other complex illnesses, there is no universal susceptibility gene for such disorders. Instead, multiple genes with small effect sizes may contribute to the disease phenotypes. Moreover, only a subset of individuals is susceptible to stress and/or trauma, while many others show a remarkable resilience to adverse experiences. Although the bed nucleus of the stria terminalis (BNST) plays an important role in the stress response and emotional and social behaviors, it has been largely overlooked with respect to its possible dysregulation in mental disorders. In this study we aimed to investigate the polygenic architecture of stress susceptibility and resilience within the BNST at the single cell level relevant to mood and anxiety disorders.

Methods: In an unbiased approach, we performed single-nucleus RNA sequencing (snRNAseq) using the inDrops technique to identify distinct neuronal subpopulations within the BNST that demonstrate resilient-specific or susceptible-specific transcriptional changes following chronic social defeat stress (CSDS) in mice. Historically, the classification of defeated mice into susceptible and resilient groups has been based on their performances in a social avoidance task. However, CSDS also leads to multiple other behavioral and physiological changes reminiscent of depressive- and anxiety-like symptoms in a subset of mice. In the past, such readouts have often been neglected in the classification of stress-resilient and stress-susceptible mice. To address these limitations, we subjected C57BL/6J male mice ($n = 56$) to a 3-week CSDS paradigm and developed a comprehensive testing battery for the characterization of CSDS-induced behavioral and physiological alterations.

Results: In the majority of animals CSDS induced not only a social avoidance phenotype, but also increased anxiety-related behavior as well as critical changes in physiological readouts relevant to HPA axis activity (social avoidance test: social interaction ratio, $t54 = 1.825$, $p = 0.07$; open field test: total distance, $t54 = 5.191$, $p < 0.0001$; elevated plus maze: number of open arm entries, $t54 = 2.005$, $p < 0.05$; baseline corticosterone: $t54 = 2.570$, $p < 0.01$). Thus, the classification into defeat-resilient and defeat-susceptible mice was based on the principal component analysis of 20 different behavioral and physiological readouts. 24 hrs after the last defeat, mice were sacrificed and BNST tissue punches of 2-4 animals of the same group were pooled based on the k-means clustering method. This resulted in 17 sample pools ($n = 5$ ctrl; $n = 4$ defeat resilient; $n = 8$ defeat susceptible) for subsequent snRNAseq. After applying QC metrics, we profiled transcriptomes of > 100.000 nuclei across all samples. We have identified 33 cell clusters, annotated into 5 major cell types of inhibitory neurons, excitatory neurons, astrocytes, microglia and oligodendrocytes. Many of these clusters express known markers of the BNST such as Sst, Tac2, Npy or Adcyap1. We have also identified populations of neurons with previously undescribed molecular signatures. In order to identify distinct neuronal subpopulations that demonstrate resilient-specific or susceptible-specific transcriptional profiles, we are currently performing differentially gene expression as well as pathway analyses of ctrl, defeat-resilient and defeat-susceptible samples with the goal to distinguish mechanisms related to stress exposure from those related to susceptibility and resilience.

Conclusions: We provide initial data that will aid in the construction of a comprehensive map of resilient-specific and susceptible-specific cell-types within the mouse BNST. To this end, we have identified both known and previously uncharacterized cell-types within the BNST. Follow up studies will assess the functional role of these cell-types in stress susceptibility and resilience.

Keywords: BNST, Single-Cell RNA Sequencing, Chronic Social Defeat, Stress Resilience and Susceptibility

Disclosure: Nothing to disclose.

P291. Transdiagnostic Prediction of Depression and Cognition Using fMRI and PET

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Background: Depression and cognitive dysfunction occur across psychiatric disorders and may have shared neurobiological underpinnings that could be targeted in a symptom- rather than disorder-specific manner. Disturbances in functional networks and the glutamate system are evident across major depressive disorder (MDD), posttraumatic stress disorder (PTSD) and bipolar disorder (BD). Investigating both network organization and the glutamate system as they relate to shared symptoms transdiagnostically will provide a more complete mechanistic understanding of transdiagnostic symptoms and could reveal novel treatment targets.

Methods: A transdiagnostic sample of 85 individuals were imaged with fMRI and [18F]FPEB PET to identify network organization and availability of the glutamate receptor mGluR5 ($n = 32$ MDD; $n = 24$ PTSD; $n = 29$ BD). Connectome-based predictive modeling (CPM) was applied to the fMRI data. Specifically, CPM with leave-one-out cross validation was used to identify functional connections ('edges') within a 268-node matrix that predicted depression severity and delayed recall memory transdiagnostically. Mean [18F]FPEB (VT) values from each of the 268 nodes were also correlated with the behavioral score for each participant.

Results: CPM successfully predicted depression severity and delayed recall memory across MDD, PTSD and BD individuals, as indicated by a significant correspondence between actual and predicted behavioral values (depression: $r = 0.25$, $p = 0.02$, $n = 85$; delayed recall: $r = 0.34$, $p = 0.02$, $n = 55$). Connections within and between medial-frontal, fronto-parietal and default-mode networks, predicted depression severity; and connections within and between dorsal attention, ventral attention and fronto-parietal networks were predictive of delayed recall. Successful prediction and a similar pattern of results was also observed when including healthy controls ($n = 35$; total $n = 90$). PET results revealed significant negative correlations between mGluR5 availability and depression severity in nodes of the PFC and cerebellum, transdiagnostically. Positive correlations were observed between mGluR5 and delayed recall in nodes of the PFC and parietal regions (p's < 0.05).

Conclusions: We were able to generate transdiagnostic predictive models of depression severity and delayed recall memory, pointing to shared mechanisms underlying these constructs across MDD, PTSD and BD. We also demonstrate associations between mGluR5 availability and depression severity/cognition. Brain-behavior relationships observed with both fMRI and PET converged on regions of the PFC. Our findings implicate distinct transdiagnostic networks underlying depression and delayed recall memory – networks that can be targeted with novel symptom-specific treatment interventions. Next steps will be to fuse fMRI and PET data and determine whether the observed predictive functional networks are underpinned by regional alterations in mGluR5 availability.

Keywords: Depression, fMRI, PET

Disclosure: Nothing to disclose.

P292. Tianeptine, but not Fluoxetine, Decreases Avoidant Behavior in a Mouse Model of Early Developmental Exposure to Fluoxetine

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Background: Depression and anxiety, two of the most common mental health disorders, share common symptoms and treatments. Most pharmacological agents available to treat these disorders target monoamine systems. Currently, finding the most effective treatment for an individual is a process of trial and error. To better understand how disease etiology may predict treatment response, we studied mice exposed to the selective serotonin reuptake inhibitor (SSRI) fluoxetine (FLX) during early development. These mice are known to show the murine equivalent of anxiety- and depression-like symptoms in adulthood, and here we report that these mice are also behaviorally resistant to the antidepressant-like effects of adult SSRI administration. The atypical antidepressant tianeptine (TIA) exerts its therapeutic effects through agonism of the mu-opioid receptor instead of directly targeting monoaminergic systems. Here, we investigated whether TIA administration in adulthood would be effective in this early developmental FLX exposure model of SSRI resistance.

Methods: C57BL/6J (C57) male and female pups were injected with either FLX (10 mg/kg, i.*p*) ($n = 39$, 18 male, 21 female) or vehicle ($n = 31$, 14 male, 17 female) from postnatal (PN) day 2 to 11, a period in which mouse brain development parallels that of the third trimester of a human pregnancy. Avoidant behavior was measured in postnatal FLX (PN FLX) or vehicle (PN VEH) exposed mice in adulthood in the open field test (OF). Cages with co-housed PN FLX and PN VEH mice were subsequently pseudorandomly organized to receive either chronic FLX or vehicle control administered as adults. FLX (18 mg/kg) was administered through the drinking water for three weeks; the vehicle control group received water alone. Behavioral testing in the OF and novelty suppressed feeding task (NSF) began after three weeks of FLX administration, which continued to be administered during testing. A two-week washout period followed completion of post-FLX behavioral testing before beginning TIA administration. Cages were again pseudorandomly reorganized into TIA and vehicle control administration groups, ensuring equal distribution of those which had received FLX or vehicle during the first drug administration period. A 30 mg/kg solution of TIA NaCl (Qingdao Sigma Chemical Co., purity verified by NMR analysis) was delivered at a dose of 0.1 ml/10 grams mouse weight via intraperitoneal injection twice per day for 14 days. TIA was dissolved in 0.9% sterile saline. The respective vehicle control group received injections of 0.9% sterile saline. The effects of chronic FLX treatment on adult PN FLX mice on a 129SvEv (129) background was also assessed using the NSF.

Results: We found that as adults, C57 PN FLX animals share a similar avoidant phenotype to that previously reported in 129 PN FLX mice, manifest as a decrease in rearing and center time in the OF relative to PN VEH mice (unpaired t-test, $p < 0.005$ and $p = 0.049$, respectively, $n = 31$ PN VEH, 39 PN FLX). Interestingly, we found that adult FLX administration did not improve, or even exacerbated, avoidant behavior in PN FLX mice. PN FLX mice that had received adult FLX administration showed decreased rearing (unpaired t-test, $p = 0.02$, $n = 17$ VEH, 20 FLX) and center time (unpaired t-test, $p = 0.03$, $n = 17$ VEH, 20 FLX) in the OF relative to PN FLX mice given vehicle (VEH), and no effect was seen on their latency to feed in the NSF (unpaired t-test, $p = 0.70$, $n = 17$ VEH, 20 FLX). Similarly, adult FLX administration increased latency to feed in the NSF in PN FLX on a 129 background (unpaired t-test, $p = 0.04$, $n = 27$ VEH, 30 FLX). By contrast, adult TIA administration increased rearing and center time in the OF in PN FLX mice relative to those treated with vehicle (unpaired t-test, $p = 0.005$ and $p = 0.03$, respectively, $n = 19$ VEH, 20 TIA). Adult TIA administration also decreased latency to feed in the NSF in PN FLX mice, relative to those receiving vehicle control (unpaired t-test, $p = 0.0001$, $n = 17$ VEH, 19 TIA).

Conclusions: Our results confirm prior work demonstrating that in mice exposure to FLX during early development produces an avoidant-like phenotype as adults. In a mouse, the brain development occurring during this period of exposure approximates the brain development occurring during the third trimester in a human. Consistent with this, human epidemiological data indicates that in utero exposure to FLX increases the risk of being diagnosed with an affective disorder in adolescence and adulthood. Our results now demonstrate that early exposure to FLX results in affective symptoms that are subsequently resistant to FLX administration in adulthood. Remarkably, the atypical antidepressant TIA remains completely effective in improving affective behaviors in these mice. Overall, these findings suggest that PN FLX mice may represent a model of SSRI-resistant affective behavioral changes and that TIA may be a promising alternative treatment from SSRIs for humans with suspected in utero early developmental exposure to SSRIs, and more broadly that it may be helpful in a subset of people with SSRI-resistant depression.

Keywords: Avoidance, Fluoxetine, Tianeptine, Mouse Models, Brain Development

Disclosure: Nothing to disclose.

P293. Mu Opioid Receptor Signaling is Required for the Cellular Effects of Ketamine Enantiomers in the Lateral Habenula

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Background: Racemic ketamine [(R,S)-ketamine] as well as esketamine [(S)-ketamine] are potent N-methyl-D-aspartate receptor (NMDAR) antagonists that are increasingly used in the clinical treatment of refractory depression. The enantiomer arketmaine [(R)-ketamine] has also demonstrated antidepressant properties in both preclinical and clinical studies. Both enantiomers display activity at a number of neuronal receptors, including μ-opioid receptors (MORs), complicating the issue of mechanism of action. Preclinical studies have linked the antidepressant effect of racemic ketamine to its ability to reduce hyperactivity in the lateral habenula (LHb), a brain nucleus involved in reward learning. We recently demonstrated that intact MOR signaling is required for the antidepressant actions of (R,S)-ketamine in rodents displaying LHb hyperactivity, a preclinical model of depression. The potency of the ketamine enantiomers to decrease LHb hyperactivity, and the requirements of MOR signaling are currently unknown. Increasing our understanding of the mechanism of action of ketamine enantiomers in reducing LHb hyperactivity may help in the development of new rapid antidepressant compounds.

Methods: We prepared acute LHb brain slices from two different rodent models of depression– congenital learned helplessness (cLH) and acquired learned helplessness (aLH)–and used calcium imaging to test the cellular effects of co-administration of ketamine enantiomers and MOR antagonists on neuronal activity.

Results: We find that acute application of either ketamine enantiomer results in a similar reduction of LHb activity in our rodent models, that is reduced by co-application of the MOR

antagonist CTAP. The NMDAR antagonist APV occludes the ability of (S)-ketamine, but not (R)-ketamine, to reduce LHb activity.

Conclusions: Our results suggest that intact MOR signaling is required for the ability of ketamine enantiomers to acutely reduce LHb neuronal activity. However, (S)-ketamine, but not (R)-ketamine, also requires intact NMDAR signaling as well. These findings suggest that the two ketamine enantiomers may have different mechanisms of action for their antidepressant effects, and that future studies of the relative contributions of NMDAR and MOR signaling to rapid antidepressant effects is warranted.

Keywords: Ketamine, Lateral Habenula, Molecular Neuroscience

Disclosure: Nothing to disclose.

P294. Early Life Stress Induces Amotivation and Alters Epigenetic Regulation in the Nucleus Accumbens in a Sex-Dependent Manner

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Background: Although many pharmaceutical therapies have been developed for treating mood disorders, over half of individuals that seek treatment do not respond positively, indicating a dire need for new treatments and thus a need for uncovering novel molecular mechanisms underlying stress-induced behaviors. Early life stress (ELS) is a well-known risk factor for the development of several psychiatric illnesses, including depression. Molecular studies on the impact of ELS on the brain have primarily focused on cognition-related brain regions and behavior, so the molecular mechanisms underlying the impact of ELS on reward-related regions, such as the nucleus accumbens (NAc), and motivated behaviors are lacking.

Here we used the Limited Bedding and Nesting (LBN) model, which consists of rearing pups in an impoverished environment during early life (PND 2-9), to assess the effects of ELS on motivated behaviors and on transcriptional and post-translational regulation in the NAc, a key region involved in depression-like phenotypes such as reduced motivation and anhedonia. In a previously published dataset we found that LBN promotes sex-specific differential expression in the NAc. Remodeling of unique histone marks may contribute to this, so in this study we performed a genome-wide screen of posttranslational histone modifications (PTM) in the NAc to assess whether epigenetic mechanisms contribute to these distinct transcriptional profiles. We also performed pathway analyses on our RNAseq dataset to identify unique upstream regulators and pathways being significantly enriched by LBN. Additionally, in our last experiment we test for the effects of LBN on motivation for male and female Long-Evans rats to access a palatable food reward using an operant self-administration task.

Methods: Experiment 1: Pups were reared in either a LBN or standard environment during PND 2-9. Nuclei were isolated from NAc punches from LBN or control adult males and females ($n = 4$-5/group) and histones were extracted. Propionic anhydride derivatization of histones and peptide extraction and subsequent global histone posttranslational modification analysis was performed.

Experiment 2: Qiagen Ingenuity Pathway Analysis (IPA) was performed on our previously published NAc RNAseq data set that included LBN and control males and females ($n = 4$-6/group) in order to assess sex differences in the effects of LBN on upstream regulators and enriched pathways in the NAc.

Experiment 3: Pups were reared in either a LBN or control environment ($n = 4$-11/group) during PND 2-9. At PND 60 rats were run through a self-administration operant task where they lever pressed for sucrose pellets over the course of 13 days (10 days training on FR1; 1 day on a progressive ratio task (PR); 1 day FR1; 1 day PR).

Statistical analysis: Exp. 1: Relative abundance of histone PTMs were calculated and PTM analysis was performed. Exp. 2: Qiagen IPA was used to assess LBN effects on upstream regulators and pathway enrichment. Exp. 3: data was normalized when appropriate followed by ANOVA testing and pairwise comparison post-hoc analyses.

Results: Our genome-wide assessment of histone PTMs revealed differing patterns of LBN-driven PTMs in the NAc of males and females (group N's = 4-5). We found that LBN significantly increased the expression of 3 histone marks in males (H3K4me3, H4K12ac, H3K18acK23ac) and decreased the expression of 1 histone mark in females (H2A1K5acK9ac) (p's < 0.05).

Using IPA we identified a sex difference in several predicted upstream regulators in our previously published LBN NAc RNAseq dataset. Notably, CREB1 was significantly enriched as an upstream regulator in LBN females. Additionally, IPA identified CREB signaling as a significantly enriched pathway in stressed females. Targets of this upstream regulator also differed between sexes. In females, CREB1 is a predicted regulator of 24 differentially expressed genes (DEG), of which 10 are activated and 14 are inhibited. In males, CREB1 is a predicted regulator of 6 DEGs, where 5 are activated and 1 is inhibited.

Preliminary data from an ongoing study shows that LBN reduces motivation for a food reward in a PR operant task in non-food deprived adult male and female Long-Evans rats ($p = 0.025$). Further analyses of our preliminary data using an FR1 schedule show a significant interaction effect between sex and stress ($p = 0.045$). Pairwise comparison analyses show that LBN females lever press less on an FR1 schedule compared to LBN males by the last day of testing ($p = 0.005$).

Conclusions: Our results identify two potential molecular mechanisms in the NAc that may drive our observed sex-specific LBN-induced phenotypes: sex-specific histone modifications and sex-specific enrichment of upstream regulators, such as CREB1. Acetylation of two of our identified histone marks (H4k12ac and Hk23ac) has been shown to be in part driven by CREB-binding protein (CBP). Previous work shows that acetylation of these histone marks in reward-related regions is altered by adult social stress models in rats. Taken together this suggests that CREB signaling and CBP-driven histone acetylation may be a driving force of stress phenotypes. In an ongoing preliminary study, we are finding that LBN reduces motivation to lever press for a food reward. Upcoming studies will assess the effects of LBN on motivation for other natural rewards, such as social rewards, as well as the role epigenetic modifications, such as CBP-driven histone acetylation, may be playing in these LBN-driven depression-like phenotypes.

Keywords: Early Life Stress, Nucleus Accumbens, Motivation, Epigenetics, Sex Differences

Disclosure: Nothing to disclose.

P295. Sidekick-1, A Novel Regulator of Stress Resilience in the Prefrontal Cortex

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Background: Depression is a leading cause of disability worldwide. Stress is a major risk factor, yet some individuals are resilient to stress. Both environmental and genetic factors contribute to the dysregulation of neural circuits in depression and genes that regulate the formation, maintenance, and plasticity of synapses

are of particular interest as molecular hubs for regulation of circuit function.

We previously identified Sidekick-1 (Sdk1) as an affective circuit hub gene in a network regulating stress resilience. Overexpression of Sdk1 in the prefrontal cortex (PFC) increased resilience to stress in male mice. Separately, several genome-wide association studies in humans found SDK1 variants associated with depression, suggesting an evolutionarily conserved role in regulation of emotional behavior.

Sdk1 is a cell surface molecule implicated in circuit formation in the retina. However, little is known about cell-specific expression patterns of Sdk1 in the adult brain and its function within affective circuits. Here we probe Sdk1 cell-type and layer-specific expression in the PFC, conservation between mice and humans, and its modulation by stress in both male and female mice.

Methods: Chronic social defeat stress generated resilient and susceptible phenotypes in 10 week old male mice ($n = 12$ control, $n = 11$ stress), and subchronic variable stress generated a susceptible phenotype in 10 week old female mice ($n = 10$ control, $n = 10$ stress). Depression-like behavior was assessed using social interaction and social preference tests. Quantitative real-time PCR determined bulk Sdk1 expression levels in the PFC, nucleus accumbens (NAc) and ventral hippocampus of control and stressed animals. The expression pattern of Sdk1 mRNA in the PFC of 10 week old mice ($n = 14$) was assessed using multiplex RNAScope fluorescent in situ hybridization. Probes for Slc17a7 (vGlut1) and Slc32a1 (vGAT) identified excitatory and inhibitory neurons, respectively. PFC layers 1, 2/3, 5 and 6 were designated by distance from the midline. PFC-NAc neurons were retrogradely labeled with AAVrg-Cre-EGFP injected into the NAc, and the PFC was immunostained for GFP and in situ hybridized for Sdk1. Publicly available scRNAseq datasets (Allen Brain Map) from mouse and human whole cortex were used to identify cell-type specific expression of Sdk1 and de novo clustering on sub-regions was performed using Seurat.

Results: We found that Sdk1 is expressed in the PFC, NAc and ventral hippocampus of adult male and female mice, with expression highest in the PFC. qPCR revealed a significant reduction in Sdk1 expression in the PFC of stressed susceptible male ($p < 0.01$) and female mice ($p < 0.05$). Given the role of the PFC-NAc pathway in stress resilience, we probed correlations in Sdk1 expression between the two regions, identifying a trending positive correlation in resilient male mice ($r2 = 0.52$ $p = 0.07$).

RNAScope in situ hybridization in the PFC showed that Sdk1 was expressed in 50.9% of vGlut1-positive neurons and 37.5% of vGAT-positive neurons. The highest level of Sdk1 expression was observed in layer 6 (55.7%, $p < 0.0001$) with moderate expression in layers 2/3 (17.4%) and 5 (23.4%). Patterns of cell-type and layer-specific Sdk1 expression were conserved in scRNAseq analysis of human PFC. De novo clustering of scRNAseq data from mouse PFC showed that Sdk1-expressing cells were found across clusters, with several clusters of excitatory or inhibitory neurons marked by high numbers of Sdk1-expressing cells. Retrograde GFP labeling of PFC-NAc projection neurons and combined in situ hybridization for Sdk1 and immunohistochemistry for GFP showed that a subpopulation of NAc projecting neurons in the PFC express Sdk1.

Conclusions: In this study we characterized the prefrontal expression of Sdk1, a novel regulator of stress resilience. We found that Sdk1 is modulated by stress in male and female mice and is expressed in subsets of both excitatory and inhibitory neurons, with a significant enrichment in deep layers of the PFC. Cell-type and cortical layer specificity was conserved between mice and humans. In addition to regulation of absolute expression in the PFC, coordinated regulation of Sdk1 expression across brain circuits may underlie resilience. Future work will address the circuit-specific effects of Sdk1 in behavioral adaptation to stress. As a cell surface receptor, Sdk1 is a potential target for pharmacological intervention. Understanding how Sdk1 confers resilience may lead to development of new mechanistically-informed treatments for depression.

Keywords: Prefrontal Cortex, Depression, Stress Resilience, Mouse Models

Disclosure: Nothing to disclose.

P296. Combined Effects of Nitric Oxidase Synthetase Intron Polymorphism and Childhood Emotional Abuse on the High-Risk Suicide Attempter Phenotype

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Background: Both genetic susceptibility and adversity in early age have been associated with the earlier onset and more severe course of suicidal behaviors. Early life adversity, especially emotional abuse has been shown to impair stress response system and cause oxidative stress. Nitrous oxide is involved in dopaminergic function modulation, and long-term memory formation, among other function. Several polymorphisms of nitric oxide synthase (NOS) genes have been associated with depression, suicide attempt (SA), though results remain inconclusive. Recent randomized trials also propose a rapid antidepressive effect of nitrous oxide, which is a donor for nitric oxide (NO), a final NOS product. Meanwhile, NOS3-knockout mice have diminished neurogenesis, impaired learning process, and show depressive-like behaviors. Stress has also been shown to rapidly increase NOS activity, possibly resulting in adequate memory consolidation, which is important for development of future response schemas. In the present study, we hypothesized that individuals with childhood adversity history and NOS polymorphisms might engage in suicidal behaviors at younger age, and more frequently.

Methods: We evaluated $N = 501$ individuals with SA history for their sociodemographic characteristics, childhood adversity using the Childhood Trauma Questionnaire (CTQ), as well as their clinical characteristics, including mental disorders according to the Mini International Neuropsychiatric Interview (MINI) screening tool, family history of mental disorders and suicide, and the characteristics of suicidal behaviors (age of first suicide attempt, number and severity of attempts). All individuals were also genotyped for the major nitric oxide synthetase 2 and 3 (NOS2 and NOS3). First, we compared all included characteristics according to the early-attempter cut-off set at 18 years age using the t-test, chi-square and appropriate nonparametric tests. Then, we performed haplotype analysis and analysis based on specific polymorphisms. Since childhood emotional abuse drove the association between childhood abuse and early suicidal behavior, and the 27-bp repeat polymorphism of NOS3 was the only genetic association, we the divided patients to four groups of either both positive, one positive, and one negative. We then performed a Cox-regression to see how these four groups differ. Finally, we applied several logistic regression models, controlling for covariates to see the independency of the associations. Level of significance was set at $p < .05$.

Results: Female sex, family history of mental disorders, CTQ total score, emotional abuse (strongest association), physical abuse, and sexual abuse, multi-attempter phenotype, severe SA history and the 27-bp repeat polymorphism of NOS3 were all statistically significantly associated with the early SA. Haplotype analysis revealed that associations were driven by the 27-bp repeat polymorphism of NOS3. When divided in four groups, we found that individuals with both childhood emotional abuse and the 27-bp repeat polymorphism of NOS3 were younger at their first SA (27.39 ± 13.21 vs 35.75 ± 16.07, $p < .001$), had more SA (31.6% vs 11.3%, $p < .001$), had more family history of SA (66.0% vs

39.3%, $p < .001$), and were more likely to have realized severe SA (30.2% vs 14%, $p < .05$) than those neither with the polymorphism nor with the emotional abuse history. Furthermore, those with either emotional abuse history or the 27-bp repeat polymorphism of NOS3 showed intermediate results. Cox regression confirmed the different distribution of the age of the first SA in four groups. Regression model showed that the effect was additive rather than synergistic. The model which included all associated factors with the age of the first SA (excluding CTQ total score and other substitutes due to their interaction with emotional abuse) had adjusted R2 of .130. It showed that emotional abuse, the 27-bp repeat polymorphism of NOS3, eating disorder history, and substance use disorder remained significantly independently associated with earlier age of the first SA ($p < .05$ in all cases).

Conclusions: Both reported emotional abuse and the 27-bp repeat polymorphism of NOS3 were associated with earlier age of the first suicide attempt in suicide attempters. These factors seem to have additive rather than interactive effect, and are also associated with a generally riskier phenotype with higher number of suicide attempts, family history of suicidal behaviors, and severe suicide attempt history. Further studies are needed to elucidate the mechanism of the presented associations, possibly targeting on the role of oxidative stress in the emergence of suicidal behaviors.

Keywords: Suicidal Behavior, Nitric Oxide, Childhood Adversity

Disclosure: Nothing to disclose.

P297. Anti-Cardiolipin Antibody: A Potential Biomarker for Depression

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Background: Remission rates for Major Depressive Disorder (MDD) are estimated to hover around 35% and about one-third of MDD patients fail to respond to conventional antidepressants. This is not surprising, as we have yet to identify the biological underpinnings of this psychiatric disorder. A promising avenue of exploration is the role of inflammation in depression, or at least in a subset of depressed patients. It is crucial to first establish which specific inflammatory biomarkers play a role in MDD's etiopathology. CRP, IL-1β, IL-6, and TNF are strong contenders. Anti-cardiolipin antibody (aCL IgM) is an inflammatory marker that has the potential to be a robust candidate but there are insufficient studies to confirm this potential. Cardiolipin is a phospholipid, which is a class of molecules important for neurotransmitter signal conduction and neurodevelopment. Abnormalities in phospholipid regulation, as seen in antiphospholipid syndrome (APS), typically lead to hypercoagulable sequalae, but there is a growing body of evidence demonstrating neuropsychiatric repercussions. APS patients have higher depressive symptoms and, inversely, depressed patients have higher antiphospholipid antibodies titers than healthy controls.

Our study focused on aCL IgM. The primary goals were to investigate the longitudinal progression of aCL IgM in MDD subjects receiving antidepressant therapy in comparison to healthy controls and to determine if changes in aCL IgM correlate to changes in depressive symptoms. Our secondary goal was to ascertain if baseline aCL IgM could predict treatment response.

Methods: MDD subjects, both male and female between the ages of 20 – 65 years, had to be in either their first or recurrent episode of MDD and otherwise physically healthy.

Upon successful completion of a series of physiological and psychiatric screens, all subjects had their blood drawn for inflammatory biomarkers and completed a series of scales (the Hamilton Rating Scale for Depression (HAMD-17), Hamilton Rating Scale for Anxiety (HAM-A), and Beck Depression Inventory (BDI)) at a baseline visit.

MDD subjects were then enrolled in one of two treatment studies. Cohort-E received a 12-week regimen of escitalopram 20 – 40 mg/day ($n = 20$). Cohort-Q received a 12-week regimen of quetiapine, up to 150 mg/day as tolerated ($n = 28$). During the follow-up visits at weeks 1, 2, 4, 8, and 12, blood draws were repeated and the subjects completed the same scales that were administered upon screening.

The screening and baseline visit for HCs ($n = 27$) were identical to the ones for MDD subjects. After the baseline visit was completed, no further blood draws were done on HCs.

Results: When Cohort-Q and Cohort-E participants were grouped together ($n = 48$), MDD subjects were found to have an elevated baseline aCL IgM (19.9 ug/ml) compared to healthy controls (8.32 ug/ml) ($p = 0.0062$). aCL IgM was found to have a statistically significant correlation with HAMD-17 scores at baseline in MDD subjects ($p = 0.0185$, $r = 0.296$).

Looking at the individual cohorts, Cohort-Q MDD subjects had a significantly elevated baseline aCL IgM level when compared to Cohort-E MDD subjects ($p = 0.0083$). On the other hand, only Cohort-E MDD subjects evidenced a significant correlation at baseline between aCL IgM level and HAM-A score ($p = 0.0392$, $r = 0.4327$); they also showed a significant inverse correlation between week 12 HAMD Item #10 (Anxiety, Somatic) and week 12 aCL IgM level ($p = 0.0268$, $r = -0.5516$).

Neither the combined nor individual cohorts displayed any significant change in aCL IgM throughout the course of the medication trials, regardless of treatment response.

When comparing the change in Cohort-E MDD subjects' aCL IgM from baseline to week 12 versus the change in Cohort-Q MDD subjects' aCL IgM from baseline to week 12, there was no statistical difference.

The two MDD groups differ significantly in sex, with 55.32% of Cohort-Q being female versus 78.05% of Cohort-E ($p = 0.0416$), and age (Cohort-Q mean age = 43.79, Cohort-E mean age = 37.56, $p = 0.0169$).

Conclusions: MDD subjects had significantly higher serum levels of aCL IgM when compared to HCs. Moreover, at baseline, the higher the aCL IgM level, the higher the depression severity, as measured by HAMD-17 score. However, this study did not demonstrate that aCL IgM levels changed significantly throughout a 12-week course of antidepressant treatment and revealed no correlation between changes in depressive symptoms and changes in aCL IgM levels. Baseline aCL IgM could not predict treatment response. We conclude that, despite its minimal predictive ability, serum levels of aCL IgM have a diagnostic potential in MDD that necessitates further exploration. Future studies would benefit from including aCL IgM in their inflammatory biomarker panels and from extending their observational period beyond the clinical trial, as the anti-inflammatory effects of antidepressants may be seen only after depressive symptoms have been stabilized.

Additionally, we speculate that the differences in each cohort's respective exclusion criteria led to the differences in results. Cohort-Q excluded treatment-resistant MDD subjects. Cohort-E did not. Being that co-morbid anxiety is a risk factor for treatment-resistant MDD, this could explain why Cohort-E subjects were more anxious, based on HAM-A score. As such, we recommend future studies identify which subjects have treatment-resistant MDD, first episode MDD, and other subsets of MDD prior to drawing conclusions.

Keywords: Depression Inflammation Cytokine, Cardiolipin, Antidepressant

Disclosure: Nothing to disclose.

P298. Predictors of Functional Impairment in Bipolar Disorder: Results From 13 Cohorts From Seven Countries by the Global Bipolar Cohort Collaborative

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Background: Persistent functional impairment is common in bipolar disorder (BD) and is influenced by a number of demographic, clinical, and cognitive features. The goal of this project was to estimate and compare the influence of key factors on community function in multiple cohorts of well-characterized samples of individuals with BD.

Methods: Thirteen cohorts from 7 countries included $n = 5{,}882$ individuals (both male and female) with BD across multiple sites. The approach was planned as purposefully simplistic with a focus on one question via systematic uniform application of analyses across sites. First, each site was asked to empirically identify how they defined 'good versus poor' functioning in their cohort. Sites were required to dichotomize whatever measure was chosen for defining function in their study, such that individuals with BD were described as having either good or poor functional status. Sites were instructed to conduct a logistic regression with the functional outcome as the dependent variable while model-level and predictor-level results were collated. The results of each site's logistic regression were then compared descriptively side-by-side, i.e., the goal herein was to find consistencies across samples and to identify where differences exist by individual site. Thus, meta-analyses were not conducted, rather a multiple cohort replication and expansion approach was used.

Results: Akin to prior work, we found high rates of functional impairment, ranging from 41-75%. Poor community functioning was associated with depressive symptoms in 10 of 12 of the cohorts that included this variable in the analysis (all p-values < 0.05). Lower levels of education (in 3 of 12 cohorts, all p-values < 0.1), a greater number of prior manic (2 of 7 cohorts, all p-values < 0.1) or depressive (2 of 8 cohorts, all p-values < 0.05) episodes, presence of a comorbid substance use disorder (2 of 8 cohorts, all p-values < 0.1), and a greater total number of psychotropic medications (in 2 of 7 cohorts, all p-values < 0.05) were also associated with poor functioning.

Conclusions: While our results are largely confirmatory of previous within-study reports, this study provided an opportunity to survey the global landscape of current data, identify the challenges inherent to conducting global collaborative research in this area, and highlight the overt need for future collaborative work that will identify specific contributors to continued functional impairment in BD to prioritize targets of intervention. The bipolar clinical research community is poised to work together to characterize the multi-dimensional contributors to impairment and address the barriers that impede patients' complete recovery. Likewise, we must also identify the core features which enable many to thrive and live successfully with BD. A large-scale, worldwide, prospective longitudinal study focused squarely on BD and its heterogeneous presentations will serve as a platform for discovery and promote major advances toward optimizing outcomes for every individual with this illness. We thank the National Alliance for Mental Illness (NAMI) for their generous support of this project (to KEB, AN, and MM).

Keywords: Functional Impairment, Depressive Symptoms, Mood Disorders, Heterogeneity

Disclosure: Nothing to disclose.

P299. Polygenic Risk for Depression is Associated With Altered Dentate Gyrus Structure in Offspring at Family Risk for Depression

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Background: Numerous studies showed an increased (2-5 fold) risk for major depressive disorder (MDD) and other psychopathology in the offspring of a parent with depression. MDD in two previous generations (grandparents and parents) is associated with even higher rates of psychopathology in the third generation. While these clinical findings are well-replicated, efforts to explain the mechanisms underlying family risk for depression are ongoing. One area of study is the dentate gyrus (DG) subfield of the hippocampus, which both rodent and human studies suggest may be important in determining which high-risk individuals are susceptible to depression. Mice susceptible to depressive behaviors have altered DG structure (e.g. decreased neurogenesis) and increasing or decreasing the activity of DG mature granule cells can respectively induce or reverse depressive-like behaviors. We have shown that humans at high family risk for depression have decreased DG structure, which predicts future, but not current or past, depressive symptoms. However, whether genetic risk for MDD may contribute to individual differences in DG structure, or that it is due to greater likelihood of an adverse childhood environment, has not been determined. Here we present data on the role of genes, measured by a polygenic risk score (PRS) for MDD, and early life trauma in our previous findings of decreased DG structure in offspring at family risk for depression.

Methods: Sample: The overall sample includes $N = 244$ children, G2, and grandchildren, G3 of probands (G1) with and without MDD. G2 and G3 offspring of G1 probands with MDD were classified as high-risk for MDD; those of probands without MDD as low risk. A subset of $N = 110$ participants underwent MRI scanning.

Imaging: MRI scanning was performed with a GE Signa 3 Tesla whole-body scanner with an 8-channel, phased array head coil. To evaluate structural differences, Freesurfer 6.0 was used on T1-weighted structural scans for (para)hippocampal segmentation "FS60" into 12 subfields, of which we evaluated the DG regions CA4 and GCMLDG. To investigate DG microstructural differences, mean diffusivity, a measure thought to reflect neural integrity, was assessed. DG mean diffusivity was assessed using diffusion MRI with MRtrix analytic pipeline.

Genotyping was done using the Global Screening Array v.3. A total of 10079k SNPs were available for analysis after quality control and imputation procedures. Depression PRS and Schizophrenia were calculated from the Broad depression GWAS summary statistics from Howard et al. and from summary statistics from the Schizophrenia Working Group of the Psychiatric Genomics Consortium et al., using PRSoS accelerated pipeline for each participant at different GWAS p-value thresholds ($p < 10\text{-}8$, $p < 10\text{-}6$, $p < 10\text{-}5$, $p < 10\text{-}4$, $p < 10\text{-}3$, $p < 0.01$, $p < 0.05$, $p < 0.1$, $p < 0.5$).

Measures: Diagnoses were assessed using the Schedule for Affective Disorders and Schizophrenia (SADS) and/or SADS for school-aged children (K-SADS), as appropriate. Depressive symptoms were measured using PHQ-9 and rumination scales. Disability was assessed using the WHODAS. Early life trauma was assessed by the K-SADS traumatic events module.

Statistical Analyses: Statistical analyses were conducted in R using generalized estimating equations (GEE) to account for family structure in the data.

Results: All analyses included adjustments for sex, and ancestry. Family history of depression was associated with higher MDD, but not schizophrenia, PRS, indicating increased cumulative genetic risk for MDD ($b = 0.43$, $p = 0.01$, thresholded at $p < 0.1$ and up). We then found that MDD (but not schizophrenia) PRS was associated with higher depressive symptoms ($b = 0.2$, $p = 0.02$, threshold $p < 0.01$), with more rumination ($b = 0.21$, $p = 0.007$ at threshold $p < 0.01$, marginal at higher thresholds) and with higher disability scores ($b = 0.2$, $p = 0.003$, threshold $p < 0.1$ and up), also adjusting for a family and personal history of depression.

Next, we found that MDD PRS predicted smaller left DG volume (left: $b = -0.16$ $p < 0.05$, threshold $p < 0.5$), and lower right DG microstructure as measured by higher mean diffusivity ($b = 0.23$, $p = 0.005$, threshold $p < 0.001$ and $p < 0.01$), also adjusting for a lifetime history of depression, family risk for depression, total brain volume and age, suggesting a genetic effect of MDD on dentate gyrus structure.

Interestingly, while early life trauma by itself was not associated with DG structure, early life trauma interacted with MDD PRS ($b = -0.33$, $p < 0.001$, thresholds $p < 0.01$ and higher), showing that DG volume was only decreased in participants who experienced early life trauma (simple slope: $b = -0.38$, $p = 0.03$), while there was no association between MDD PRS and DG volume in those who had not (simple slope: $b = 0.09$, $p = 0.62$).

Conclusions: These findings suggest that genetic variation associated with depression predisposes to smaller dentate gyri, particularly in individuals who experience trauma early in life. Findings of smaller DG volumes in depression are therefore likely not just a consequence of disease, but determined by a combination of genetics and increased early adversity. A better understanding of the mechanisms of depression susceptibility can identify individuals most likely to develop depression early, and, as existing interventions can regulate the DG, can ultimately guide interventions for those at particular risk.

Keywords: Hippocampus, Depression, Polygenic Risk Scores

Disclosure: Nothing to disclose.

P300. Translational Profile of Genes in Prefrontal Parvalbumin Interneurons Following Chronic Stress

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Background: Hypofunction of the prefrontal cortex (PFC) contributes to stress-related neuropsychiatric illnesses. Previous finding from our lab indicates chronic stress enhances spontaneous miniature inhibitory neurotransmission in the PFC. Although it is clear that GABAergic transmission in the PFC undergoes significant alterations during chronic stress, little is known about the mechanisms leading to prefrontal hypoactivity. Mounting evidence suggests that chronic stress leads to an increase in activity of parvalbumin (PV) expressing GABAergic interneurons (INs) in the PFC. As a result, the goal of this study was to elucidate the gene expression profile of parvalbumin interneurons in the prefrontal cortex following exposure to chronic stress.

Methods: Transgenic mice Rosa26L10a-eGFP/L10a-eGFP; PVcre/+ were generated that expressed an EGFPL10a ribosomal fusion protein under control of the PV promoter. Male mice were subjected to the chronic variable stress (CVS) paradigm which comprised of a series of randomly alternating stressors administered twice daily over a period of 14 days or served as non-stressed controls. 24 hours after cessation of the stress protocol, we isolated translating ribosomes specifically from prefrontal parvalbumin interneurons using the Translating Ribosome Affinity Purification (TRAP) technique followed by RNA-seq analysis. Data were analyzed using targeted pathway analysis using Enrichr and full transcriptome analysis using GSEA. Potential drug interactions across the top 1000 gene signatures ('perturbagens') were analyzed using ILINCs.

Results: Over 442 genes were ≥1-fold enriched in parvalbumin interneurons following chronic stress compared with the control no stress condition, including targets with known relevance to oxidative stress, extracellular matrix, phospho-lipid metabolism and neurotransmitter binding and activity. At pathway level, we have found changes in oxidative phosphorylation (mitochondrial electron transport), neurotransmission and changes in lipid metabolism and transport. Our drug-target analysis supports the involvement of neurotransmission and growth factor related changes following chronic stress. The analysis of leading-edge genes revealed differentially regulated set of genes relevant to cell binding/growth and mitochondrial function (encoding respiratory chain complex and electron transport chain proteins) following chronic stress in prefrontal PV INs.

Conclusions: Collectively, these results provide new insight into the potential molecular mechanisms and druggable targets associated with chronic stress induced changes in prefrontal GABA-ergic parvalbumin interneurons and how that may lead to stress-related illnesses. Further experiments are needed to validate these findings in stressed vs controls animals and to determine whether alterations in specific target genes in PV INs may lead to stress related phenotypes.

Keywords: Gaba Neuron, Parvalbumin Neurons, Chronic Stress, Medial Prefrontal Cortex, RNA Sequencing

Disclosure: Nothing to disclose.

P301. Expression of the Astrocyte-Specific Extracellular Matrix Gene Htra1 Regulates Susceptibility to Stress in a Sex-Specific Manner

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Background: While the underlying pathophysiology of major depressive disorder (MDD) is poorly understood, convergent evidence from pre-clinical and clinical research supports the notion that MDD is related to impaired structural plasticity in key limbic regions. The extracellular matrix (ECM) of the brain represents a novel domain for study as it not only provides structural support, but also is intimately involved in regulating synaptic plasticity and remodeling.

Methods: We analyzed transcriptional profiles of ECM-related genes from the nucleus accumbens (NAc) in postmortem brain tissue of both male and female patients with MDD as well as in mice exhibiting depression-like behavioral abnormalities after exposure to 21 days of chronic variable stress (CVS). After identifying ECM-related genes that were similarly dysregulated across species, we confirmed cell-type-specificity using in situ hybridization in male and female mice exposed to CVS. Next, to determine causal links, we utilized viral vectors designed to overexpress or knockdown the expression of target genes in a

cell-type specific manner and assessed behavioral reactivity to sub-threshold exposure to stress. In a separate cohort of mice, we assessed for changes in the electrophysiological properties of DA neurons recorded in vitro in mice following cell-type specific manipulation of target genes combined with sub-threshold exposure to stress.

Results: We identified Htra1, an astrocyte-enriched secreted serine protease, as being significantly down-regulated in the NAc of males and up-regulated in females across both species. We found that selective manipulation of the Htra1 gene in astrocytes within the mouse NAc bidirectionally controls susceptibility to stress in a sex-specific manner. Furthermore, direct manipulation of Htra1 in astrocytes of the NAc, in conjunction with sub-threshold stress, influences medium spiny neuronal signaling.

Conclusions: Our findings reveal a pivotal role of astroglia as well as the brain's ECM in mediating stress vulnerability that is impacted in a sex-specific manner and set the stage for novel therapeutic approaches for MDD.

Keywords: Chronic Stress, Major Depressive Disorder (Mdd), Extracellular Matrix, Astrocyte, Sex Differences

Disclosure: Nothing to disclose.

P302. Exploring Genetic Variation of Cytochrome C Oxidase Subunit IV as Potential Marker of Increased Mitochondrial Function Associated With Antidepressant-Induced Mania

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Background: Mechanistically, the Wnt signaling pathway has been postulated to mediate antidepressant response; similarly, individual's bioenergetic status may also impact treatment response. In vitro and in vivo studies have also shown that antidepressants can influence electron transport chain (ETC) complex activity, either by increasing (Mito+) or decreasing (Mito-) mitochondrial energetics. A preclinical antidepressant-induced mania-like phenotype has been associated with an increased respiratory control ratio (State 3/State 4), catalyzed by cytochrome C oxidase (COX). COX is involved in the final step of the ETC to generate ATP and is encoded by ten nuclear and three mitochondrial (mt) DNA. We hypothesize that the rate of antidepressant induced mania (AIM+), a standardized biobank clinical phenotype, will be higher in antidepressants that increase mitochondrial energetics (Mito+), in comparison to Mito- antidepressants. We additionally sought to investigate whether genetic variants in nuclear genes that encode for the COX enzyme are associated with AIM + .

Methods: Based on existing literature, we classified antidepressants as Mito + (venlafaxine, paroxetine, nortriptyline, bupropion) or Mito- (escitalopram, amitriptyline) and identified genotyped patients from the Mayo Clinic Bipolar Biobank, with history of use of Mito+ or Mito- antidepressants. Patients were then categorized and analyzed based on retrospective assessment of AIM and by antidepressant Mito status (+ or -). In a subset analysis, using logistic regression adjusting for the first principal component of ancestry, we tested whether genetic variations in 10 nuclear genes (1895 SNPs in total) encoding for COX (COX10, CO14, COX15, COX20, COX4I2, COX6A1, COX6B1, COX7B, Fastkd2, PET100) was associated with AIM + with antidepressant Mito+ activity. To reduce multiple testing, we combined the SNP-level results into gene-level tests of association using MAGMA for generalized gene analysis of GWAS data. Additionally, GWAS results were combined with eQTL data for cross-tissue expression using FUSION to assess whether genetically predicted gene expression of the candidate genes is associated with AIM + .

Results: Bipolar participants with AIM + ($n = 129$) and AIM- ($n = 597$) phenotypes were further classified into Mito + ($n = 481$) and Mito- ($n = 245$). While accounting for patient overlap in the two groups, AIM + was more frequent with Mito+ vs. Mito- antidepressant use (21.1% vs. 10.6%; OR = 2.1; $p < 0.001$). Gene-level results showed no significant association between genes that encode the COX enzyme and AIM + in response to use of antidepressants with Mito+ activity. Further, eight COX genes, estimated in the cross-tissue eQTL FUSION analysis (COX20, COX6B1, COX6A1, COX14, PET100, COX10, COX15, Fastkd2) showed no significant gene-trait association with AIM when combined with the gene-level results.

Conclusions: These data suggest categorizing antidepressants based on mitochondrial energetics, and not conventional mechanism of action (SSRI, TCA, etc), may be of value in future larger clinical and pharmacogenomic studies of antidepressant-induced mania. Despite evidence showing a possible link between mitochondrial function and bipolar disorder, these data did not provide evidence to support the hypothesis of mitochondrial abnormalities, as defined by variations in COX-related nuclear genes, in AIM + participants with antidepressants that positively impact mitochondrial bioenergetics. No genome-wide significant loci in any of the GWAS was identified but were underpowered for detecting hypothetical effect size. Future assessments focusing on different ETC complexes and analysis of mtDNA genes may provide more insights into the relationship between AIM + and mitochondrial energetics.

Keywords: Bipolar Disorder, Mitochondrial Respiration, Antidepressant Induced Mania, Genetic Variation

Disclosure: Nothing to disclose.

P303. In Vitro Modeling of Depression Neurobiology: Differentiating Blood-Derived Neural Progenitor Cells into Hippocampal-Like Neurons

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Background: In vitro modeling of mood disorders deploying recent advances in stem cell reprogramming offers a unique alternative to postmortem brain studies. Few laboratories to date have examined induced pluripotent stem cells (iPSC)- derived neurons from patients with mood disorders, in part due to the complexity of the method. Our UCSD laboratory has worked closely with collaborators at the Salk Institute who have developed protocols for differentiation of iPSCs to different neuronal types. In particular, we have studied the response of stem cell-derived hippocampal granule cells from patients with bipolar disorder to lithium in vitro. In this study, we aimed to reproduce this protocol and generate dentate gyrus hippocampal-like neurons from neural progenitor cells (NPCs) derived from patients with major depressive disorder for further in vitro experimentation modeling pharmacological response.

Methods: NPCs available from a UCSD-stored cellular bank were transfected with a Prox1:GFP lentiviral vector, plated on Poly-L-Ornithine/Laminin coated 6-well plates, and cultured for 22 days in differentiation and maturation medium. Cytosine arabinose was added to the half of cell colonies prior to FACsorting to help select well-differentiated dentate gyrus (DG)-like neurons.

Results: We have received well-differentiated colonies of DG hippocampal-like neurons which were able to generate electrical

signals on the microelectrode plate. Adding cytosine arabinose improved the output of the FACsorting procedure.

Conclusions: Successful reproduction of the suggested differentiation protocol validates the use of UCSD-stored NPC bank for further in vitro experimentation to answer clinical questions.

Keywords: Stem Cells, Neuron, Major Depressive Disorder, Neural Progenitor Cells, In Vitro Neuronal Differentiation

Disclosure: Nothing to disclose.

P304. Amygdala, Orbitofrontal, and Medial Prefrontal Activation During Implicit Facial Emotion Processing Distinguish Young Adults With Low and High Levels of Negative Urgency From Those With Bipolar Disorder

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Background: Bipolar disorder (BD) is a debilitating condition manifested during late adolescence through early adulthood and marked by difficulty regulating emotions and emotion-triggered impulsivity, which is prevalent during hypo/manic and mixed episodes and persists during inter-episode periods. Alterations to medial-lateral prefrontal cortical and subcortical emotion-processing and emotion-regulation neural circuitry have been shown to characterize BD. Neuroimaging may additionally identify objective markers of BD risk in this circuitry. The negative affective components of hypo/mania and mixed states are characterized by negative urgency (NU), the tendency to impulsively respond to negative affect. High NU is evident in adults with BD, and one study to date has shown that NU statistically mediates the relationship between reward-related neural circuitry and measures indicative of hypo/mania in transdiagnostic individuals at-risk for BD. The relationship between NU and emotion-processing neural circuitry has not been examined in these individuals. The present study compares transdiagnostic young adults ($n = 224$) to individuals diagnosed with BD ($n = 59$) during a functional magnetic resonance imaging (fMRI) task assessing implicit emotion processing to determine the extent to which emotion-processing neural circuitry can distinguish individuals with differing risk levels for BD, based on NU.

Methods: 283 young adults (22.34 ± 2.92 years, 65.37% female) were scanned by 3T fMRI while viewing angry, fearful, sad, and happy faces. The Urgency, Premeditation (lack of), Perseverance (lack of), Sensation Seeking, Positive Urgency (UPPS-P) Impulsive Behavior Scale assessed NU in the transdiagnostic sample, which was median-split into low NU ($n = 113$) and high NU ($n = 111$) groups. Two different scanners were used during data collection, thus, fMRI data were harmonized using ComBat, a statistical harmonization method that mitigates measurement differences attributable to scanner effects while preserving inter-subject variability. Emotional face-related activity was examined using two region-of-interest (ROI) approaches: anatomical and meta-analytic. The anatomical ROIs consisted of a priori regions known to be involved in emotion-processing in BD, such as the caudate, putamen, ventral striatum (VS), amygdala, orbitofrontal cortex (OFC), medial and ventrolateral prefrontal cortices (MPFC, VLPFC), and the ventral and dorsal anterior cingulate. Meta-analytic ROIs were selected from a recent meta-analysis of cognitive, emotional, and resting-state BD functional neuroimaging studies conducted by our lab that identified the anterior caudate, VS, posterior cingulate, ventral anterior cingulate, amygdala, superior temporal gyrus, OFC, and VLPFC. Parameter estimates for regions within each ROI for each emotional face were separately extracted. Separate regularized regression analyses were completed for the anatomical and meta-analytic ROIs to assess group differences in a multinomial logistic framework with age and sex covariates included in the model given significant differences ($p < 0.001$ and $p = 0.01$, respectively).

Results: Elastic net regression cross-validation identified 5 neuroimaging and 1 demographic (age) non-zero predictor variables that optimized model fit. A Nagelkerke pseudo r-squared indicated that 19.1% of the variance in group was explained by these predictors, and the final model was significant at $p < 0.001$. The 5 neural activity variables were: 2 variables to angry faces (right amygdala and left lateral OFC), 1 variable to fearful faces (right lateral OFC), one variable to sad faces (left MPFC), and 1 variable to happy faces (right medial OFC). Post-hoc Tukey HSD-corrected tests individually comparing low NU and high NU to BD indicated that low NU and high NU had significantly lower amygdala activity to anger versus BD ($p < 0.001$ and $p = 0.004$, respectively); low NU and high NU had significantly lower left lateral OFC activity to anger versus BD ($p < 0.001$ and $p = 0.004$, respectively); high NU had significantly lower left MPFC activity to sadness versus BD ($p = 0.019$); and low NU and high NU had significantly greater right medial OFC activity to happiness versus BD ($p = 0.003$ and $p = 0.004$, respectively). The elastic net model with meta-analytic ROIs did not yield any non-zero predictor neuroimaging variables.

Conclusions: These findings provide preliminary evidence suggesting that emotion-processing alterations in limbic and prefrontal circuity in individuals with both low and high trait NU may be emotion-triggered impulsivity-based objective vulnerability markers for BD that are prominent during young adulthood.

Keywords: Bipolar Disorder, fMRI Biomarkers, Negative Urgency, Facial Emotion Processing, Impulsivity

Disclosure: Nothing to disclose.

P305. Comparison of Interleukin-8 Levels in Major Depressive Disorder and Bipolar Depression After Treatment With Escitalopram

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Background: Depressive symptomatology in Major Depressive Disorder (MDD) and Bipolar Disorder (BD) can have severely negative effects on patients' quality of life and can lead to very harmful consequences. Although much progress has been made in the understanding and treatment of affective disorders, a significant number of patients are nonresponsive or partially responsive to current treatment modalities. Neuroinflammation and cytokine dysregulation appear to be key contributors to the pathophysiology of affective disorders, including MDD and BD. Interleukin-8 (IL-8) is a pro-inflammatory chemokine that is activated during periods of acute inflammation. Growing evidence supports IL-8 dysregulation in MDD and BD. We present findings on IL-8 from two studies of patients with MDD and treatment resistant bipolar disorder depression (TRBDD) who were treated with escitalopram (ESC), a selective serotonin reuptake inhibitor. While we assessed a panel of various inflammatory biomarkers for these studies, in this poster we discuss our analysis of IL-8. We present data on only those subjects who received ESC treatment either alone or in combination with an anti-inflammatory agent.

Methods: Data from two separate studies were used for this analysis. In the first study, participants ($n = 66$) ages 20-65, who had been diagnosed with MDD by DSM-IV criteria were included in the study. Study participants with MDD were treated with either escitalopram (ESC) or quetiapine (QTP) for a total of 12 weeks. Serum inflammatory biomarkers were measured at baseline and week 12. The Hamilton Depression Scale (HAM-D) 7, 17, and 21were administered at those two time points. Additionally, baseline serum IL-8 levels of 24 participants without a significant past medical history were also included in this study as healthy controls (HC). Paired sample t-tests were conducted to analyze IL-8 levels for those treated with ESC in this study. Pearson correlation coefficients were calculated to analyze the relationship between IL-8 and depressive symptomatology at week 12 for study completers. In the second study, participants ($n = 47$) who had been diagnosed with TRBDD (Bipolar Disorder I or II) were included in this randomized, double-blind trial. Participants were randomized to receive ESC + placebo or the combination of ESC + celecoxib (CBX) for an 8-week treatment period. HAM-D scores and serum inflammatory biomarker levels were determined at baseline and week 8. HC subjects ($n = 30$) also had baseline serum IL-8 levels assessed. Paired sample t-tests, Kruskal Wallace test, and linear effects models were conducted.

Results: For the MDD study, baseline IL-8 values were significantly different between MDD (Mean ± SEM) (4.02 ± 0.71) and HC (1.86 ± 0.34) participants ($p = 0.007$). In study completers, there was no statistically significant correlation between IL-8 levels and HAM-D scores at week 12 of treatment. Furthermore, for study completers who received ESC ($n = 17$), there was no statistically significant difference in serum IL-8 values between baseline (6.10 ± 2.14) and week 12 (4.85 ± 1.36) of treatment ($p = 0.653$). In the BDD study, baseline serum IL-8 levels were significantly different between the BDD participants (3.92 ± 0.53) and the HC subjects (2.21 ± 0.47) ($p = 0.0021$). There was no statistically significant difference between serum IL-8 levels or HAM-D scores over the 8-week treatment period. In those who received ESC, there was no statistically significant difference between baseline (4.15 ± 0.85) and week 8 values (3.77 ± 0.70) ($p = 0.394$). Likewise, in those who received ESC + CBX, there was no statistically significant difference between baseline IL-8 values (3.73 ± 0.73) and week 8 IL-8 values (3.09 ± 0.29) ($p = 0.363$).

Conclusions: Baseline serum IL-8 values were significantly elevated in both the MDD and BDD participants compared to the HC participants. This supports the inflammatory dysregulation hypothesis in patients with depressive mood disorders. Overall, there was no statistically significant correlation between IL-8 levels and depressive symptomatology in patients with MDD or BDD after being treated with escitalopram. Baseline IL-8 levels did not predict treatment response. This suggests that IL-8 may not play a critical role in the antidepressant effect of escitalopram.

Keywords: Neuroinflammation, Interleukin-8, Major Depressive Disorder, Bipolar Depression, Escitalopram

Disclosure: Nothing to disclose.

P306. Longitudinal Increase in CSF sTREM2 is Associated With Increased Markers of CSF AD Pathology in Late-Life Major Depression Potentially Reflecting a Compensatory Mechanism

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Background: Triggering receptor expressed on myeloid cells 2 (TREM2) is a transmembrane innate immune receptor of the immunoglobulin family. In the brain, TREM2 is found exclusively on microglia and its stimulation has been associated with anti-inflammatory and protective effects. Activation of TREM2 also results in the formation of a proteolytic soluble product (sTREM2). Higher baseline CSF sTREM2 concentrations have been associated with a slower rate of cognitive decline and decreased longitudinal brain amyloid deposition in Alzheimer's disease (AD). Thus, it has been proposed that CSF sTREM2 might reflect an anti-inflammatory state. In a previous study by our group (ACNP presentation 2017), cognitively unimpaired individuals with late-life major depression (LLMD) which is associated with increased risk for AD, showed significant reductions in CSF sTREM2 levels and a lack of significant correlations with CSF AD biomarkers compared to controls, consistent with the aforementioned hypothesis and that TREM2- mediated anti-inflammatory microglia activation might be impaired in this disorder. In the current report, we examined the relationship between longitudinal changes in CSF sTREM2 during a 3-year period and their relationship to LLMD diagnosis and changes in AD and inflammatory markers.

Methods: Our baseline sample consisted of 51 subjects aged 60 years and older who completed a longitudinal observational study over three years and an optional lumbar puncture (LP). 38 of these individuals completed the LP at year 3 (20 with LLMD and 18 controls). We evaluated the effects of time on CSF TREM2 with related-samples Wilcoxon Signed Rank Test and the effect diagnosis on change in CSF sTREM2 with Mann Whitney U test. Correlations between change in CSF sTREM2 and CSF markers of AD (Aβ42, Aβ40, total-tau, p-tau181), inflammation (Il-6, Il-8), and Complement component 3 (C3) markers were run with Spearman's Rank test.

Results: Baseline CSF sTREM2 was significantly lower in the LLMD group vs controls ($p = 0.03$). There were no group differences in CSF sTREM2 from baseline to Year 3 (LLMD $p = 0.82$, Controls $p = 0.18$), nor did Year 3 differ between the LLMD and control group ($p = 0.35$). No differences were observed between controls and LLMD for the longitudinal change in CSF sTREM2, AD biomarkers and inflammatory markers. In the whole group, change in sTREM2 was significantly moderately correlated with change in CSF Aβ40 (rho = 0.54, $p < 0.001$), Aβ42 (rho = 0.48, $p = 0.003$), and PTau181 (rho = 0.34, $p = 0.04$).

In the control group, change in sTREM2 was significantly correlated with change in CSF Aβ40 (rho = 0.58, $p = 0.01$) and Aβ42 (rho = 0.56, $p = 0.02$).

In the LLMD group, change in sTREM2 was significantly correlated with change in CSF Aβ40 (rho = 0.50, $p = 0.03$), Tau (rho = 0.44, $p = 0.05$) and *P*-Tau181 (rho = 0.52, $p = 0.02$) but not with change in CSF Aβ42, a more specific marker of cerebral amyloidosis.

Change in inflammatory markers (i.e., IL-6, IL-8) were not significantly correlated with change in sTREM2 ($p > 0.05$) for LLMD or controls, or the whole group. Change in sTREM2 was significantly correlated with C3 (rho = 0.35, $p = 0.04$) in the whole group.

Conclusions: There were no group differences in change in CSF sTREM2 during a 3-year period, nor any difference between baseline and year 3. The longitudinal increase in CSF sTREM2 during a 3-year period and its association with CSF AD biomarkers may reflect increased anti-inflammatory microglia activation and phagocytosis in response to pathological forms of AD biomarkers Aβ, tau, and p-tau 181.

Interestingly, the CSF sTREM2 increase was associated with the increase CSF Aβ42 in controls, but not in LLMD. This finding suggests that upregulation of anti-inflammatory microglia and phagocytosis of brain amyloid deposits may be less efficient in LLMD.

Similarly, the positive correlation between the longitudinal increase in CSF sTREM2 and the increase in CSF T-tau and P-tau181, which we found in the LLMD group but not in controls is also consistent with an upregulation of anti-inflammatory microglia in response to increased tau and neurofibrillary tangles, markers of neurodegeneration and AD, respectively.

However, the change in CSF sTREM2 was correlated with the change in CSF C3 in the whole cohort; our group and others have also found positive correlations between CSF sTREM2 and CSF neurofilament light (NFL) protein, a biomarker of neuroaxonal damage.

Taken together these results suggest that higher CSF sTREM2 concentrations may reflect not only upregulation of anti-inflammatory microglia and phagocytosis in response to increased brain amyloid and tau pathology, but also increased neurotoxic effects which are possibly related to its reported intrinsic pro-inflammatory effects.

Keywords: CSF sTREM2, Microglia, Aβ, Depression, Alzheimer's Disease

Disclosure: Nothing to disclose.

P307. High Psychiatric Comorbidity Denotes a Candidate Bipolar Disorder Sub-Phenotype Marked by Low Prefrontal GABA Levels and High Impulsivity: A Preliminary 2x2 (Anxiety Disorder X Alcohol Use Disorder) Factorial MRI Investigation

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Background: Though nearly 80% of individuals with bipolar disorder (BD) experience anxiety (ANX) and/or substance use (SUD) disorders at some point in their lives, relatively few studies have focused on the impact of co-occurring ANX and/or SUD on BD pathophysiology or treatment. Over the past decade, we have focused on characterizing and manipulating disturbances in glutamatergic (excitatory) and GABAergic (inhibitory) neurotransmission and neurobehavioral control that are shared between individuals with BD and Alcohol Use Disorder (AUD). We have found that individuals with co-occurring BD + AUD have uniquely-low dorsal anterior cingulate cortex (dACC) GABA and glutamate levels, uniquely-low activation to alcohol cues in the right inferior frontal gyrus (rIFG; i.e., the main locus of inhibitory control in the brain), and the highest levels of impulsive choice (i.e., delay discounting) relative to healthy-volunteer, BD or AUD alone comparator groups. However, despite emerging evidence that ANX are independently associated with dysfunctional GABAergic/glutamatergic transmission and impulsivity, we have failed to date to account for the potential moderating impact of ANX on our findings. To address this oversight, we re-analyzed our published GABA, glutamate (1H-MRS; Prisciandaro, 2017), alcohol cue reactivity (fMRI; Prisciandaro, 2019), and delay discounting (behavioral task; Mellick, 2019) data, with ANX moderating the effects of AUD on these variables in individuals with BD (+/-AUD; $n = 56$) from the lead author's K23 study (AA020842).

Methods: All K23 study participants were required to demonstrate ≥1 week of abstinence from alcohol and drugs via serial ethyl glucuronide and urine drug screen testing. General exclusions included serious medical illness, history of head injury, severe co-occurring Axis I disorders, benzodiazepine/antidipsotropic use, or history of complicated alcohol withdrawal. Co-occurring drug use disorder was exclusionary for BD but not BD + AUD participants. Participants with substantial medication dose changes ≤1 week before MRI were excluded. Participants completed a baseline diagnostic visit including the Structured Clinical Interview for DSM-IV Axis I Disorders and a delay discounting computer task. Participants then returned approximately 4 days later for an MRI including 1D/2D J-resolved 1H-MRS of dACC and a well-established fMRI visual alcohol cue exposure paradigm (MRI methods detailed in primary publications). For the present re-analysis, we divided the BD +/-AUD subsample by current diagnosis of ≥1 ANX (i.e., Panic Disorder, Agoraphobia, Social Anxiety Disorder, or Generalized Anxiety Disorder), resulting in 4 participant groups nested within a 2x2 (AUDxANX) factorial design matrix (BD [$n = 17$], BD + ANX [$n = 14$], BD + AUD [$n = 13$], BD + ANX + AUD [$n = 12$]). For each dependent variable, a 2x2 general linear model was estimated. Significant main effects and/or interactions were followed by pairwise comparisons.

Results: There were significant interactions between AUD and ANX in BD participants on levels of GABA ($F = 4.38$, $p < 0.04$), alcohol cue reactivity (i.e., in a cluster encompassing the rIFG; $F > 4.03$, $p < 0.05$ FWE), and delay discounting (i.e., LogK; $F = 5.05$, $p = 0.03$). Post-hoc mean testing found that BD participants with both co-occurring ANX and AUD had uniquely-low, while BD participants with co-occurring ANX but not AUD had uniquely-high, levels of GABA and rIFG activation to alcohol versus neutral beverage images. Delay discounting was only elevated in BD participants with both co-occurring ANX and AUD, with remaining groups statistically indistinguishable from one another. Preliminary glutamate data suggested a main effect of AUD, though analyses of complex relationships among the diagnostic groups are ongoing.

Conclusions: Results demonstrated that co-occurring ANX moderated previously reported effects of co-occurring AUD on neuro-chemical/behavioral variables in individuals with BD. In most cases, abnormalities previously attributed to co-occurring AUD in BD individuals were either specific to, or substantially more severe in, BD individuals with both co-occurring ANX and AUD. We hope to replicate and extend these findings in a larger sample with the goals of providing a neuroscience foundation for the development and evaluation of personalized treatments for "real-world" individuals with BD and alternative psychiatric classification systems that overcome the limitations inherent to the existing DSM system.

Keywords: Bipolar Disorder, Psychiatric Comorbidity, Impulsivity, Proton Magnetic Resonance Spectroscopy, Functional MRI (fMRI)

Disclosure: Nothing to disclose.

P308. Pharmacological Targeting of FGF14 Modulates Motivated Behavior by Increasing Medium Spiny Neuron Firing

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Background: Serving as the central hub of the mesocorticolimbic (MCL) circuit, the nucleus accumbens (NAc) confers prominent regulatory effects on motivated behaviors. In the principal cell type of the NAc, namely medium spiny neurons (MSN), protein:protein interactions (PPI) between voltage-gated Na + (Nav) channels and Nav channel auxiliary proteins play a central role in generating their electrical outputs. In particular, the PPI between the Nav1.6 channel and fibroblast growth factor 14 (FGF14) is central to action potential (AP) discharge of MSNs and, when the PPI is altered, there are resultant neuromodulatory effects. Given this prominent role of FGF14:Nav1.6 complex assembly in generating electrical outputs of MSNs, the central role of the NAc in regulating the MCL circuit, and the latter conferring important regulatory effects on motivated behaviors, we hypothesize that pharmacological targeting of the FGF14:Nav1.6 complex could exert regulatory effects on motivated behaviors.

Methods: We employed medicinal chemistry to synthesize and optimize, as well as chemoinformatics, the split-luciferase assay (LCA), surface plasmon resonance (SPR), and whole-cell patch-clamp electrophysiology to validate, pharmacological probes in silico to ex vivo. Compounds displaying promising in silico to ex vivo pharmacodynamic profiles were investigated in vivo using single-unit electrophysiological recordings and for effects on motivation in an operant behavior paradigm.

Results: Starting with a candidate pool of ~45,000 commercially available compounds, we employed an amalgam of complementary and orthogonal primary in-cell screening assays, in-cell counter-screening assays, and protein:ligand binding studies to identify small molecule modulators of FGF14:Nav1.6 complex assembly. Using this integrated screening platform, we identified a class of three compounds with conserved pharmacodynamic profiles suggestive of potent and selective modulatory effects on the FGF14:Nav1.6 complex. Of these 3 compounds, 7605 displayed the most favorable drug-like properties for CNS drug development, including a molecular weight less than 400 g/mol and a topological surface area less than 75 Å2. To improve the potency and aqueous solubility of 7605, substituents of the parental scaffold were replaced, producing the optimized analog PW1028. Notably, PW1028 modulated FGF14:Nav1.6 complex with an IC50 of ~250 nM according to the LCA and displayed nanomolar binding affinity to FGF14 as determined by SPR. In heterologous cells, PW1028 was shown to exacerbate FGF14-mediated regulatory effects on Nav1.6 channel inactivation. In MSNs of the NAc, these FGF14-dependent modulatory effects of PW1028 on Nav1.6 channel inactivation were recapitulated, as voltage-clamp recordings revealed that PW1028 caused a ~15 mV depolarizing shift in the voltage-dependence of steady-state inactivation of the transient Na+ current (INa) of MSNs from wild-type mice without affecting MSNs from Fgf14-/- mice. As anticipated, this hampering of Nav channel closure and prolonged INa conferred by PW1028 was shown to potentiate the intrinsic excitability of MSNs of NAc as determined using whole-cell current-clamp electrophysiological recordings. The findings of these ex vivo studies were recapitulated in vivo using single-unit electrophysiological recordings, as IP injection of 5 mg/kg PW1028 increased the firing rates of accumbal neurons. At the behavioral level, these electrophysiological changes were found to be accompanied by systemic administration of PW1028 maintaining motivational states in a satiated state, and, therefore, suggesting that the compound affects hedonic states in situations lacking motivation.

Conclusions: These results demonstrate that the FGF14:Nav1.6 complex is a novel target for the development of first-in-class PPI-targeting neuropsychopharmacological agents.

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Keywords: Neuropharmacology, Ion Channels, Reward, Neural Circuits

Disclosure: IonTx Inc.: Founder (Self)

P309. Social Dominance Hierarchies Negotiate the Response to Psychosocial Stress in an Adult Female Mouse Model of Depression

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Background: Depression is one of the most common psychiatric conditions of modernity, and it disproportionately affects women. Recently, there has been a generational trend of increased prevalence in adults. It has been postulated that this is due to the demands of competitive work environments, socioeconomic stress and relative social isolation. Why some people thrive in such environments and others develop depression is poorly understood. Rodent models of social hierarchies can be studied to investigate the psychosocial stressors and neurobiology contributing to the depressive milieu.

We hypothesize that the protective effects of social partnerships contribute to stress susceptibility in a rank-based manner. We perform our investigation in two aims – first to determine the role of rank in reward salience, and second to investigate differences in vulnerability to depression induced by social instability stress (SIS).

Methods: To investigate whether novel social contact is inherently rewarding, we pair-housed adult female C57/Bl6J mice ($n = 28$). Mice underwent open field test (OFT) to identify differences in anxiety, exploration, or locomotion. Social rank was assigned via competitive exclusion (CE) and home cage behavioral analysis. After hierarchies stabilized, mice underwent the three-chamber social approach (3-CSA) to gauge sociability and interest in social novelty. To investigate rank differences in biological markers of stress, fecal samples were processed for corticosterone metabolites (FCM) via ELISA. Finally, immunohistochemistry (IHC) was performed on whole brains to examine c-Fos expression after exposure to a novel conspecific ($n = 12$) or an empty cage ($n = 8$).

Remaining mice ($n = 8$) underwent an experiment investigating motivation and anxio-depressive responses to social isolation (SI) stress. Dyads were re-tested for rank, then subjected to OFT and elevated plus maze (EPM). They underwent appetitive conditioning (AC) to identify rank differences in reward learning. After, mice underwent 8 weeks of SI, followed by repeat OFT and EPM. The development of a depressive phenotype was monitored through weekly coat state score (CSS), nest building test (NBT), and FCM. In the 7th week sucrose preference test (SPT) was performed. After SI, mice were re-introduced to their original social partner ($n = 4$) or to an identical empty environment ($n = 4$). IHC was performed on brains for c-Fos, co-labeled for GAD67 or ChAT.

Results: Dyads rapidly formed stable hierarchies (>85% within 3 days), and rank was independent of innate anxiety or locomotor characteristics. However, subordinates expressed more supported rears during the OFT. Subordinates also spent more time exploring novel contexts in 3CSA, regardless of whether the stimulus was social or inanimate. In contrast, dominants prioritized time in social chambers.

Subordinates also demonstrated higher FCM at baseline and during hierarchy formation. As hierarchies stabilized, FCM became comparable between ranks. IHC revealed differential c-Fos expression in brain regions involved in social recognition, reward salience, and threat evaluation, including the medial prefrontal cortex, lateral septum, claustrum, nucleus accumbens, and basolateral amygdala. Cell counting is ongoing to determine if a rank-based difference exists.

Ranks learned comparably in AC training and displayed equivalent motivation to perform the task. Subordinate CSS deteriorated during SI and exploratory behavior diminished in the OFT. Dominants consumed less sucrose in the SPT ($< 60\%$ preference) compared to subordinates. These differences were not attributable to rank differences in locomotion or anxiety in OFT and EPM. All mice experienced a progressive increase in FCM during SI. More mice ($n = 20$) will be tested to determine the significance of these trends.

Conclusions: Here we show that adult female dyads form stable hierarchies rapidly upon pair housing. Furthermore, we identified distinct behavioral and biological traits which predict future rank in paired mice. Notably, subordinate animals expressed more exploratory but not anxiety behaviors and higher FCM status at baseline, while prioritizing novelty exploration in the 3CSA. This is similar to male hierarchies, in which subordinates

engage in patrolling behavior. To our knowledge, this trait has not been demonstrated in females.

Post-SI, the lack of a sucrose preference in dominants is interesting when compared to the deterioration in CSS, higher FCM and diminished exploratory behavior demonstrated by subordinates. This indicates that subordinates become more stress-responsive after SI, whereas dominants become anhedonic. An alternative interpretation is that subordinates consume more sucrose in response to stress, similar to 'binge-eating' behavior. These findings collectively demonstrate a distinction between stress and anxiety, and indicate an integrated, adaptive function for rank, stress status and role assignment.

The next aim examines the influence of rank on vulnerability to psychosocial stress produced by an unstable social environment. A cohort of adult female C57/Bl6J mice ($n = 36$) is currently undergoing 7 weeks of SIS, in which cage mates are randomized every 3 days to disrupt hierarchies as they become established. CE is performed during SIS to identify rank, and FCM is taken. After, mice will undergo behavioral testing for anxio-depressive phenotypes and changes in attention and cognition.

Keywords: Social Behavior, Anxiety and Depression, Females, Social Stress, Social Dominance

Disclosure: Nothing to disclose.

P310. Increased Effort Expenditure in Major Depression Following a TNF-Alpha Antagonist is Mediated by Change in Systemic Immunometabolism

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Background: Evidence suggests that increases in inflammation and alterations in systemic immunometabolism may contribute to the pathophysiology of motivational anhedonia in major depressive disorder (MDD). MDD patients have been shown to exhibit elevated levels of peripheral blood inflammatory biomarkers, particularly C-reactive protein (CRP), tumor necrosis factor (TNF)-alpha, interleukin-6 (IL-6), and IL-1-beta, which have in turn been linked to motivational deficits in both animals and humans. Specifically, stimulation of cytokines has been shown to reduce willingness to expend effort for rewards. Further, these effects have been found to be strongest in patients with both high inflammation and altered protein and genomic signaling within pathways related to glucose metabolism. These metabolic changes have been hypothesized to reflect an increased reliance on glycolysis (as opposed to oxidative phosphorylation) within activated immune cells. Taken together, these data raise the possibility of an immunometabolic-dependent pathway that contributes to the development of motivational anhedonia for depressed patients with high inflammation. To date, however, only a few studies have explored the impact of pharmaceuticals that target inflammation on motivational anhedonia in depressed patients. Here, we present novel results from a newly completed study (NCT03006393) aimed at identifying the mechanisms by which inflammation and immunometabolism contribute to motivational anhedonia in depressed patients.

Methods: Effort-based decision-making and peripheral inflammatory markers were assessed in 60 patients with current MDD at a baseline session, after which 37 patients 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. Motivated behavior was using an effort-based decision-making task (EBDM) during which participants made a series of choices about how much physical effort (rapid button presses) they were willing to expend in exchange for varying amounts of monetary reward. Effort discounting was assessed by examination of choices at each effort level (20%, 50%, 80%, or 100% of a participant's maximum press rate) as well as by a well-validated computational model. For this model, a free parameter 'k' is fit to each participant's choices and represents the extent to which effort reduces the value of reward. Plasma samples were also collected at each time point, and were assayed for TNF soluble receptor II (TNFRII) and five markers of systemic glucose metabolism: adiponectin, resistin, leptin, glucose, and insulin. These six markers were combined to form a composite measure of TNFRII and glucose metabolism.

Results: At baseline, the overall proportion of effortful choices as well as the k discounting parameter were associated with measures of anhedonia and fatigue, including the apathy-motivation index, and the anhedonia subscale from the IDS-SR (ps < .05). Following randomization, there was a significant treatment X time interaction for TNFRII with a reduction for patients receiving infliximab relative to placebo ($p = 0.024$), but not glucose metabolism. We also observed a significant group by time interaction such that patients receiving infliximab showed a decrease in effort discounting (lower k parameter) relative to placebo ($p = 0.045$). We also observed a treatment by time by effort interaction ($p < .05$) such that this effect was most pronounced at the higher levels of effort (80% and 100%). Further, we observed that post-infusion decreases in plasma TNFRII and glucose metabolism predicted increases in effortful choices at the highest effort level (controlling for age and sex, $p = 0.009$) as well the k discounting parameter ($p = 0.033$). Finally, a bootstrapped mediation analysis found that change in plasma TNFRII and glucose metabolism significantly mediated the relationship between treatment assignment (infliximab or placebo) and change in effortful choice ($p < 0.05$).

Conclusions: The results of the study indicate that administration of the anti-inflammatory agent infliximab to patients with current MDD and high inflammation increases the willingness to expend effort, a hallmark of motivational anhedonia. Moreover, this effect appears to mediated by changes in markers of TNF signaling in combination with markers of glucose metabolism. These results highlight the potential for the combination of inflammatory and immunometabolic biomarkers and anti-inflammatory treatment strategies to identify and treat motivational impairments.

Keywords: Precision Medicine for Mood Disorders, Immune Modulation, Effort Based Decision Making Task, Anhedonia, Computational Models of Decision-Making

Disclosure: Blackthorn Therapeutics: Consultant (Self)

P311. Zelquistinel (GATE-251): Results of a Phase 1 Single Ascending Dose Study to Evaluate the Safety, Pharmacokinetics, and EEG Effects of a Novel Rapid Acting, Orally-Bioavailable NMDA Receptor Modulator

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Background: Major depressive disorder (MDD) is a common disabling and potentially life-threatening condition estimated to affect > 300 million people worldwide. Glutamatergic mediated plasticity and modulation of NMDA receptor activity have been employed in developing rapid-acting treatments for MDD. GATE-251 (also known as AGN-251751) is a NMDA receptor modulator

that binds to a unique site on the receptor. In animals, zelquistinel has oral bioavailability greater than 90%, and provides efficacy in several animal models of depressive-like behavior. This Phase 1 first-in-man study evaluated safety, pharmacokinetics and GATE-251 as well as EEG measures of NMDAR activation in healthy volunteers.

Methods: This double-blind, randomized, placebo-controlled study evaluated healthy male or female subjects aged 18-55 years, with supine heart rate of 50-100 bpm who used no other drugs for at least 14 days prior to this study and were negative in drug of abuse screens. Part A of the study evaluated safety, plasma pharmacokinetics and EEG following single fasting doses of 100 microg, 1 mg, 3 mg, 10 mg, 25 mg, or 50 mg GATE-251 as an oral solution, compared to a water placebo (randomization 6:2 drug: placebo). A separate group studied a 1 mg dose in female subjects. Part B evaluated safety and CSF pharmacokinetics of single doses of 1 mg or 10 mg fasted and 1 mg fed ($N = 5, 3, 3$) in subjects with indwelling lumbar catheters. Part C evaluated plasma PK in fasted male subjects compared to subjects who received a high-fat breakfast ($N = 5, 3$).

Results: Safety: In this study no serious adverse events were reported, nor were any clinically significant changes in plasma chemistry, hematology or urinalysis observed. No subjects experienced clinically significant changes in vital signs or ECG. There were no treatment emergent psychotomimetic symptoms (as measured by changes in C-SSRS, BPRS + or CADSS) from predose baseline to end of study. Treatment-related adverse events (TRAEs) were few: in Part A ($N = 14$ placebo, $N = 42$ GATE-251) there were no TRAEs in the placebo group and 1 in the 1 mg GATE-251 group: headache. In Part B ($N = 4$ placebo, $N = 12$ GATE-251), there were no TRAEs in the placebo group and 2 in the 10 mg group: 1 lumbar puncture syndrome, 1 disturbance in attention. In Part C ($N = 0$ placebo, $N = 6$ GATE-251), there were no TRAEs.

Pharmacokinetics: In Part A, GATE-251 exhibited rapid absorption and dose-related increases in exposure assessed as Cmax or AUC. T1/2 was similar across doses at 0.5 hr, and was not different in male vs female subjects. In Part B, transport into the CSF was significant, with a Tmax of 4 h. Too few sampling times prevented calculation of T1/2 in CSF. A high fat meal slowed absorption of GATE-251 compared to fasted, with reduced Cmax exposure, although AUC exposure was not affected.

EEG: GATE-251 increased resting alpha EEG power, indicative of effective central modulation and enhanced NMDAR activation. While Gate-251 induced alpha power showed an inverted U-shaped dose response relationship at higher doses, doses that optimally enhanced alpha EEG demonstrated CSF drug concentration that corresponded to concentrations that enhance NMDAR activity in vitro.

Conclusions: GATE-251 was well-tolerated oral NMDA modulator with few treatment-related AEs. There was no evidence of psychotomimetic effects over the dose range evaluated. GATE-251 was rapidly absorbed into the plasma and exposure was dose-related. No gender-associated differences were observed. In plasma, AUC exposure was not affected by a high fat meal whereas in CSF AUC exposure appeared to be enhanced. The CSF levels achieved within this dose range were robust and corresponded the concentrations necessary to modulate NMDA receptors in vitro. Translational pharmacodynamics of qEEG/ERPs and the plasma/CSF pharmacokinetics demonstrate that the assessed doses meet and/or exceed drug concentrations that are predicted to be maximally efficacious in humans.

Keywords: Fast-acting Antidepressant, NMDA Glutamate Receptors, Translational Approaches to Drug Development, Synaptic Plasticity

Disclosure: Gate Neurosciences, Anagin, Vasculonics, MindX: Founder (Self)

Karuna Therapeutics: Advisory Board(Self)

Johnson and Johnson: Grant (Self)

P312. Target Trial Emulation: Evaluating a Treatment for Metabolic Depression

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Background: Previous evidence suggests that pioglitazone, an insulin-sensitizing medication, in patients with major depressive disorder (MDD) and insulin resistance (IR) can be useful in ameliorating non-remitted MDD. To investigate this claim, we conducted two emulated target trials (ETTs) of the effect of adjuvant pioglitazone on antidepressant response among people with type 2 diabetes (DM2). Pioglitazone was compared to two other classes of non-insulin sensitizing DM2 medications, sulfonylureas and dipeptidyl peptidase 4 (DPP4) inhibitors. It was hypothesized that the combination of antidepressant medication and pioglitazone would elicit a superior antidepressant treatment response compared to adjuvant DPP4 inhibitors or sulfonylureas over a one-year follow-up period.

Methods: The two ETTs were designed using health insurance claims from the Optum Clinformatics® Data Mart version 3.0 (Optum) (Optum Insight, Eden Prairie, MN). Poorer antidepressant response was measured by an increase of new prescriptions for antidepressants one year after the study start date. A treatment was defined as new if it had never been prescribed, or if it had not been prescribed for at least one year plus the number of days' supply of the most recent prescription.

Results: The participants in ETT1 who took pioglitazone ($n = 1308$) were more likely to be younger, male, and use statin medications when compared to users of DPP4 inhibitors ($n = 1634$). In ETT2 pioglitazone users ($n = 1{,}639$) were more likely to take statins, use insulin, and take a greater average of antidepressant medications in the prior year compared to those who used sulfonylureas ($n = 4{,}879$). After matching, the standardized mean difference for all covariates in both ETTs was <0.15, with exception of age for ETT1 (SMD = 0.17). In ETT1, instrumental variable analysis found that pioglitazone users added a new antidepressant or antipsychotic treatment 1.3 times compared to 1.7 times among DPP4 users over a 1-year follow-up period. In ETT2, similar analysis found that pioglitazone users added a new antidepressant or antipsychotic treatment 0.8 times compared to 1.4 times among DPP4 users over a 1-year follow-up period.

Conclusions: These findings lend evidence to the hypothesis that adjuvant pioglitazone lead to a stronger antidepressant treatment response than sulfonylureas or DPP4 inhibitors among individuals with MDD and DM2 - as measured by fewer treatment shifts and/or additions of a new antidepressant or antipsychotic. Coupled with previous evidence on the role of pioglitazone in MDD patients with IR, this evidence suggests that pioglitazone may prove useful in ameliorating treatment MDD response among people with comorbid DM2.

Keywords: Major Depressive Disorder, Emulated Target Trial, Insulin Resistance

Disclosure: Nothing to disclose.

P313. Go/NoGo EEG Data Associations Depression Severity in Depressed and Suicidal Patients

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Background: Impaired inhibition has been observed in depressed and suicidal patients. The Go/NoGo task assesses cognitive and

motor inhibition, as well as behavioral control. Increased average amplitude of event-related potentials (ERPs) is linked to increased behavioral control and healthier levels of brain activity. In this study, we aimed to examine the mean ERPs associated with successful response inhibition and its relationship with depression severity and suicide risk.

Methods: Three groups of adult depressed patients of both genders: i) after a suicide attempt in the past 72 hours ($n = 6$); ii) currently endorsing suicidal ideation ($n = 10$); and iii) non-suicidal ($n = 9$). Participants subjects underwent an electroencephalogram (EEG) while participating in a Go/NoGo task during 420 trials. EEG data was recorded utilizing the ActiveTwo BioSemi system, using a 34 electrode mesh. Participants were instructed to press a mouse button when the arrow was oriented vertically (without tilt, $n = 340$) and not to press the button when the arrow was tilted (15 degrees tilt, $n = 80$). EEG mean ERP amplitude during the correct NoGo trials.

Results: There were no ERP activity differences between groups. ERP activity recorded during the correct NoGo stimuli was positively correlated with depression severity ($p = 0.016$), with a non-significant trend with age ($p = 0.052$).

Conclusions: Cognitive inhibition was correlated with depression severity and age, rather than with suicide risk status in adult depressed suicidal patients.

Keywords: Suicide, EEG, Behavioral Inhibition

Disclosure: Neurostar: Grant (Self)

P314. Theta-Gamma Coupling Vs Clinical Diagnosis in Separating Older People With Mild Cognitive Impairment With or Without Major Depressive Disorder on Cognitive Function

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Background: Individuals with mild cognitive impairment (MCI) and major depressive disorder (MDD), including when MDD is in remission, are at higher risk for cognitive decline and Alzheimer's dementia (AD) than those with either diagnosis alone. Prefrontal cortex theta-gamma coupling (TGC) may be an early marker of prefrontal cortical dysfunction. The aim of this study was to determine whether TGC can discriminate individuals' cognitive performance better than their diagnosis (i.e., MCI vs. MCI + MDD). We hypothesize that TGC at a specific cut-off will create more distinct groups with regard to global cognition than clinical diagnoses.

Methods: We analyzed baseline data from 128 participants with MCI (mean age: 71.8, SD: 7.3) and 85 with MCI + MDD (mean age: 70.9, SD: 4.7) who are participating in a dementia prevention trial (NCT02386670). Participants with MDD were in remission. Participants with MDD were in remission. Participants were assessed clinically and cognitively with a comprehensive neuropsychological battery. TGC was measured during the performance of a working memory task, the N-back. We determined a sample-derived TGC cut-off using performance on the 2-back condition among 50% of our sample (training sample, randomly selected). We then tested that cut-off on the other 50% of the sample (validation sample), to examine how the cut-off separated individuals on global cognition as ascertained using a composite global cognitive measure from the neuropsychological battery. Both of these steps were repeated 1000 times to generate stable estimates of the differences between groups. We compared the Cohen's d between the TGC groups and the diagnostic groups.

Results: Cohen's d for the TGC groups (Cohen's d = 0.31) was larger than the Cohen's d for the diagnostic groups (Cohen's d = 0.13). There were no significant differences in performance on any of the cognitive domains between the MCI and MCI + MDD groups. In contrast, the high vs. low TGC groups differed significantly in terms of global cognition ($t(210) = -2.45$, $p = 0.02$), processing speed ($t(207.9) = -2.78$, $p = 0.006$), working memory ($t(210) = -2.91$, $p = 0.004$), and executive function ($t(209) = -2.04$, $p = 0.04$).

Conclusions: Global cognition differed in groups based on low vs. high TGC but not in groups based on MCI vs. MCI + MDD diagnosis. The TGC groups (but not the diagnostic groups) also differed in terms of cognitive domains depending on prefrontal cortex. Our results suggest that TGC might be an early marker of prefrontal cognitive dysfunction. Future work should focus on determining if individuals with low TGC are at an increased risk for cognitive decline and progression to AD.

Keywords: EEG Biomarkers, Mild Cognitive Impairment Due to AD, Major Depression Disorder

Disclosure: Nothing to disclose.

P315. No Meaningful Opioid Abuse Liability of REL-1017 (Esmethadone; D-Methadone), a Rapid-Acting Antidepressant in Clinical Development: A Human Abuse Potential Study

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Background: Opioid abuse is widespread, with an estimated 57,550 overdose deaths from synthetic opioids in the U.S. in 2020. REL-1017, a promising, rapid-acting, antidepressant candidate, is the dextrorotary isomer of methadone (esmethadone; d-methadone). Although previous studies indicate that d-methadone is opioid inactive and lacks meaningful abuse potential, its close chemical similarity to the opioid-active isomer l-methadone prompted further evaluation. Therefore, we performed a human abuse potential (HAP) study using current state-of-the art methodology to further ascertain the abuse liability of REL-1017.

Methods: The study was a single-dose, randomized, double-blind, double-dummy, active- and placebo-controlled, 5-way crossover HAP study of REL-1017 in experienced recreational drug users. The study consisted of 4 phases: Screening, Qualification, Treatment, and Follow-up. Within 28 days of Screening, eligible subjects participated in a 4-day Qualification Phase to ensure that they were able to discriminate the effects of the positive control, oral oxycodone 40 mg, when compared to placebo. During Qualification, all subjects underwent a naloxone challenge test to confirm that they were not opioid-dependent. Qualified subjects entered the Treatment Phase. During the Treatment Phase, composed of 5 treatment periods, each subject received the following oral treatments in a randomized, double-blind, double-dummy, crossover fashion with >11 days of washout between treatments: REL-1017 25 mg (25R)(proposed therapeutic daily dose), REL-1017 75 mg (75R) (proposed loading dose), REL-1017 150 mg (150R)(maximum tolerated dose), oxycodone 40 mg (OX)(standard active control), and placebo (PL). Subjects then completed a safety follow-up approximately 1 week after the last treatment. The primary

endpoint of this study was the maximum effect (Emax) for Drug Liking ("at this moment"), assessed with a bipolar (0 to 49 = dislike; 50= neutral; 51-100 = like) visual analog scale (VAS). The key secondary endpoints were VAS for Overall Drug Liking and for Take Drug Again. The primary treatment comparisons to assess the abuse potential of REL-1017 included oxycodone versus placebo (study validity), REL-1017 versus oxycodone, and REL-1017 versus placebo, using the primary endpoint (Drug Liking VAS Emax). Data were analyzed using a one-sided Student's *t* test for paired data for oxycodone vs. REL-1017, and using equivalence margins: oxycodone vs. placebo (□ = 15) and REL-1017 vs. placebo (□ =11). Statistical analyses were performed on "modified completers", defined as subjects completing all 5 treatments, and excluding subjects with similar Drug Liking Emax scores (<5 points difference) across all study treatments or subjects with an Emax for placebo >60 and ≤5 difference between Emax for oxycodone and placebo.

Results: A total of 50 subjects were randomized; 3 subjects did not complete all 5 treatments, and 3 subjects did not fulfill criteria for "modified completers." A total of 44 subjects fulfilled the criteria for "modified completers" and were evaluated in the statistical analyses. The mean age of the 44 "modified completers" was 36.6 (SD 9.24); 8 (18.2%) subjects were females, and 36 (81.8%) were males. The mean and median Emax for the five drug conditions are shown below.

Mean	PL: 51.7	25R: 53.0	75R: 58.2	150R: 64.9	OX: 85.0
Median	PL: 50.0	25R: 50.0	75R: 50.0	150R: 58.0	OX: 89.0
P-value vs. OX	<0.001	<0.001	<0.001	<0.001	
P-value vs. PL		<0.001	<0.001	<0.10	

The Emax for 40 mg oxycodone was significantly greater than placebo, confirming study validity, and it was greater than all 3 doses of REL-1017, also with a high degree of statistical significance. Consistent, statistically significant differences between all tested doses of REL-1017 and oxycodone were seen for the two key secondary endpoints ("Overall Drug Liking" and "Take Drug Again"). Comparison of REL-1017 to placebo using the FDA suggested equivalence margin analysis indicated similarity to placebo at $p < 0.001$ for the 25 mg and 75 mg doses of REL 1017 and at $p < 0.10$ for the 150 mg dose.

Conclusions: REL-1017 doses of 25 mg (daily therapeutic dose), 75 mg (loading dose) and 150 mg (maximum tolerated dose and 6X the daily therapeutic dose) exhibited at least a 20-point difference in Emax Drug Liking compared to 40 mg oxycodone with a highly significant difference ($p < 0.001$). The similarity of 25 mg and 75 mg doses of REL-1017 to placebo were also highly significant ($p < 0.001$), with similarity of 150 mg REL-1017 to placebo at $p < 0.1$. Low-level liking is commonly seen in HAP studies at high doses of the test substance and is consistent with unscheduled substances and with controlled substances in U.S. DEA Schedule V or IV. While DEA scheduling of new molecules is based on the analysis of multiple factors, including in vitro studies, animal studies, and human studies, the current HAP study design is considered the single most predictive and important study to determine abuse potential. This study showed no meaningful opioid abuse potential for REL-1017 in healthy recreational opioid users. Ongoing Phase 3 trials to confirm the efficacy and safety of REL 1017 are registered with ClinicalTrials.gov (NCT03051256).

Keywords: REL-1017, Antidepressant, Abuse Potential Study

Disclosure: Pinney Associates: Consultant (Self)

P316. Stress During Adolescence, Inflammatory Status and Depression Vulnerability: Potential Role of the Gut Microbiome

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Background: Exposure to early adverse experiences induces persistent changes in physiological, emotional and behavioral functions predisposing the individual to an enhanced vulnerability to develop different psychiatric disorders during lifespan. The adverse outcomes depend upon the timing of the stressful experiences, and in this contest, adolescence represents a key sensitive period for brain development. Among the biological systems involved, the gut microbiota has recently been proposed to act on the interplay between the stress response, brain functions and immune system, through the gut-brain axis communication.

Methods: We used a combined preclinical and clinical approach. At preclinical level we used the model of social isolation in rats during adolescence, based on the lack of all social contacts, for four weeks after weaning, followed by re-socialization until adulthood. We collected fecal samples at different post-natal days to investigate the short- and long-lasting effects of social isolation on gut microbiota composition by running the 16S metagenomics sequencing and we collected brain areas (dorsal and ventral hippocampus and prefrontal cortex) samples at killing to measure a panel of inflammatory and microglia activation markers by Real Time PCR. Both male and female animals were analyses. To evaluate the impact of stress on depression vulnerability and to identify possible peripheral biomarkers blood samples from a group of adolescents exposed to childhood trauma and also characterised for depressive symptoms have been tested for transcriptomic analyses (by running both GeneAtlas and RNAseq techniques).

Results: 16S metagenomics sequencing analysis revealed that microbial changes were influenced by age in both isolated and controls rats, regardless of sex, whereas social isolation impacted the microbial composition in a sex-dependent manner. A multivariate analysis was used to identify stress-related genera associated with social isolation condition. In brain areas we found a specific inflammatory pattern, in dorsal and ventral hippocampus, that significantly correlated with gut microbiota composition, with a more pronounced effect in male animals. In particular, SI female rats showed significant increased expression of TGFβ (+30%, $p = 0.039$) in the dorsal hippocampus and of IL1β and IL6 in the ventral hippocampus (+31% and +38%, $p < 0.044$) compared to CTRL animals (Fig. 3 and Supplementary fig. 4). SI male rats reported increased expression of MIF and TGFβ (+91% and +45%, $p < 0.015$) in the dorsal hippocampus and IL1β and IL6 in the ventral hippocampus (+53% and +41%, $p < 0.005$) compared to controls. Regarding microglia activation markers, social isolated male rats showed a significant increased expression of IBA1, CX3CL1, CX3CR1, CD40 and CD68 compared with controls in dorsal hippocampus (+57%, +72%, +61%, +61%, +56%, $p < 0.014$) as well as increased expression of CD40 (+ 21%, $p < 0.049$) in the ventral hippocampus. In females, SI-related genera were associated with inflammation primarily in the ventral hippocampus. In particular, the association involved IL1beta, IL6 and all but CD68 for the microglial activation biomarkers ($|rho| > 0.64$, $p < 0.096$). In males, the association of SI-related genes with inflammation were comparable in both dorsal and ventral hippocampus and mainly involved the genera Christensenellaceae R-7 group ($|rho| > 0.68$, $p < 0.090$).

Transcriptomic analyses in blood samples of adolescents (males and females) revealed alterations in several pathways related to the immune system, with an effect that was driven by male and not female adolescents.

Conclusions: Overall, we reported a novel sex-specific association between gut microbiota composition and inflammatory response related to social isolation paradigm during adolescence. A more pro-inflammatory status was also observed in a group of adolescents that were exposed to childhood trauma and that developed depression, with an effect that, similarly to the animal model, was more pronounced in males. This overall suggests that stressful experiences early in life could have a long-lasting impact on the development of the immune system, of the gut microbiome and of their interplay, that could in turn influence the vulnerability to develop mental disorders later in life.

Keywords: Early Life Stress, Adolescence, Depression, Inflammation, Gut Microbiome

Disclosure: Nothing to disclose.

P317. Sex Influences the Effects of Social Status on Socioemotional Behavior and Serotonin Neurochemistry in Rhesus Monkeys

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Background: Sex differences in the prevalence rates of many psychiatric disorders highlight the importance of including sex as a biological variable in animal studies focused on understanding mechanisms underlying psychopathology. One translational animal model that has been leveraged to understand the impact of chronic psychosocial stress exposure on behavioral health is social subordination in rhesus macaques. Hierarchical dominance relationships maintained by agonistic interactions govern social interactions in rhesus monkeys, similar to many other mammalian species. Importantly, social dominance is associated with resistance to the physiological consequences of social stress while social subordination is associated with vulnerability to stress-related, adverse phenotypes. While serotonergic pathways play a critical role in the etiology and treatment of stress-related psychiatric conditions, the contribution of biological sex in these processes remain unclear. Thus, the current study was designed to assess sex differences in the effects of social status on socioemotional behavior and serotonin neurochemistry in socially housed rhesus monkeys. We hypothesized that sex and social status interact to influence socioemotional behaviors as well as 5HT1A receptor binding potential (5HT1A-BP) in regions of interest (ROIs) implicated in the regulation of aggressive, affiliative, and anxiety-like behaviors.

Methods: Gonadally intact adult female ($n = 14$) and male ($n = 13$) rhesus monkeys housed in small social groups of three to six monkeys each were subjects in the current study (6 dominant and 8 subordinate females; 6 dominant and 7 subordinate males). Behavioral observations were collected for each group using a standard monkey ethogram over five weeks to capture rates of aggression, submission, affiliation, and anxiety-like behavior. A positron emission tomography (PET) scan for 5HT1A receptor binding potential was conducted using 4-(2′-Methoxyphenyl)-1-[2′-(N-2′′-pyridinyl)-p[18F]fluorobenzamido]ethylpiperazine (MPFF). Structural MR images were obtained within three weeks of the PET scan for co-registration to assist in region placement and evaluation of white matter volumes. ROIs included sub-regions of the prefrontal cortex (PFC), the hypothalamus, the dentate gyrus of the hippocampus, and the dorsal raphe nucleus (DRN). The Emory University Institutional Animal Care and Use Committee approved all procedures in accordance with the Animal Welfare Act and the U.S. Department of Health and Human Services "Guide for Care and Use of Laboratory Animals." ANOVAs were used to determine the effects of sex (males vs. female), social status (dominant vs. subordinate), and their interaction on socioemotional behaviors and 5HT1A-BP. Post-hoc analyses were conducted when necessary and significance was set at $p \leq 0.05$ for all tests.

Results: Submission was significantly greater in subordinate versus dominant animals ($F = 11.637$, $p = 0.003$), regardless of sex ($F = 1.006$, $p = 0.327$). Aggression was not impacted by sex, status, or their interaction (p's > 0.05). Rates of affiliation were impacted by an interaction of sex and status ($F = 4.243$, $p = 0.05$). Dominant females engaged in more affiliation than did dominant males ($F = 10.134$, $p = 0.004$). Anxiety-like behavior was significantly greater in females than in males ($F = 6.909$, $p = 0.015$), regardless of social status ($F = 0.122$, $p = 0.122$). Structural MRIs showed that the ROI volumes were significantly larger in males than in females (p's < 0.05), except for the DRN ($p > 0.05$). Because of these sex differences, further analyses assessing impacts of sex, status, and their interaction on 5HT1A-BP were adjusted for age and structural volume of ROI. 5HT1A-BP in the straight gyrus was significantly greater in females than in males ($F = 4.937$, $p = 0.037$) regardless of social status ($F = 0.381$, $p = 0.544$). Hypothalamic 5HT1A-BP was also significantly greater in females than in males ($F = 26.989$, $p < 0.001$), regardless of social status ($F = 1.590$, $p = 0.221$). 5HT1A-BP in the dentate gyrus of the hippocampus was significantly impacted by a sex by status interaction ($F = 5.862$, $p = 0.025$). 5HT1A-BP in the dentate gyrus was greater in dominant compared to subordinate females ($p = 0.027$) but was not different between dominant and subordinate males ($p = 0.569$). There were no effects of sex, status, or their interaction on 5HT1A-BP in the DRN (p's > 0.05).

Conclusions: Taken together, the current data show that sex impacts the effects of social status on socioemotional behavior and 5HT1A-BP in ROIs implicated in the regulation of aggressive, affiliative, and anxiety-like behaviors in rhesus monkeys. These data have important implications for the treatment of stress-related behavioral health outcomes, as they suggest that sex and social status are important factors to consider in the context of serotonergic drug efficacy.

Keywords: Sex Differences, Social Status, Serotonin 1A Receptors, Behaviors

Disclosure: Nothing to disclose.

P318. Using Machine-Learning on Proteomics and Lipidomics Data to Improve Individual Prediction of Chronicity in Major Depressive Disorder

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Background: Depressive disorder is a heterogenous condition that differs widely in both therapy response and prognosis. The ability to individually predict disease course early on is essential for optimal treatment planning. Here, we use a data-driven machine learning approach to test the potential of combining two sets of -omics data in addition to easy-to-acquire clinical baseline variables for the prediction of two-year chronicity in major depressive disorder.

Methods: Proteomics (243 analytes) and lipidomics (231 variables) assays were performed on whole blood serum at baseline in patients with baseline depression ($n = 611$ for proteomics, $n = 790$ for metabolomics, $n = 608$ for combined data) from the NESDA cohort (1). Two classes of outcome (two-year chronicity or remission) were balanced (49% vs 51%) and showed no significant differences in possible confounding factors

(age, gender, bmi, years of education, antidepressant use at baseline, months of antidepressant use between baseline and follow-up). XGboost implementation in R was used with an 80-20 train-test split. An inner 10 times repeated 10-fold cross validation loop was used to optimize hyperparameters, mitigate overfitting issues and select for the best generalizable model (1000 hyperparameter-grid combinations were used) in the train set. Final models using a) clinical, psychological, and demographical data only (10 variables), b) added proteomics data and c) added lipidomics data were tested on an outheld test set and evaluated by their respective AUROC. Variable importance analysis was performed using SHAP (2).

Results: Using non-biological data only, our model was able to predict two-year chronicity (AUROC = 0.63, accuracy = 63%.) Addition of proteomics yielded substantially increased predictive performance (AUROC = 0.75, accuracy = 67%). Using lipidomics in combination with the non-biological data resulted in low predictive value (AUROC = 0.57, accuracy = 53%). Adding proteomics data to the lipidomics and non-biological data slightly increased model performance, but still showed poor performance (AUROC = 0.59, accuracy = 58%). The proteomics-only trained model showed comparable performance metrics to the proteomics and clinical variables trained model (AUROC = 0.73, balanced accuracy = 70%). Variable importance analysis indicated that combining clinical with protein data resulted in a different ranking of the most predictive proteomic analytes compared to the proteomics-only model. For the model trained on both proteomic and non-biological data, symptom severity was the most important predictor, followed by proteomic analytes involved in hemostasis and the immune system. Repeated analysis on proteomics data only with a penalized logistic regression model (elastic net) showed lower performance and limited overlap of most important variables, indicating the importance of detection of non-linear patterns in the included biological data.

Conclusions: Proteomics but not lipidomics data were able to augment the performance of clinical variables regarding chronicity of Major Depressive Disorder 2 years later. Even though the model that used a combination of proteomics and clinical variables showed good performance (AUROC = 0.75), improvement in accuracy and feasibility of data-acquirement are needed to warrant clinical implementation.

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Keywords: Biomarker Prediction, Machine Learning, Proteomics

Disclosure: Nothing to disclose.

P319. Effect of Lumateperone (ITI-007) on Quality of Life and Functional Disability in the Treatment of Bipolar Depression

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Background: People with bipolar disorder have a more impaired quality of life and functional disability compared with both the general population and those with other mood or anxiety disorders. Residual symptoms of depression account for a greater degree of reduced quality of life than symptoms associated with hypomania or mania in patients living with bipolar disorder. Lumateperone (lumateperone tosylate, ITI-007), a mechanistically novel antipsychotic that simultaneously modulates serotonin, dopamine, and glutamate neurotransmission, is FDA-approved for the treatment of schizophrenia and is currently being investigated for the treatment of bipolar depression

In a recent phase 3 clinical trial (Study 404, NCT03249376) in people with bipolar I or bipolar II disorder experiencing a major depressive episode (MDE, bipolar depression), lumateperone 42 mg monotherapy significantly improved symptoms of depression compared with placebo. This analysis of Study 404 investigated improvements in functional disability and quality of life as measured using the prespecified secondary outcome measure the Quality of Life Enjoyment and Satisfaction Questionnaire-Short Form (Q-LES-Q-SF). In addition, a post hoc analyses looked at the individual items of the Q-LES-Q-SF.

Methods: Patients in this study (18–75 years) had a confirmed diagnosis of bipolar I or bipolar II disorder based on Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) criteria. Participants had to be experiencing an MDE (Montgomery-Åsberg Depression Rating Scale [MADRS] Total score ≥ 20 and Clinical Global Impression Scale-Bipolar Version-Severity [CGI-BP-S] score ≥ 4). Patients were randomized to receive lumateperone 42 mg or placebo orally, once daily in the evening for 6 weeks. The primary endpoint was the change from baseline to Day 43 in MADRS Total score, analyzed using a mixed-effects model for repeated measures (MMRM) approach in the intent-to-treat (ITT) population.

The Q-LES-Q-SF, a measure of quality of life and aspects of function, evaluates patient satisfaction with quality of life using a 5-point rating scale (from 1 [very poor] to 5 [very good]). This post hoc analysis evaluated the mean change from baseline to Day 43 in the Q-LES-Q-SF individual item scores using an analysis of covariance with last observation carried forward (ANCOVA-LOCF) in the ITT population. An ANCOVA-LOCF was also used to analyze the psychosocial factor composed of items 2 (mood), 4 (household activities), 5 (social relationships), 6 (family relationships), 7 (leisure time activities), 8 (ability to function in daily life), 9 (sexual drive, interest and/or performance), 10 (economic status), 11 (living/housing situation), and 14 (overall sense of well-being) and the physical factor composed of items 1 (physical health), 12 (get around physically without dizziness), and 13 (vision in ability to do work).

Results: The ITT population comprised 376 patients (lumateperone 42 mg, 188; placebo, 188). Mean baseline MADRS Total score and CGI-BP-S depression subscore of 30.6 and 4.6, respectively, indicate the ITT population had moderate-to-severe depression symptoms at baseline. Patients in the lumateperone 42-mg group had significantly greater mean improvement on MADRS Total score change from baseline to Day 43 compared with placebo (least squares mean difference vs placebo [LSMD], −4.585; 95% confidence interval [CI], −6.344 to −2.826; effect size vs placebo [ES], −0.56; $P < .0001$). Lumateperone 42-mg treatment significantly improved Q-LES-Q-SF Total score from baseline to Day 43 compared with placebo (LSMD, 2.9; 95% CI, 1.15 to 4.59; $P = .001$). There were significant improvements at Day 43 with lumateperone vs placebo for the Q-LES-Q-SF psychosocial factor (ES, +0.38; $P = .0005$); differences vs placebo did not reach statistical significance in the physical factor (ES, +0.20; $P = .0627$).

The Q-LES-Q-SF items with the lowest mean scores at baseline (very poor to poor ratings) suggested that the domains that were the most impaired in patients with bipolar depression were mood (lumateperone, 1.8; placebo, 2.0), leisure time activities (lumateperone, 2.0; placebo, 2.2), and sexual drive, interest, and/or performance (lumateperone, 2.0; placebo, 1.9). By Day 43, lumateperone treatment significantly improved 8 of the 14 items in the Q-LES-Q-SF: mood ($P = .0012$); household activities ($P = .0049$); social relationships ($P = .0075$); family relationships (P

= .0034); leisure time activities ($P = .0020$); ability to function in daily life ($P = .0022$); sexual drive, interest, and/or performance ($P = .0468$); overall sense of well-being ($P = .0283$). Overall life satisfaction also significantly improved with lumateperone treatment ($P = .0016$).

The largest improvements with lumateperone 42 mg compared with placebo (ES > + 0.3,) were seen in items representing the ability to function in daily life (+0.34), family relationships (+0.32), household activities (+0.31), leisure time activities (+0.34), mood (+0.35), and overall life satisfaction (+0.35; all LSMD, 0.3; all $P < .01$).

Conclusions: In patients with bipolar I or bipolar II disorder experiencing an MDE, treatment with lumateperone 42 mg compared with placebo significantly improved patient quality of life and functional impairment. These results support lumateperone 42 mg as treatment of MDEs associated with bipolar I or bipolar II disorder.

Keywords: Bipolar Depression, Lumateperone, Functional Disability

Disclosure: Intra-Cellular Therapies, Inc.: Employee, Stock/Equity (Self)

P320. Impaired Coping in Rats Exposed to Chronic Mild Stress: Modulatory Effect of Chronic Lurasidone Treatment

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Background: Exposure to stress represents a major risk factor for the onset of psychiatric disorders. Accordingly, exposure of adult rats to chronic stress represents a valuable experimental tool to characterize the mechanisms that may contribute to the vulnerability, or resilience, to stress exposure. One key trait in psychiatric patients is represented by disturbances in emotional control. While emotional control is a human feature, exposure to acute challenges in rodents can be used to investigate key pathways and systems that may be relevant in emotional control. Here, we used the chronic mild stress (CMS) paradigm to identify changes in the responsiveness to an acute stress and we investigated the potential impact of a chronic treatment with the antipsychotic drug lurasidone in ameliorating the functional changes produced by CMS exposure.

Methods: Adult male rats were initially exposed to CMS for 2-weeks and sucrose intake was measured as a proxy for anhedonia. Animals that showed reduced intake (vulnerable) were randomized to receive vehicle or the antipsychotic drug lurasidone (3.0 mg/kg, per os) for 5 more weeks while continuing CMS exposure. A sub-group of animals was also used to investigate the responsiveness to an acute challenge, as a proxy of emotional control, by exposing them to a 30 min-immobilization stress, whereas control rats were left undisturbed in their home cages. Blood and brain tissues were collected for the molecular analyses. Statistical significance was ascertained by two-way ANOVA followed by Tukey's and Sidak's multiple comparison test, when appropriate. Significance was set at $p < 0.05$

Results: CMS exposure caused a significant reduction in sucrose intake, which was normalized by lurasidone treatment ($p < 0.001$), with an effect that develops progressively during the treatment. CMS exposure produced significant changes in molecular systems associate with emotionality, including neuroplasticity-related genes, GABAergic function, and redox mechanisms, with a beneficial effect exerted by chronic lurasidone. Next, we investigated the modulation of brain-derived neurotrophic factor (BDNF), a trophic molecule linked to the pathophysiology of depression, as a proxy of functional plasticity in response to an acute challenge. We found that exposure of control animals to an acute stress up-regulates Bdnf expression in the prefrontal cortex, as a protective mechanism to cope with the challenging event. Such modulation is impaired in rats exposed to CMS, but it is restored by sub-chronic lurasidone administration. This data prompted us to map BDNF transcriptional changes specifically in parvalbumin (PV) and calcium calmodulin kinase II (CAMKII) positive neurons, the major inhibitory and excitatory neuronal populations of the PFC. For control animals, we localized acute stress-induced increase of BDNF transcripts in PV cells ($p < 0.05$), an effect not observed in CMS rats. However, we observed a significant increase of BDNF expression in pyramidal cells of CMS rats treated with lurasidone ($p < 0.05$), but not in PV cells.

Conclusions: In summary, we provide new insights on the mechanism of action of lurasidone in the context of stress-related dysfunction, suggesting that the antipsychotic drug may improve specific psychopathological domains. Moreover, our results suggest that pharmacological intervention produces adaptive changes leading to alterations of brain circuits that correct the functional activity and responsiveness of key brain regions involved in stress response and that may ultimately promote resilience.

Keywords: Chronic Unpredictable Mild Stress, Neuroplasticity, Stress Resilience, BDNF

Disclosure: Angelini, Otsuka, Lundbeck, Recordati: Honoraria (Self) Sunovion: Grant (Self)
Sumitomo Dainippon Pharma: Consultant, Grant (Self)

P321. Cerebellar Transcranial-Magnetic Stimulation as a Potential Treatment in Bipolar Disorder

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Background: Bipolar Disorder (BD) symptoms include impairment in the coordination of cognitive processes and emotions. Recent work has implicated cerebellar (ceb) abnormalities in the pathophysiology of BD, including dysfunctional interactions between the ceb and the medial frontal cortex (MFC). As the ceb-MFC interactions are important for cognitive and mood regulation, the present work sought to boost ceb-PFC connectivity through cerebellar transcranial-magnetic stimulation (ceb-TMS). Additionally, ceb-TMS has recently been used to improve cognitive and mood symptoms in patients with schizophrenia. As schizophrenia and BD have phenomenological overlap, including the target symptoms of cognitive deficits and depressed mood, there is reason to be optimistic that patients with BD will benefit from the same treatment protocol.

Methods: In the present double blinded clinical trial, the safety and efficacy of using ceb-TMS in BD was examined. Both men and women suffering from BD ($n = 30$) were pseudorandomly divided into active and sham treatment groups with optional crossover at the end of the trial. Neuro-navigated TMS of the cerebellar vermis or sham treatment was provided 2x/day for 5 days (total of 10 treatments). A theta-burst stimulation protocol was utilized, consisting of 50 Hz triplets repeated at 5 Hz delivered for 2 s and followed by an 8 s pause. Neurobiological mechanisms underlying cognitive symptoms in BD were investigated via electroencephalography (EEG - Aim 1). Effects of TMS on cognitive symptoms were evaluated via clinical scales and cognitive tasks including the supra-second interval timing task (Aim 2).

Results: Contrary to our hypothesis, we found that participants who received active ceb-TMS showed reduced trial-wide low frequency activity (delta and theta bands) compared to those who

received sham treatment. Reduction in low-frequency oscillations were specific to interval-timing task performance, as this effect was not observable during rest. Event-locked spectral analyses indicate that while participants who received sham treatment had an increase in cue-elicited low frequency power, this increase was prevented by the active ceb-TMS treatment. Despite oscillatory differences between sham and active groups, no significant differences in interval timing and cognitive/mood scales were detected.

Conclusions: The present work suggests that ceb-TMS is a safe and well-tolerated treatment for individuals with BD. EEG analyses indicate that ceb-TMS leads to a reduction in MFC low-frequency activity. However, effects on mood/cognition were not detected.

Keywords: TMS EEG, Cerebellum, Bipolar Disorder

Disclosure: Nothing to disclose.

P322. A Multi-Omics Approach to Understanding ATP Utilization Deficits in Major Depression

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Background: Bioenergetic dysregulation is an established facet of severe mental illnesses like major depression (MDD). ATP is the body's primary energy carrier and is required to maintain most cellular processes. Dysregulation of ATP generation via glycolysis and mitochondrial respiration is implicated in MDD but much less is known about how ATP utilization is altered and its effect on downstream biological processes in disease.

Methods: To assess the expression and activity of the complement of proteins that utilize ATP (the ATPome) in the brain in MDD, we carried out bioinformatic and biochemical analysis. For preliminary biochemical analysis, ATP-binding enzymes were enriched in postmortem dorsolateral prefrontal cortex (DLPFC) from age, sex and PMI matched MDD and non-psychiatrically ill subjects ($n = 3$/group). Using a desthiobiotin-ATP probe, protein samples were enriched for ATP-binding enzymes and were analyzed by mass spectrometry (5600 TripleTOF, ABSciex). The activity of a subset of ATPome enzymes, serine/threonine kinases, were analyzed using the array-based Pamgene12 (Pamgene Int) in male and female dorsolateral prefrontal cortex (DLPFC) from age, sex and PMI matched MDD subjects and non-psychiatrically ill controls (pool $n = 3$-10 subjects/group). Z-score +/- 2 following permutation analysis by Kinase Random Sampling Analyzer (KRSA) indicates that a kinase was mapped to significantly altered peptides (log fold change +/-0.4) more or less frequently than expected by chance. For bioinformatic analysis, a targeted "ATPase" gene list was generated and filtered from Gene Ontology (GO) Annotation using "ATPase" and "Kinase" as search terms. Significant differentially expressed genes from this list were extracted from publicly available male and female postmortem MDD transcriptomic datasets. Pathway enrichment (GO, KEGG, Reactome) and pathway clustering by parent-child analysis were conducted.

Results: Biochemical enrichment of ATP-binding proteins resulted in the positive identification of ATPase enzymes, protein kinases and known ATPase-interacting proteins in enriched samples compared to negative (no probe) control for each subject. Approximately 200 ATPome proteins, including Na + /K + ATPase subunits, mitogen activated protein kinases (MAPK) and protein kinase C (PKC) were differentially expressed (FC expression > ±1.2) in the DLPFC in MDD compared to control. Analysis of serine/threonine kinase activity identified a significant increase ($p < 0.05$) in global signal intensity in MDD. Altered MAPK family activity was also identified by kinome analysis. MAPKs were predominantly found in female MDD subjects, conversely, a greater number of dark kinases were identified in male subjects. Commonly identified kinases include AKT and PKC, suggesting disease and sex-specific changes in expression and activity of protein kinases in the DLPFC in MDD. Bioinformatic analysis identified approximately 300 significantly differentially expressed "ATPase" genes ($p < 0.05$) in each MDD dataset. Clustering of GO pathways (FDR < 0.1; density index minimum 0.5) found significant enrichment of metabolic and signal transduction pathways in MDD. Stress-response pathway clusters were enriched in male subjects, suggesting sex-specific differences in ATP utilization pathways in MDD.

Conclusions: Bioinformatic and biochemical analysis of post-mortem datasets and tissue confirm transcript, protein and activity-level perturbations of ATP utilization pathways in MDD. Preliminary proteomic analyses found significant ATPase and kinase enzyme expression changes in the DLPFC in MDD. These findings were complemented by kinome analysis, which found kinases with altered activity in male and female MDD subjects compared to controls. Pathway analysis integrating data from multiple MDD transcriptomic datasets highlighted both common and unique enriched biological pathways that are perturbed in males and females in MDD. Integrating omics and bioinformatic data provides insight into the mechanisms underlying ATP utilization deficits and its contribution to the pathophysiology of MDD.

Keywords: Proteomics, Major Depression Disorder, Bioenergetics, Kinomics

Disclosure: Nothing to disclose.

P323. Changes in Functional Connectivity Following Intermittent Theta Burst Stimulation (iTBS) are Proportional to the Electric-Field at the Cortex

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Background: Noninvasive neuromodulation is increasingly being applied to effectively treat mental illness. Transcranial magnetic stimulation (TMS) is currently approved by the FDA for the treatment of depression, obsessive compulsive disorder, and smoking cessation. The FDA-approved stimulation target for depression is the left dlPFC, and stimulation at this site is thought to alleviate symptoms by modulating functional connectivity with the stimulation site. Efforts to quantify the changes in connectivity following neuromodulatory TMS have largely focused on specific downstream targets of the stimulation site, potentially ignoring larger scale shifts in whole brain connectivity. Furthermore, this literature lacks a comprehensive model aimed at predicting large scale shifts in functional connectivity following neuromodulatory TMS. One simple approach to building such a model might be to assume that changes in connectivity are proportional to the energy delivered to the cortex, as operationalized by electric- (e)-field modelling. Accordingly, the purpose of this work is to predict changes in connectivity following TMS by modelling the electric field induced at the site of stimulation in a sample of depressed individuals recruited for a connectivity-based rTMS brief intervention.

Methods: Resting state functional magnetic resonance imaging (rs-fMRI) was collected from 16 depressed patients before and after they received 3 days of neuronavigated intermittent theta burst stimulation (iTBS) aimed at the left dlPFC delivered in 2 rounds of 1200 pulses each (2400 stimulations per session). The left dlPFC targets were chosen a priori based on connectivity between the left dlPFC and the subgenual anterior cingulate

cortex (sgACC). NxN correlation matrices were constructed from the cleaned, parcellated (Gordon atlas) resting state timeseries. E-field models were parcellated using the Gordon atlas, vectorized, thresholded, and converted to NxN matrices reflecting the mean e-field norm for each pair of regions in the matrix. These e-field matrices were then used to predict pre-post changes in functional connectivity. For the primary analysis, e-field models were conducted at the site of stimulation and at a control region (contralateral motor cortex). For the secondary analysis, e-field models were conducted at 24 evenly spaced orientations for sites along a either an anterior-to-posterior (dlPFC) or inferior-to-superior (motor cortex) vector centered on the stimulation/control site from the primary analysis.

Results: For the primary analysis, we found that the e-field model based on the stimulation site predicted ~1.01% of the variability (v) in the pre-post functional connectivity changes, which was significantly greater than the variability predicted by the control site ($v = 0.27\%$; $t(15) = 2.4115$; $p = 0.029$). For the secondary analysis, site x orientation heatmaps were created for both the stimulation and control site. For the heatmap centered on the stimulation site, there was a clear relationship between both site and orientation, and v-scores. As distance from the stimulation site increased, v-scores decreased. Similarly, as eccentricity from the optimal orientation increased, v-scores decreased. This pattern was not apparent in the heatmap centered on the control site. Permutation tests were conducted across site/orientation pairs in the heatmap with a cluster centered on the stimulation site/optimal orientation showing the strongest difference.

Conclusions: These results suggest that functional connectivity following iTBS changes systematically as a function of the energy reaching the cortex, and that this relationship can be described using e-field modelling. More importantly, this finding lays the groundwork for future studies that individualize TMS targeting based on how predicted functional connectivity changes might impact symptom expression. In a recent paper in NPP, we put forth a model aimed at doing just that. This model makes two assumptions. First, changes in network connectivity should be proportional to the TMS dose at the cortex, which is supported by the current work. Second, symptom change following TMS should be proportional to the association between network changes and symptoms. Although our NPP paper uses this model to make confirmatory predictions regarding left dlPFC stimulation and depression, prospective treatment using this targeting approach is necessary to support the second assumption of the model. However, if supported, this model could provide a domain-general whole-brain TMS targeting individualization approach applicable across mental disorders.

Keywords: TMS, Resting State Functional Connectivity, Electric Field Modeling

Disclosure: Nothing to disclose.

P324. Dissociable Effects of Cognitive Effort and Cognitive Flexibility on Anhedonia and Apathy

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Background: Anhedonia and apathy are transdiagnostic features of neuropsychiatric illness, predicting worse functional outcomes and diminished antidepressant response. Decreased willingness to apply cognitive effort may play a key role in these debilitating symptoms and interfere with current treatments such as cognitive-behavioral therapy. Cognitive flexibility, the ability to fluidly switch between mental states, goals or tasks, is also impaired in disorders with high levels of apathy and anhedonia, and decreased flexibility is associated with an increased risk for suicide. However, how cognitive flexibility relates to cognitive effort, and how these decision-making constructs jointly relate to critical depressive symptoms remains unknown. One possibility is that changes in flexibility are mediated partially or fully by changes in the subjective value of cognitive effort. Alternatively, cognitive flexibility and effort may contribute independently to the decreased motivation and reward sensitivity in anhedonia and apathy. Establishing a quantitative link between cognitive effort and flexibility and anhedonia and apathy would support the development of novel biomarkers and interventions for Major Depression and other neuropsychiatric disorders. Here, we make use of new developments in computational psychiatry to make quantitative, model-based measurements of cognitive effort and flexibility and connect them to core depressive symptoms.

Methods: 210 online participants recruited through Prolific (64 female, 146 male) completed a task measuring cognitive effort valuation, the Cognitive Effort Discounting Task (COGED) and a task measuring cognitive flexibility in an explore/exploit framework, a three-arm restless bandit task, along with inventories of neuropsychiatric symptoms including the PHQ-9, Snaith Hamilton Pleasure Scale (SHPS) and the Apathy Motivation Index (AMI). For the COGED data we computed the total area under the discounting curve (AUC) for each participant, a summary measure of the subjective value of cognitive effort. For the three-arm bandit behavior, we fit both a Hidden Markov Model (HMM) of exploration/exploitation and a novel foraging model of exploration/exploitation decisions to measure aspects of cognitive flexibility. While the HMM labels each trial as corresponding to either a state of exploration or exploitation, the foraging model decomposes behavior into a learning parameter, a decision threshold and an inverse temperature parameter that captures the amount of randomness in decisions, one measure of flexibility. We related the SHPS, AMI and PHQ-9 scores to these model-based task behavioral measures with generalized linear models.

Results: Participants consistently discounted increased levels of cognitive effort in the COGED task and performed better than chance on the bandit task. The median PHQ-9 score was 10, implying that approximately 50% of the sample displayed symptoms of moderate or severe depression. The cognitive effort AUC was weakly correlated with the bandit HMM probability of exploration (Spearman's $rho = 0.14$, $p = 0.055$) but not with the inverse temperature parameter from the explore/exploit foraging model ($rho = 0.05$, $p = 0.51$). In separate linear regression, cognitive effort AUC predicted the SHPS score ($beta = -0.21$, $p = 0.02$) and the AMI social motivation sub-scale ($beta = -0.1$, $p = 0.02$); the inverse temperature parameter of our bandit foraging model predicted the SHPS score significantly ($beta = -0.5$, $p = 0.014$) and at a trend level the AMI emotional sensitivity ($beta = -0.2$, $p = 0.1$) and the PHQ9 ($beta = -0.15$, $p = 0.06$). The HMM probability of exploration and the other foraging parameters did not predict these symptom scores. In multiple linear regression, the cognitive effort AUC and inverse temperature both predicted SHPS score ($p = 0.006$ and 0.005, respectively) and the interaction was significant ($p = 0.04$); cognitive effort and inverse temperature also predicted the PHQ9 score ($beta = -1$, $p = 0.02$ and $beta = 100.5$, $p = 0.002$, respectively, interaction $p = 0.01$). Multiple regression results for AMI sub-scales were not significant. These results were confirmed with multiple ordinal logistical regression with quantile binned symptom scores.

Conclusions: We found that computational model-based measures of cognitive effort in the COGED task and cognitive flexibility in an exploration/exploitation bandit paradigm predict levels of anhedonia, apathy, and overall depressive symptom burden. Our results do not suggest a mediating role for cognitive flexibility in the relationship between effort and symptoms. Rather cognitive effort and flexibility appear to independently contribute

specifically to anhedonia and interact more in explaining more severe symptoms and overall depression level. Measuring both cognitive effort and flexibility may thus be important to fully understanding individual variability in core symptoms of impaired motivation and reward sensitivity. These results support ongoing work in our group on the neural circuit correlates of effort and flexibility that may give rise to novel biomarkers and treatment targets for anhedonia and apathy.

Keywords: Anhedonia, Cognitive Flexibility, Effort Discounting, Computational Psychiatry, Depression

Disclosure: Nothing to disclose.

P325. Neural Response to Peer Feedback Moderates Effects of Social Stress on Depression Symptoms Among Adolescents

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Background: Peer interactions become increasingly salient during adolescence, a time for increasing onset of internalizing disorders. Social mechanisms are a critical component of internalizing disorders risk – potentially blunted response to peer acceptance in depression and increased response to rejection in anxiety. Alterations in the neural underpinnings of social responsiveness are likely most salient in the context of acute social stress. In line with diathesis-stress models, we hypothesized that potentially deleterious effects of increased neural sensitivity to peer rejection would only manifest when individual are faced with peer rejection in daily life. The integration of neural and prospective stress measures to understand psychiatric risk has been lacking in the neuroscience literature.

Methods: Towards filling this gap, data were examined from $N = 90$ psychiatrically healthy adolescents (12-14 years old), either with ($n = 26$) or without ($n = 64$) a maternal history of depression. Teens completed the Chatroom Task during fMRI and provided self-report on stress experience (Adolescent Life Events Checklist) and depression symptoms (Mood and Feelings Questionnaire) up to nine times over the subsequent two years. Voxel-wise linear mixed effects models were fit to test our hypothesis that neural sensitivity to peer feedback would moderate stress effects on depression symptoms.

Results: While peer-related social stress was a strong predictor of depression symptoms longitudinally ($\beta = 0.34$, $t(626) = 10.02$, $p < .001$, $\eta p2 = 0.14$), this interacted with brain response to peer acceptance vs. rejection in scanner in 70 regions (cluster corrected for multiple comparisons, voxel-wise $p < .001$). This included the bilateral anterior insula extending in the right posterior insula, bilateral caudate, dorsal anterior cingulate cortex, right putamen, and left amygdala. Parsing the interaction with activity in the left anterior insula ($\beta = 0.12$, $t(623) = 5.366$, $p < .001$, $\eta p2 = 0.13$), we see a strong positive association between peer stress experience and depression symptoms among those with greater reject versus accept activity but this was not present in those with exhibiting greater accept versus reject activity.

Conclusions: These data highlight the combined influence of neural diatheses and social stress in understanding normal variability in subclinical depression symptoms during adolescence. This provides a critical framework for examining brain activity as a moderator (rather than an outcome variable) in whole-brain analyses to examine different models of the neuroscience of mental health. Currently, we find that differential neural sensitivity to peer feedback in key social processing regions is a risk/resilience factor to the experience of typically occurring social stress.

Keywords: fMRI, Social Rejection, Adolescent Depression, Psychosocial Stress

Disclosure: Nothing to disclose.

P326. Impact of Transcutaneous Vagus Nerve Stimulation on the Central Autonomic Network and Anxiety in Response to Stress in Major Depression

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Background: Alterations of cardiac autonomic function in response to negative stressful stimuli have been implicated in the comorbidity of major depression (MDD) and cardiovascular disease (CVD). Further, there is often considerable concomitant anxiety symptomatology in MDD which contributes to greater affectation of cardiovagal regulation and increased cardiovascular risk. It has been proposed that alterations of brain regions involved in stress response and emotion regulation are responsible for the cardiovagal dysfunction observed in MDD, with women presenting greater affectation compared to men. Given the significant impact of the comorbidity between MDD and CVD, it is necessary to develop novel treatments at the neural-cardiac interface. Recently, transcutaneous auricular vagus nerve stimulation (taVNS) has emerged as a safe, non-invasive alternative to implanted VNS, with promising beneficial effects on regulation of depressive symptoms and cardiac autonomic function. Neuroimaging studies suggest that taVNS effects are mediated by modulation of the nucleus tractus solitarii (NTS) from which projections synapse with regions involved in cardiac autonomic regulation. Further, as NTS is highly influenced by respiration (receiving facilitatory inputs during exhalation), an optimized respiratory-gated approach (RAVANS) has been proposed by our team. The present study evaluated the effects of RAVANS on modulation of anxiety symptoms and brain circuitry involved in stress response and autonomic regulation in women with active recurrent MDD. MDD women were studied initially given they have twice the risk than men.

Methods: Twenty premenopausal women (age: 30.3 ± 4.7 years) with recurrent MDD in an active episode were included in an experimental cross-over study that included two functional MRI visits within one week. Subjects were randomized to receive 30 minutes of exhalatory-gated (e-RAVANS) or inhalatory-gated (i-RAVANS) stimulation during the first or second scanning session. Surface MR-compatible electrodes were placed in the left cymba concha of the auricle and stimulation consisted of monophasic rectangular pulses (300 usec pulse width, 30 Hz frequency, 0.8s duration) with current intensity set to achieve moderate (but not painful) sensation. Before and after the stimulation period, subjects underwent three runs (360s each) of a mild visual stress challenge task in the scanner comprising presentation of blocks of negative valence/high arousal, neutral valence/low arousal and fixation images adapted from the International Affective Picture System (IAPS). Whole-brain fMRI data were collected on a 3T scanner with a 32-channel head coil and a simultaneous multislice sequence (TR = 1250ms, TE = 33 ms, flip angle = 65°, FOV = 200 x 200 mm2, 75 axial slices, voxel size = 2 mm3). A piezoelectric transducer was used to collect heart rate data during the task and an adaptive point-process algorithm was applied to compute variations in instantaneous estimates of the high frequency component of heart rate variability (HF-HRV, 0.15 to 0.4 Hz). The State-Trait Anxiety Inventory (STAI)

was collected at baseline and after the experimental sessions to evaluate changes in anxiety symptoms.

fMRI data were preprocessed using the CONN toolbox and included realignment, slice timing, motion correction, spatial smoothing and normalization and artifact detection. Comparisons of interest in the fMRI analysis (negative vs neutral, post-stimulation vs pre-stimulation) were tested using linear contrasts and results were submitted to a second level multiple regression analysis with changes in HF-HRV power as a covariate. We restricted the analysis to specific regions of interest (ROIs) from the central autonomic network (e.g., hypothalamus, amygdala, hippocampus, cingulate and prefrontal cortex) using a small volume correction approach. Changes in HF-HRV were included in regression analyses to investigate the link with changes in anxiety symptoms (STAI score difference: Post - Pre stimulation).

Results: e-RAVANS was associated with increased cardiovagal modulation in response to the visual stress task when compared to i-RAVANS (HF-HRV % change = 46.3 ± 33.8% vs 22.1 ± 30.7%, $p = 0.03$). Changes in HF power in response to e-RAVANS were related with increased activation of right dorsolateral prefrontal cortex (314 voxels, $t = 4.98$, $p < 0.001$) and anterior cingulate cortex (1672 voxels, $t = 2.72$, $p < 0.01$) and reduced activation of left amygdala (172 voxels, $t = 3.60$, $p < 0.001$). e-RAVANS also resulted in a significant reduction of anxiety symptoms compared to baseline (STAI = 47.8 ± 7.49 vs 55.2 ± 6.29, $p < 0.01$), which was significantly associated with increased cardiovagal modulation (HF-HRV change) ($\beta = -1.93$, t (16) $= -2.73$, $p = 0.017$, Adj $R2 = 0.27$).

Conclusions: Our results indicate that exhalatory-gated stimulation of the auricular vagus nerve effectively modulates brain circuitry involved the regulation of physiological response to stress in major depression. Further, our study revealed significant effects on anxiety levels in association with increased cardiovagal modulation. These results provide initial evidence supporting a novel neuromodulation approach with beneficial effects in acute relief of anxiety symptomatology and regulation of cardiovascular function that may be effective in the management of patients with comorbid MDD and CVD.

Keywords: Transcutaneous Auricular Vagus Nerve Stimulation (taVNS), Major Depressive Disorder, Central Autonomic Network, Functional MRI (fMRI), Heart Rate Variability

Disclosure: Nothing to disclose.

P327. Estradiol Mediates Stress-Susceptibility in the Male Brain

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Background: Depression is the leading cause of disability worldwide afflicting ~16% of the population. Although, women are diagnosed with depression twice as often as men, there are approximately 109 million men worldwide suffering from the disorder. In addition, men suffering from depression are at higher risk to lose their life from suicide, one of the most common symptoms of depression, with suicide rates being four times higher in men than women. In susceptible populations, stress is a major risk factor for the development of mental disorders, including depression. While the role of estrogen receptors in the pathophysiology of depression and as treatment targets in females are widely elucidated, their role in males is not well understood.

Methods: We used a subthreshold social defeat stress model which consists of three cycles of two-min physical stress and fifteen-min of sensory stress, in combination with immunohistochemistry, neuronal tracing, RNAscope, in vitro electrophysiology, in vivo optogenetics, in vivo fiber photometry and hormonal manipulations to investigate the role of estradiol and its receptors in the development of social avoidance and anhedonia in male mice ($n = 10$-15). Statistical analysis was performed with ANOVAs followed by Holm-Sidak post-hoc analysis. If criteria for parametric analysis were not met, Kruskal-Wallis test was performed followed by two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli.

Results: We found that absence of estrogen receptor-β (ERβ) is associated with stress susceptibility in male mice following exposure to a mild stressor. Activity of a subpopulation of ERβ-expressing neurons projecting to the nucleus accumbens (NAc) is reduced in male mice lacking gonadal hormones subjected to mild stress, while activation of this circuit reverses stress-induced maladaptive behaviors and induces stress resilience. Estradiol (E2), often considered a female specific hormone, is distributed in the male brain via aromatization of testosterone. We identified that absence of E2, but not testosterone per se, underlies susceptibility to develop maladaptive behaviors following exposure to mild stress in males. Using brain-selective delivery of E2 through administration of a prodrug, which offers a viable treatment option in males, we demonstrated that E2 prevents the development of depression-related behaviors following acute/mild stress in hypogonadal male mice.

Conclusions: Overall, our findings provide evidence for an estrogen-based mechanism underlying stress susceptibility and suggest a novel therapeutic strategy for treating depression in males.

Keywords: Depression, Estradiol, Estrogen Receptor, Circuits

Disclosure: Nothing to disclose.

P328. Application of a Bayesian Adaptive Randomization Design to Optimize Intravenous Ketamine Dosing for Late-Life Treatment-Resistant Depression

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Background: Evidence of the antidepressant efficacy of glutamate modulating drugs, especially the non-competitive N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine (and its S-enantiomer), has grown rapidly in recent years. Especially important is their rapid onset of action compared to other antidepressive agents, and their apparent effectiveness in patients not responding to established treatments. The antidepressant effects of a single intravenous (IV) dose of ketamine (0.5 mg/kg) typically begin within 24 hours of infusion, and persist for up to two weeks. The biological mechanisms of this prolonged impact are attributed to sustained patterns of increased excitation/inhibition (E/I) balance which outlast the short elimination half-life of ketamine. However, the clinical and biological effects of IV ketamine for late-life treatment-resistant depression (LL-TRD) have not been investigated, acting as a barrier to safe treatment of a growing clinical concern in geriatric medicine.

The aim of our investigation was to study the sustained clinical and neurophysiological effects of several doses of IV ketamine (KET) relative to midazolam (MID) in patients with LL-TRD. The simultaneous study of clinical and physiological data was

designed to clarify the dose-related effects of ketamine on high-level changes in synaptic transmission, and how these changes might be related to the clinical outcomes at 7 days post-infusion.

Methods: Thirty-three veteran patients (55-72 years, mean age: 62.9 ± 5.86, 30.3% female), were enrolled in a randomized, double-blind, placebo-controlled design, and randomized to either a single infusion of IV ketamine (0.1, 0.25, or 0.5 mg/kg) or midazolam (0.03 mg/kg) condition. Randomization was achieved using Bayesian adaptive randomization (BAR) with an initial allocation ratio of 1:1:1:1. The allocation ratio was updated as patients completed the study to prioritize the comparison of the most clinically effective conditions. Patients completed evaluations on six visits to assess clinical effectiveness and durability of the infusion intervention (baseline and 1, 2, 3, 7, and 28 days post infusion).

We used Bayesian adaptations of generalized linear models to study the effects of time, drug condition, and time*drug interaction on clinical and physiological outcomes (EEG gamma oscillations). Our primary endpoint was the change in Montgomery-Åsberg Depression Rating Scale (MADRS) score between baseline and 7 days post-infusion. Durability was evaluated by estimating the probability of a clinical response to a single infusion at the 28 day visit for patients who demonstrated a clinical response to infusion at day 7. Gamma power was evaluated at 3 different time scales (-1 to +4 hours relative to infusion; 24 hours post-infusion; 24 hours to 7 days post-infusion). All analyses controlled for data values at the baseline time point. Kendall's tau correlations were performed to draw inferences on the relationship between gamma power and change in MADRS.

Results: Infusions in all conditions were safe and well tolerated. The BAR stopping rules terminated randomization to 0.1 and 0.25 mg/kg ketamine conditions at $N = 4$ and $N = 5$, respectively, due to inferior clinical performance. The inferior conditions were explored in the analysis of our primary endpoint (MADRS change) but were omitted from the physiological analyses due to low power.

Sixteen patients (48.5%) achieved a clinical response at 7 days (KET 0.5 = 8, KET 0.25 = 2, MID = 6). Ketamine 0.5 mg/kg demonstrated a strong absolute probability (ap) of achieving a response at 7 days (ap = 0.7, 95% credible interval (95%-CrI) = 0.43 – 0.90), and was consistent with a higher response at 7 days than MID (Posterior Probability (pp) [KET 0.5 > MID] = 0.89). Probability of a clinical response was lower for ketamine 0.25 (ap = 0.42, 95%-CrI = 0.11 – 0.78) and 0.1 mg/kg (ap = 0.13, 95%-CrI = 0.01 – 0.53). Response durability at 28 days among responders at 7 days was greatest for KET 0.5 ($N = 7$, ap = 0.82, 95%-CrI = 0.52 – 0.97), and reduced proportionally with lower doses; KET 0.25 ($N = 1$, ap = 0.5, 95%-CrI = 0.09 – 0.90), MID ($N = 2$, ap = 0.37, 95%-CrI = 0.10 – 0.71).

Our data showed evidence of a significant time*condition interaction amounting to divergent effects of drug on gamma power over the course of metabolism (pp [KET 0.5 > MID] = 0.92). Gamma power rapidly increased during the infusion period for KET 0.5 and then gradually returned to baseline levels approximately 4 hours post-infusion (pp = 0.92), while the MID infusion was associated with a continuous upwards trend in gamma power over the duration of the recording (pp = 0.91). At 24 hours, there was low evidence of differences in gamma power between the conditions (pp [KET 0.5 > MID] = 0.37). Time* group changes in gamma power between 24 hours and 7 days post-infusion could not be determined by the model. Peak gamma power during infusion was associated with greater change in MADRS score at 7 days for KET 0.5 ($\tau = -0.5$, $p = .08$), but not for MID ($\tau = 0.05$, $p = 0.84$).

Conclusions: Our results suggest that IV ketamine at 0.5 mg/kg is clinically effective in LL-TRD. Our EEG findings provide preliminary evidence that the reactivity of gamma power might reflect susceptibility to enhanced clinical effects; precision medicine optimization of ketamine therapy will require further study.

Keywords: Ketamine, Treatment-Resistant Late Life Depression, Neuropsychiatry, Clinical Trial

Disclosure: Nothing to disclose.

P329. CCR2 Monocytes Repair Cerebrovascular Damage Caused by Chronic Social Defeat Stress in Mice

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Background: Mental disorders such as major depressive disorder (MDD) have a complex etiology. Genetic, biological, and environmental factors contribute to their manifestation. The great disease burden of MDD inspires attention to factors that might be studied to achieve basic understanding and potential therapeutic treatments. A well-studied contributing environmental factor is psychosocial stress. In animals, psychosocial stress can be studied using a paradigm called chronic social defeat (CSD). CSD in mice elicits depressive-like and anxiety-like behaviors, and it also produces elevated activity of microglia, oxidative stress responses in microglia and neurons, and scattered cerebral microbleeds with associated local blood-brain barrier (BBB) breakdown. Such physical events lie at the core of many brain disorders featuring cerebrovascular injury, such as stroke or traumatic brain injury. The innate peripheral immune system plays an important role in response to and resolution of such injuries. We therefore investigated the participation of innate immune cells—monocytes—during CSD and after cessation of CSD.

Methods: We measured activated CCR2hi monocyte trafficking to the vascular injury sites in Ccr2wt/rfp reporter mice both during two weeks of CSD (pairing of subject C57BL/6 mouse with a dominant CD-1 mouse in divided cage housing; barrier lifted for 5 min each day) and one week following CSD cessation (CSD recovery). Monocytes were tracked by immunohistochemistry/confocal microscopy and by flow cytometry of whole brain preparations. Fibrinogen (an inflammatory blood product) was both tracked and administered i.c.v. in some experiments. Clodronate liposomes were administered i.v. to deplete monocytes. Behavior was assayed in the urine scent marking (USM) task that reveals stress-induced asocial behavior.

Results: We replicated our earlier finding that CSD induces rare microhemorrhages distributed stochastically but somewhat more prevalently in cerebral cortex. The microbleeds were microscopically identified by the presence of i.v.-injected tracers, plasma immunoglobulin and fibrinogen, and erythrocytes present in perivascular spaces and adjacent parenchyma. Nevertheless, CSD did not recruit monocyte infiltration at these sites or anywhere else—the distribution and density of adhered CCR2-rfp+ monocytes was the same in home-cage control and CSD brains. However, after recovery from CSD, when fibrinogen is being cleared, many newly adhered CCR2 + cells were detected in perivascular spaces, and brain endothelial cells showed elevated gene expression of the CCR2 chemokine receptor ligands CCL7 and CCL12, but not CCL2. Flow cytometry showed that adhered CCR2 + cells were mostly the non-classical, anti-inflammatory Ly6Clo type, and confocal microscopy showed they phagocytosed fibrinogen. In Ccr2rfp/rfp mice lacking CCR2, fibrinogen was not cleared in CSD recovery. Fibrinogen infused intracerebroventricularly in unstressed mice induced CCR2 + cells to adhere to the vasculature in Ccr2wt/rfp but not Ccr2rfp/rfp mice. Depletion of monocytes with i.p.-injected clodronate liposomes during CSD

recovery prevented fibrinogen clearance and blocked behavioral recovery in the USM task.

Conclusions: We hypothesize that peripheral CCR2 + monocytes play no role during chronic psychosocial stress because they lack key chemokine signals that might permit an inflammatory response to stress-induced microbleeds. However, during the stress recovery period, chemokine signaling permits anti-inflammatory monocytes to lodge in perivascular spaces, phagocytose blood products, and support vascular repair, promoting normalization of behavior post stress. These findings suggest that therapeutic administration of anti-inflammatory drugs during acute periods of stress recovery may hinder repair of vascular micro-damage and be counterproductive in the healing process.

Keywords: Neuroimmune, Brain Microvascular Endothelial Cells, Psychosocial Stress

Disclosure: Nothing to disclose.

P330. Ex Vivo Pharmacological Characterization of Selective M5 Acetylcholine Muscarinic Receptor Modulators on Dopamine Release in the Mouse Striatum

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Background: The type-5 muscarinic acetylcholine receptor (M5) is almost exclusively expressed in midbrain dopaminergic neurons, and thus is of particular interest for precise modulation of the mesolimbic pathway that is central to multiple neuropsychiatric diseases. Decreasing abnormally high dopamine transmission in the striatum has been a core approach in treating psychotic and manic symptoms of schizophrenia and bipolar disorders, while enhancing dopamine tone would be instrumental for the treatment of anhedonia, found in major depressive disorder. Indeed, M5 antagonists are promising candidates for treatment of drug misuse disorder based on their activity in preclinical models of drug abuse with cocaine, opioids, and alcohol. Although cloned over twenty years ago, and in contrast to the other four muscarinic receptor subtypes, functional pharmacological studies undertaken against a native tissue response exclusively mediated by M5 receptor activation are lacking. Here we characterize the capacity of M5-selective allosteric modulators to directly modulate dopamine release in the striatum, at the axon terminal level of the mesolimbic pathway in nucleus accumbens/caudate putamen slices.

Methods: The effects of ML375 (selective M5 negative allosteric modulator) and VU 0365114 (selective M5 positive allosteric modulator) on striatal dopamine release were evaluated using ex vivo fast-scan cyclic voltammetry (FSCV) in brain slices from wild-type and M5 knockout mouse. The significance of compound effect on dopamine release was tested using either a paired t-test or a linear mixed effects model for repeated measures.

Results: ML375 alone (10 μM) significantly decreased electrically-evoked dopamine release in the dorsal striatum of mouse brain slices ($n = 7$, $p < 0.0001$). Preliminary recordings show that in the presence of the acetylcholine nicotinic receptor blocker dihydro-β-erythroidine (DHβE, 1 μM), pre-incubation of the slice with ML375 (20min, 10 μM) completely abolished the increase in dopamine release induced by Oxotremorine-M (10 μM), a non-selective acetylcholine muscarinic receptor agonist ($n = 5$).

VU 0365114 alone (10 μM) significantly increased electrically-evoked dopamine release in the dorsal striatum of mouse brain slices ($n = 6$, $p = 0.0016$). Preliminary experiments show that in presence of DHβE (1 μM), pre-incubation of the slice with VU-0365114 (20min, 10 μM) augmented and prolonged the increase in dopamine release induced by oxotremorine-M (10 μM, $n = 3$).

Lastly, preliminary recordings in M5 knockout mice showed that oxotremorine-M did not significantly increase dopamine release in the dorsal striatum ($n = 2$).

Conclusions: In this study we established a functional pharmacological response exclusively mediated by M5 receptor activation in native brain tissue slices. Using FSCV in native tissue, selective M5 allosteric modulators, ML375 and VU0365114, both were capable of regulating dopamine signaling as measured by their impact on (1) electrically triggered dopamine release and (2) oxotremorine-M induced increase of dopamine release in the dorsal striatum. The effect of oxotremorine-M on dopamine release (at the axon terminal level) was absent in knockout mice assessing the specific effect on M5. Taken together these results highlight the important role of M5 in regulating dopamine release in the striatum. Muscarinic M5 receptors are an ideally located target capable of bidirectionally shaping dopamine signals in the mesolimbic pathways and thus, might be promising for the treatment of mood and other neuropsychiatric disorders.

Keywords: M5 Muscarinic Receptor, Dopamine, Allosteric Modulator, Striatum

Disclosure: Janssen Research and Development: Employee (Self)

P331. Distinct VIP Interneurons in the Cingulate Cortex Encode Anxiogenic and Social Stimuli

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Background: A hallmark of higher-order cortical regions is their functional heterogeneity, but it is not well understood how these areas encode such diverse information. The anterior cingulate cortex (ACC), for example, is important in both emotional regulation and social cognition. Previous work shows activation of the ACC to anxiety-related and social stimuli, but it is unknown how subpopulations or microcircuits within the ACC simultaneously encode these distinct stimuli. One type of inhibitory interneuron, which is positive for vasoactive intestinal peptide (VIP), is known to alter the activity of many cells in local cortical microcircuits, but it is unknown whether the activity of VIP cells in the ACC (VIPACC) encodes anxiety-related or social information.

Methods: Experimental mice were male postnatal day 60-120 VIP-Cre mice ($N = 6$, Vip-IRES-cre, #010908). We used miniscopes to perform in vivo single-cell resolution calcium imaging of VIP interneurons in the ACC (VIPACC) to investigate their functional heterogeneity while mice performed tasks to assay anxiety-like behaviors, general sociability and social novelty. Behavior was analyzed using DeepLabCut, an open-source program that uses machine learning to track body parts in behavioral videos. Ca2+ imaging data were processed using CaImAn written in Python (https://www.python.org/). Responses of individual cells during different behavioral conditions were assessed within each behavioral trial using receiver operating characteristic (ROC) analysis. To determine whether VIPACC receive projections from other brain areas involved in anxiety and social behavior, we used rabies virus-mediated trans-synaptic mapping.

Results: Using in vivo calcium imaging and miniscopes in freely behaving mice to monitor VIPACC activity, we identified distinct, non-overlapping subpopulations of VIPACC that preferentially activated to either anxiogenic, anxiolytic, social, or non-social stimuli. We determined that stimulus-selective cells encode the

animal's behavioral states and VIP interneuron clusters may co-activate, improving this encoding. Finally, we used trans-synaptic tracing to show that VIPACC receive widespread inputs from regions implicated in emotional regulation and social cognition.

Conclusions: These findings demonstrate not only that the ACC is not homogeneous in its function, but also that there is marked functional heterogeneity even within disinhibitory interneuron populations. This work contributes to our understanding of how the cortex encodes information across diverse contexts and provides insight into the complexity of neural processes involved in anxiety and social behavior.

Keywords: VIP Neurons, Social Behavior, Anxiety Circuitry, Anterior Cingulate Cortex (ACC), Network Activity

Disclosure: Nothing to disclose.

P332. Subjective Response to Alcohol in Young Adults: Interactions With Bipolar Disorder and Associated Anterior-Paralimbic Structure and Recent Alcohol Use

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Background: Alcohol use disorders are more prevalent in bipolar disorder than the general population. Mechanisms that contribute to elevated risk for alcohol use disorders in bipolar disorder are not known. Structural differences in anterior-paralimbic networks may predate and increase risk for problematic drinking in bipolar disorder. Moreover, recent evidence suggests medial orbitofrontal cortex (mOFC) structural differences may relate to variation in subjective response to alcohol in young adults with bipolar disorder. To extend these findings, we investigated subjective response to alcohol in young adults with bipolar disorder, compared to healthy young adults, and relations with mOFC structure and recent alcohol use.

Methods: To date, 22 young adults (13 with bipolar disorder type 1; mean age + standard deviation = 23 + 2 years, 50% women) have completed assessments of mood symptoms, recent alcohol use, and a structural MRI scan. Participants then completed placebo-controlled alcohol administration sessions in which they were dosed to a .08g% breath alcohol concentration and also completed a placebo drinking session (sessions counterbalanced). During beverage sessions, participants completed the Subjective Effects of Alcohol Scale (SEAS) at baseline (before beverage consumption) and at peak breath alcohol concentration of .08g%. The SEAS was filled out at a comparable time following beverage consumption during the placebo session. Changes in subjective response to alcohol were calculated for alcohol and placebo sessions. Group, session (alcohol, placebo), and group by session interactions were modeled, with beverage session as a repeated within subject variable, and SEAS subscale scores as the dependent variable. Age, sex, baseline heart rate, depression, and anxiety symptoms were covaried. Significance was defined as $p < 0.05$. Relations between subjective response to alcohol with mOFC structure and recent alcohol use in bipolar disorder were explored.

Results: Young adults with bipolar disorder, compared to healthy young adults, reported greater stimulation during the alcohol than placebo session (within bipolar disorder $p = .004$; group by condition interaction $p = .03$). Both groups reported an increase in sedation during alcohol administration compared with placebo (main effect of beverage session $p < .0001$). Greater stimulation reported during alcohol administration was associated with greater mOFC gray matter volume (pFWE(SVC) = 0.02) and lower frequency and quantity of recent alcohol use in bipolar disorder (p's < .05).

Conclusions: Preliminary results from this ongoing study (registered at ClinicalTrials.Gov; NCT04063384) suggest greater stimulating effects of alcohol in young adults with bipolar disorder, compared to healthy young adults. Variation in development of anterior-paralimbic systems early in illness course may contribute to differences in subjective response to alcohol and greater alcohol use. Longitudinal study examining how neural progression relates to subjective response to alcohol and development of alcohol use disorders in bipolar disorder is the next step in this work.

Keywords: Bipolar Disorder, Alcohol Sensitivity, Young Adults, Medial Orbitofrontal Cortex, Alcohol Consumption

Disclosure: Nothing to disclose.

P333. Qualitative Analysis of Culture and Depression Treatment Among US Latinx Adults

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Background: Despite consistent evidence of antidepressant effectiveness for depression management, early discontinuation is common in the US. Early discontinuation of antidepressants is associated with relapse, return of symptoms, and poor health outcomes. Latinx adults are at greater risk of early discontinuation than other groups; 54% of Latinx adults discontinue antidepressants within the first 30 days compared with 41% ($P < 0.04$) of non-Latinx white adults. Improving antidepressant use among Latinx adults could reduce mental health disparities. Previous qualitative studies have assessed Latinx adults' perceptions, barriers, and knowledge of depression treatment. However, the themes identified by researchers such as stigma, familism, and discrimination could be difficult to extrapolate to clinical practice. The National Institute of Mental Health's Strategic Plan requires that intervention studies identify targets or mechanisms that account for changes in clinical outcomes. The overall goal of this study is to identify potential targets or mechanisms for a future intervention to address early discontinuation of antidepressants among Latinx adults with depression.

Methods: We conducted guided focus groups of Latinx adults with depression recruited from 2 primary care clinics. Participants were asked open-ended questions about depression and depression treatment in the context of their Latinx culture such as: How did you decide you had depression? How did you seek and get treatment for depression? How does being Latino/a affect these decisions? and how does being Latino/a affect how someone will take medications for depression? Focus group meetings instead of phone interviews were selected because group dynamics often facilitate identification of cultural phenomenon. Meetings were conducted until we identified saturation among codes and themes. Participant responses were digitally recorded and transcribed verbatim in the original language (English or Spanish) using an online automated transcription service (trint.com) then edited by bilingual researchers for accuracy.

Qualitative data thematic text analysis was conducted using transcripts from focus group meetings. A team of researchers independently and iteratively analyzed and coded the data. We developed a preliminary codebook using a conceptual framework (adapted from Murray et al, 2004) to guide coding into major themes and subthemes. However, based on our meta-synthesis findings (completely separately), the codes and themes were revised to self-efficacy, resilience, and social determinants of health and their respective definitions. This study received an exempt determination by the University of Michigan Institutional Review Board (IRB; HUM00163233).

Results: We conducted 9 focus group meetings (median $n = 3$ per group) of 24 Latinx adults with depression. Four groups met from January to March 12, 2020, and five met from August to September

2020. Most groups ($n = 6$) met in person and 3 groups were conducted over Zoom. Focus group meetings were on average 1.5 hours long (range: 45 minutes to 2 hours). Most participants were female (83%; $n = 20$), of Mexican descent (58%; $n = 15$), born outside the US (46%; $n = 11$), preferred to speak Spanish (63%; $n = 15$), and were an average 41 years old. Key themes identified were:

Self-efficacy through verbal/social persuasion: encourage or discourage ambivalence towards depression treatment

- "My mother-in-law was like, 'Yeah, I've been on Wellbutrin and that's [for] my anxiety and my depression. Maybe you should give it a try.'"
- "If they think if you're on treatment, it kind of makes you like the weirdo, the one that they kind of whisper about. So it's kind of like, just don't take it then, you don't want to be talked about. You don't want to be looked at as…crazy."

Resilience through altruism: coping mechanism to overcome depression symptoms

- "Especially being a mother, you're supposed to be the caregiver, the provider. You don't have time to be sad or angry. You gotta take care of the child. You gotta cook, you gotta clean. You got to be happy for everybody else."

Mental health literacy: disagreement between facts and beliefs with a deference to negative beliefs over supportive facts about depression treatment

- "If you rely so much on the medicine, it also becomes an addiction. Then it is like another disease that you have to try to cure."

Antidepressant and treatment access: lack of understanding of the US health care system

- "Well, for me it was also a little difficult because I did not know the language, but thanks to the fact that when my son was born, he was born with a little difficulty and a social worker took care of me…She gave me addresses, telephone numbers and well, thank God I have already started on [treatment]."

Conclusions: Findings were analyzed using the constructs of self-efficacy, resilience, mental health literacy, and treatment access. Unlike previous qualitative analyses of similar populations, all constructs are measurable, can be compared, and could be changed. The proposed constructs could be further explored as possible targets or mechanisms of antidepressant use in this population. We will use these findings to develop a culturally tailored intervention with the goal of reducing early discontinuation of antidepressants among Latinx adults with depression.

Keywords: Qualitative Research, Hispanic/Latinos, Depression, Antidepressants, Culture

Disclosure: Nothing to disclose.

P334. Impact of SER 109, an Investigational Microbiome Therapeutic on Health-Related Quality of Life in Patients With Recurrent Clostridioides Difficile Infection: Results From ECOSPOR III, a Placebo-Controlled Clinical Trial

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Background: Clostridioides difficile infection (CDI) has a negative impact on health-related quality of life (HRQoL), particularly in patients with recurrent disease. In a randomized-controlled trial of 182 patients with recurrent disease (ECOSPOR-III, NCT03183128), SER-109, an investigational microbiome therapeutic, was demonstrated to be superior to placebo in reducing the risk of recurrent CDI (rCDI) (12.4% vs 39.8%, respectively; $p < 0.001$). The Cdiff32, a disease-specific HRQoL measure, was administered to assess the impact of SER-109 treatment on physical, social, and mental health domains, including anxiety as an exploratory outcome.

Methods: The Cdiff32 was administered in the ECOSPOR-III study at baseline, week 8, early termination, or recurrence of CDI. The Cdiff32 includes 32 questions with five levels of response measuring 3three domains (physical, social and mental). Twelve questions are specific to the mental health domain which includes mental (M), mental anxiety future (MAF), and mental anxiety current (MAC) subdomains. Domain items are scored from 0 (worst score) to 100 (best score) and aggregated by domain and globally. In the intent-to-treat population of ECOSPOR III, comparisons were made between SER-109 and placebo and by clinical outcome (ie, recurrence vs non-recurrence). The between treatment group comparison analysis controlled for age, sex, prior antibiotic use, and number of prior CDI episodes.

Results: Patient demographics and mean Cdiff32 scores were comparable between SER-109 and placebo at baseline, except the SER-109 arm had more females (67.4%). Patients achieved significant improvement in all Cdiff32 domains/subdomains at week 8 regardless of treatment group. When examining recurrence status within treatment arms, placebo patients with non-recurrence had significantly better scores on the mental health subdomains (M $p < 0.001$, MAF $p < 0.001$, and MAF $p < 0.05$) compared to those with recurrence. Notably, SER-109 patients with non-recurrence and recurrence showed improvement in all domains compared to baseline. Specifically, mental subdomains scores improved significantly from baseline in SER-109 subjects with recurrence (M $p < 0.05$, MAF $P < 0.05$, MAC $P < 0.05$). Therefore, patients in the SER-109 arm saw significant improvements in the mental health subdomains regardless of outcome whereas placebo only saw improvement in patients with non-recurrence.

Conclusions: In a Phase 3 trial in patients with rCDI, treatment with SER 109, an investigational microbiome therapeutic, led to significant improvement in Cdiff32 HRQoL scores. In contrast to placebo, SER-109 was associated with improved mental scores, regardless of clinical outcome. Future studies should evaluate whether SER-109 may provide mood and anxiety enhancing properties through modulation of the gut-brain axis.

Keywords: Microbiome, Microbiota-Gut-Brain Axis, Microbiota, Gut-Brain Axis, Fecal Microbiota Transplant

Disclosure: Tempus Labs, Seres Therapeutics: Employee (Self)

P335. Waist Circumference and its Association With Premenstrual Food Craving: The Premenstrual Hormonal and Affective State Evaluation (PHASE) Longitudinal Study

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Background: The premenstruum is marked by an increase in food craving in a significant number of reproductive age women, who are particularly vulnerable to weight gain. Functional magnetic resonance tomography studies show higher brain responses to food stimuli in the luteal vs. follicular phase, particularly in the corticolimbic areas involved in homeostasis and reward. The DSM-5 lists food craving as one of the symptoms (out of 11 total) for premenstrual dysphoric disorder (PMDD) and premenstrual syndrome (PMS) diagnoses. No study to date has established an association between either the PMS/PMDD diagnosis, or a particular premenstrual symptomatology, and central adiposity.

The proposed study evaluated whether food craving experienced during the premenstrual period is associated with waist circumference. Based on the literature showing that women's food craving increases premenstrually, and that there is an association between food craving and waist circumference in women in general, we hypothesized that there would be a positive association between premenstrual food craving and abdominal obesity.

Methods: Forty-six healthy women (mean BMI = 24.36), free of Axis I mental disorders, who were not taking medications, using drugs, drinking heavily, or smoking, prospectively provided daily ratings of food cravings across two-three menstrual cycles (122 cycles total). Their premenstrual rating of food craving was contrasted against food craving in the follicular phase to derive a corrected summary score (continuous variable) of the premenstrual food craving increase. Study groups were divided into normal ($n = 26$) and obese ($n = 20$) based on the 80 cm waist circumference cutoff, signifying an increase in a metabolic abnormality risk.

Results: The participants' diagnoses were: PMS ($n = 19$), PMDD ($n = 7$), and healthy ($n = 20$). Waist circumference category was significantly associated with premenstrual food cravings ($F(1,44) = 5.12$, $p = 0.028$). Post hoc comparisons using the Tukey HSD test (95% family-wise confidence level) showed that the mean score for the premenstrual food craving effect size was 0.35 higher for the abdominally obese vs. normal study groups (95% CI: 0.039 to 0.67). The result was statistically significant even following an inclusion of BMI in the model.

Conclusions: In accordance with our hypothesis, the present study shows a positive association between premenstrual food craving and waist circumference. This association remained significant even after adjusting for BMI, which points to a particularly dangerous process of central fat accumulation. This is the first study to link food craving in the premenstruum with central adiposity. A potential relationship between premenstrual amino acid levels, reward processing, food craving, eating patterns and central adiposity should be investigated in future studies, with the overarching goal of integrating the premenstruum as a viable intervention target for this at-risk sex and age group.

Keywords: Premenstrual Dysphoric Disorder, Food Craving, Premenstrual Syndrome, Waist Circumference, Abdominal Obesity

Disclosure: Nothing to disclose.

P336. Stress, Genetics and Mood: Impact of COVID-19 on a College Freshman Sample

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Background: To study the interplay between genetic risk for depression and psychosocial stress, including the COVID-19 pandemic, in the emergence of symptoms of depression and/or anxiety in college freshmen. We distinguish between genetically- and non-genetically based types of resilience and their role during major life stressors.

Methods: University of Michigan freshmen (2015-2020) were characterized at baseline along clinical variables. They were genotyped and polygenic risk score for depression (MDD-PRS) was calculated. Their daily physical activity was captured, and they were sampled at multiple time points throughout the freshman year on clinical rating scales including GAD-7 and PHQ-9 for anxiety and depression, respectively. To assess the impact of the pandemic, the 2019-2020 cohort was analyzed separately.

Results: Across various years, 25%-57% of college freshmen developed significant symptoms of anxiety or depression. In the 2019-2020 cohort, measures of anxiety, depression and physical activity were all altered significantly after the onset of COVID-19. Physical activity, which differed between those who did vs. did not meet criteria for a mood disorder, was dramatically reduced by the pandemic.

High MDD-PRS conferred higher relative risk for depression/anxiety during a typical freshman year. Surprisingly, the pandemic had the clearest impact on the Low MDD-PRS group, which lost any evidence of genetic advantage. Conversely, psychological predictors of resiliency emerged as key factors in protecting the High MDD-PRS subjects who did not develop a mood disorder post-stress.

Conclusions: Although the pandemic increased mood disorders across the board, it eliminated the protection of genetic resilience. Psychological indices of resiliency were associated with protecting those with higher genetic risk.

Keywords: Stress, Depression, Genetics

Disclosure: Nothing to disclose.

P337. Holographic Stimulation of Distinct Amygdala Ensembles Responsive to Opposing Valences Bidirectionally Modulates Consummatory Behavior

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Background: A central unanswered question in neuroscience is how can the heterogeneous and often opposing patterns of activity generated by genetically similar populations of neurons give rise to coordinated, complex behavior. Understanding whether these heterogeneous neural responses are necessary and sufficient to promote or suppress behavioral output is critical to determining how the brain produces behavior. Recent advances in both optogenetics and optical imaging approaches has made it possible to record endogenous stimulus-evoked neural activity patterns from individual neurons and holographically manipulate this naturally-defined activity pattern in select neurons. For the first time we harness this spatial light modulation (SLM) approach for reading and writing neural activity in a deep brain, subcortical structure, the basolateral amygdala (BLA). The BLA contains populations of excitatory principal neurons with diverse activity profiles that may encode the valence of salient stimuli. We posit that if these neurons encode the valence of a stimulus, select activation of discrete appetitive or aversive ensembles will bidirectionally modulate consummatory behavior.

Methods: Male ($n = 8$) and female ($n = 3$) C57BL/6 mice were injected with AAV8-Camk2a-ChRmine-mScarlett mixed 1:3 with AAVDJ-Camk2a-GCaMP6f into the BLA. A 7.3mm long, 0.6mm diameter GRIN lens was then lowered just above the viral injection. Mice were water restricted (1ml/day/mouse, 90% of original body weight) for one week prior to behavioral training. Mice were then habituated to head-fixation as well as trained to lick for 10% sucrose (in drinking water) solution for several days. Mice next underwent several two-photon (Bruker Ultima 2p+) imaging sessions during which they were exposed to random presentations of an appetitive (10% sucrose, 75% of trials) or aversive (2mM quinine, 25% of trials) tastant. Data were then processed (custom MATLAB and Python scripts) to identify BLA ensembles with preferential increases in activity to either the appetitive or aversive tastant. Subsequent sessions used holographic stimulation to directly play back this endogenous activity selectively to either appetitive (sucrose-activated) or aversive (quinine-activated) ensembles randomly on 50% of all trials. All

experiments were carried out in accordance with the NIH Guide and approval of the University of Washington IACUC.

Results: Consummatory licking was significantly greater following delivery of appetitive 10% sucrose (75% of trials) compared to 2mM aversive quinine (25% of trials; $p < 0.0001$). In the BLA separable populations of sucrose-responsive (67/450) and quinine-responsive neurons (56/450) were identified (mean activity in response to sucrose > quinine, $p < 0.05$), as well as neurons that reliably responded to both tastants (88/450). Within individual mice, sucrose and quinine cells were spatially intermixed throughout the field of view. High resolution two-photon imaging allowed for reliable tracking of individual neurons across multiple days of recording, and appetitive and aversive ensembles were found to be highly stable across days, suggesting a consistent role in the processing of these stimuli. Having identified separable populations of appetitive and aversive principal neurons in the BLA with stable response properties, we conducted parameterization sessions in which holographic stimulation was matched to endogenous tastant-evoked activity. Stimulation reliably and specifically evoked activity only in targeted neurons and not nearby neurons. Tastant-evoked activity during trials with no stimulation was not significantly different from trials with stimulation ($p > 0.05$), suggesting that our stimulation intensity recapitulates natural physiological responses. During sessions in which quinine-activated ensembles were specifically targeted on 50% of trials ($\mu = 6.4$ cells/mouse), a significant reduction in licking was observed on sucrose trials with stimulation compared to without stimulation ($t(4) = 9.43, p = 0.0025$). No change in licking was observed when stimulation occurred during quinine trials ($p > 0.05$). By contrast, when sucrose-activated ensembles were selectively stimulated on 50% of trials ($\mu = 7.75$ cells/mouse), no change in licking behavior was observed when stimulation occurred during sucrose trials ($p > 0.05$). Critically, on trials in which quinine was delivered, stimulation of sucrose ensembles significantly increased licking compared to trials without sucrose ensemble stimulation ($t(5) = 6.27, p = 0.003$). In mice only expressing GCaMP6f (no ChRmine expression) targeting sucrose or quinine ensembles for stimulation had no effect on neuronal activity or on licking behavior ($p > 0.05$).

Conclusions: Gaining a mechanistic insight into how the brain encodes valence is necessary for understanding neuropsychiatric disorders where hedonic tone is often disrupted. The BLA has been identified as an important brain region for the regulation of emotion and integration of valence specific information across mammalian species and primates and is often implicated in a variety of psychiatric diseases. Here we demonstrate that separable populations of genetically similar BLA principal neurons encode the hedonic value of rewarding and aversive stimuli. This sets the stage for future investigation of how these stable opposing ensembles are affected during state-manipulations such as stress and drug exposure.

Keywords: Amygdala, Two-Photon, Optogenetics, Valence

Disclosure: Nothing to disclose.

P338. Cyclocreatine: A Bioenergetic Molecule Which Improves Depression-Like and Anxiety-Like Behaviors in an Animal Model of Treatment-Resistant Depression at Altitude

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Background: Rates of major depressive disorder (MDD) and anxiety increase significantly with chronic hypoxia exposure at altitude (hypobaric hypoxia) or with chronic hypoxic disorders (COPD, asthma, cardiovascular disease or smoking). In a novel translational animal model, we find that depression-like and anxiety-like behaviors increase in rats housed at moderate altitude (4,500ft, 10,000ft) vs. at sea level, with sex-based differences. When housed at altitude, both sexes fail to respond to most selective serotonin reuptake inhibitors (SSRIs), the most widely prescribed of antidepressants. Living at altitude causes brain bioenergetic deficits in preclinical and clinical studies, and MDD is linked to brain hypometabolism. We therefore tested the potential of energetic compounds for the ability to correct altitude-related brain energy deficits and for antidepressant function at altitude. Creatine, an ergonomic compound widely used by athletes for decades, improved brain energetics in both sexes, but was only antidepressant in female rats at altitude. Creatine access to the brain is limited by the need of a specific transporter to cross the blood brain barrier, and the sex differences observed may be due to restricted access. Cyclocreatine (CyCR) is a synthetic lipophilic analog of creatine, which crosses the blood brain barrier without a transporter and can replace creatine in brain energetic pathways. CyCR exhibits higher brain bioavailability and greater stability vs. creatine. In this study, we tested CyCR as a therapeutic for depression-like and anxiety-like behaviors at altitude.

Methods: Rats housed at 4,500ft were given dietary CyCR in powdered food (3wks, 1%w/w), or un-supplemented food (controls). Rats were then tested for depressive symptoms in the forced swim and the sucrose preference tests. Another set of animals was assayed in a battery of tests including open field, marble burying, light/dark box and elevated T-maze. Brain regions linked to MDD (prefrontal cortex, striatum, hippocampus, brain-stem) were harvested after behavioral testing, and assayed for CyCR and serotonin. Forced swim test data were analyzed by one-way ANOVA of CyCR, desipramine (positive control) and food controls, while other studies were analyzed by Student's t-test for CyCR vs. food controls.

Results: A. Tissue Analyses: (1) CyCR: Dietary CyCR-treated animals exhibit serum CyCR levels of 64 ± 13mg/ml (females) or 120 ± 25mg/ml (males), while regional brain CyCR ranged from 515-765mg/g in females and 675-866mg/g in males ($n = 7$-15). Both serum and brain CyCR levels were negligible in food controls (0.03-1mg/ml, $n = 8$). (2) Serotonin: CyCR corrected the serotonin deficit in the female prefrontal cortex at altitude (Student's t-Test, $p = 0.02$), but reduced striatal serotonin in males ($p = 0.04$, $n = 11$-18). B. Depression-like Behavior: (1) Forced Swim: CyCR showed antidepressant efficacy in both sexes, reducing immobility and increasing latency to immobility (LTI) by >20% vs. controls. In females, one-way ANOVA showed a significant effect of treatment on swimming ($F2,88 = 5.3$, $p = 0.007$), climbing ($F2,93 = 8$, $p = 0.0005$), immobility ($F2,93 = 13.1$, $p < 0.0001$) and LTI ($F2,89 = 15.7$, $p < 0.0001$). In males, one way ANOVA showed significant effects of treatment on climbing ($F2,60 = 16$, $p < 0.0001$), immobility ($F2,63 = 10.7$, $p < 0.0001$) and LTI ($F2,63 = 4.97$, $p = 0.009$), but not on swimming ($F2,60 = 2.20$, $p = 0.12$). CyCR significantly increased female swimming and male climbing, and increased LTI and reduced immobility in both sexes ($p < 0.05$). (2) Sucrose Preference: CyCR reduced anhedonia by increasing sucrose preference in both females (Student's t-Test, $p = 0.0009$) and males ($p = 0.02$, $n = 10$-12). (3) Open Field: In both sexes, motor behavior did not change with CyCR treatment. C. Anxiety-like Behavior: CyCR also showed anxiolytic effects ($n = 12$). (1) Marble-burying: Rats given dietary CyCR exhibit lower anxiety-like behavior by burying fewer marbles vs. food controls (females: $p = 0.047$; males: $p = 0.02$). (2) Light/Dark Box: In females, CyCR significantly increased the latency to move from the bright side to dark side of box ($p = 0.01$), while a similar trend was seen in males ($p = 0.08$) (3) Elevated T-Maze: CyCR shows a trend to reduce general anxiety-like behavior and panic-like behavior in both sexes ($p = 0.06$).

Conclusions: Cyclocreatine is a novel bioenergetic compound which shows efficacy for depression-like and anxiety-like behaviors at altitude. Rates of MDD and anxiety increase with altitude of residence or chronic hypoxic disorders. Our preclinical studies indicate that chronic hypoxia exposure at altitude may alter brain physiology to worsen depression, anxiety and antidepressant-resistance. SSRIs are the primary antidepressants and long-term anxiolytics used in the US, but housing at altitude causes SSRIs to lose efficacy. This study shows that improving brain bioenergetics may provide a crucial therapeutic approach for treatment-resistant depression and anxiety. This is the first study to investigate the impact of CyCR as a treatment for depression and anxiety.

Keywords: Hypobaric Hypoxia, Altitude, Bioenergetics, Depression and Anxiety, Animal Models

Disclosure: Nothing to disclose.

P339. Intersection of Opioid and Nociceptive Networks in the Cingulate Cortex

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Background: Pain is an unpleasant emotional experience. Recent work from our lab and others have identified stable populations of neurons activated by acutely noxious stimuli in the amygdala and in the anterior cingulate cortex (ACC) key to the affective facet of pain. The action of opioids, the current standard of care for chronic pain, in the ACC plays a role in ameliorating the perception of the aversive quality of pain through mu opioid receptors (MOR). We hypothesize that MOR-expressing and nociception-active neurons in the ACC represents an overlapping population crucial to affective nociceptive behavior. We used single nucleus RNA sequencing (snRNAseq) to identify nociception-active cell types in the ACC that also express MOR. We also localized nociception-active ACC neurons and tested the influence of ACC MOR activity during nociception to gain further insight into the ACC MOR and nociception-active network.

Methods: ACC tissue for snRNAseq was collected in neuropathic pain or control mice ($n = 5$/condition, male). All mice experienced a noxious thermal stimuli prior to tissue collection to induce expression of immediate early genes (IEG). Next, single nuclei were isolated, cDNA libraries prepared, sequence, and aligned. Single nuclei clustering was performed in Seurat_v3. Well-characterized, a priori markers of cortical cell types were used to identify the subpopulations represented by each cluster (Slc17a7 for excitatory neurons, Gad1/2 for inhibitory interneurons). 14,220 individual nuclei (7,011 control, 7,209 neuropathic) were analyzed. MOR and nociception-active populations were identified by Oprm1 and 139 different IEGs, respectively.

To localize ACC nociception-active neurons, neurons activated by a noxious stimulus were labeled with tdtomato using a TRAP2 (Targeted Recombination in Active Populations):Ai9 line ($n = 6$, male and female); ACC nociception-active neurons were quantified with ImageJ.

In a preliminary study, ACC MOR neurons were chemogenetically inhibited during evoked behavioral responses to mechanical and noxious thermal stimuli (Oprm1-Cre line; $n = 5$-7; male and female).

Results: We identified 22 unique clusters from all major cell types (neurons and glia), including 8 glutamatergic neuron (Slc17a7) and 4 GABAergic interneuron (Gad1/2) clusters. We found Slc17a7 neuron clusters were the most transcriptionally active to the noxious stimuli, notably three clusters with single genetic identifiers: Otof, Figf, Npr3. The Figf cluster was also Oprm1 +, suggesting this cell type is well-positioned to influence nociceptive affective processes. Next, we determined the location of nociception-active neurons within the ACC. TRAP2:Ai9 histology revealed that nociception-active neurons are present throughout the ACC, with prominent labeling in layer 2/3 as well as 5. Finally, we assessed the role ACC MOR neuron activity on affective nociceptive behavior. Chemogenetic inhibition of ACC MOR neurons reduced responding to acute mechanical, but not noxious thermal, stimuli in control and neuropathic pain mice, suggesting ACC MOR activity can shift less valenced behavior.

Conclusions: We provide insight into the cell type and location of nociception-active and MOR neurons in the ACC, and insight into the behavioral effect of MOR ACC activity during nociception. Future studies will use intersectional approaches to manipulate MOR/nociception-active neurons during behavior, which we hypothesize mediates the affective analgesic effects of opioid therapies. Identifying specific networks underlying the therapeutic effects of opioids can aid the development of analgesics with improved selectivity.

Keywords: Mu-Opioid Receptors, Affective Components of Pain, Anterior Cingulate Cortex, Single-cell RNA Sequencing

Disclosure: Nothing to disclose.

P340. Sex Differences in Glucocorticoid Regulation of Dopamine Circuit Function

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Background: Stress is a significant risk factor for the development, and exacerbation, of numerous psychiatric disorders, including major depressive disorder (MDD). Corticosterone (cortisol in humans; CORT) is the primary 'stress hormone' in the body. In MDD, normal circadian rhythms of high and low CORT are flattened, leading to dysregulated and chronically elevated levels. Previous studies have found that static, chronically elevated levels of CORT induce anxiety-like behavior and impair reward-guided operant behaviors, but have only reported effects on male mice. We explicitly compared male and female mice and found that the effects occur exclusively in male mice. In the studies reported here, we explore the reasons for this sex difference in CORT-induced behavioral changes, which are potentially important for understanding sex differences in the prevalence of MDD and other stress-related disorders, as well as for designing appropriate therapeutics for both sexes.

Methods: We characterized CORT-induced behavioral changes using operant paradigms and open field tests. We then went on to determine how CORT differentially influences the function of the dopamine system in males and females using molecular biology and pharmacology to measure and manipulate dopamine transporters, and using the fluorescent dopamine sensor dLight1.3b to measure dopamine signals in the dorsomedial striatum and nucleus accumbens both in vivo during behaviors and in acute brain slices to further characterize how changes in the dopamine transporter influence dopamine release and reuptake.

Results: We found that chronic CORT administration selectively reduces operant reward-seeking in male mice. The effects in males were paralleled by decreases in dopamine transporter (DAT) function in the dorsomedial striatum (DMS) and nucleus accumbens core (NAcc). Using the DAT inhibitor GBR12909 (20 mg/kg), we found that chronic CORT treatment eliminated the increase in locomotion normally observed after DAT inhibition, but only in males. These results demonstrate a striking sex difference in how chronic CORT influences the dopamine system. We are currently working to further characterize the molecular

mechanisms underlying this difference. We are also comparing the effects of chronic CORT exposure to the effects of a chronic mild unpredictable stress paradigm in males and females to better understand the different mechanisms by which stress may impair female behavior.

Conclusions: We conclude that chronically elevated CORT affects male and female mice differently, particularly with regard to DAT function. DAT function is crucial for dopamine homeostasis and transmission in vivo, thus its regulation by CORT may have profound effects on reward-guided behaviors in males and may be important for understanding the underlying causes of MDD and other stress-related disorders. However, it remains unclear how CORT affects DAT function in males, and why females are resilient to such an effect. Our future studies will address these questions and seek to translate our findings into better diagnoses and treatment options for MDD that take sex differences into account.

Keywords: Chronic Corticosterone, Corticosterone Response to Stress, Dopamine, Chronic Unpredictable Mild Stress, Sex Differences

Disclosure: Nothing to disclose.

P341. Abnormal Developmental Patterns of Neurocognition in Adolescents With Elevated Mood Symptoms

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Background: Adolescence is critical period of neurocognitive development, including striking changes in executive functioning abilities and reward sensitivity. Adolescence is also a time of increased prevalence of mood pathology, and bipolar and unipolar symptoms have been linked to abnormalities in the same neurocognitive domains undergoing development during this period. However, it is unknown whether neurocognitive developmental patterns manifest differently for adolescents with elevated symptoms. This cross-sectional study replicated developmental patterns of neurocognitive functioning using a latent variable approach, and tested whether mood symptoms moderated developmental effects.

Methods: Participants were 606 adolescents (mean age = 19.38, SD = 2.44, 63% cisgender female) across two research sites recruited for high variance in current and past mood disorders. Participants completed three reward learning and three executive functioning tasks, and reported on age, pubertal stage, and (in n = 357, 55% with clinical mood disorders) severity of mania and anhedonic symptoms. Neurocognitive functioning was estimated using a methodologically superior latent variable approach. Structural equation models tested linear and curvilinear developmental (age, puberty) patterns of executive functioning and reward sensitivity. Manic symptom severity and anhedonic symptom severity were entered as moderators of developmental patterns. Sex was considered as a biological variable of interest.

Results: Structural equation modeling revealed a quadratic relationship between puberty and reward sensitivity that was moderated by mood symptoms: in early puberty, adolescents reporting higher mania exhibited heightened reward sensitivity whereas adolescents reporting higher (especially unipolar) anhedonia showed blunted reward sensitivity. Results also showed a linear relationship between age and executive functioning that was moderated by mania, in which adolescents reporting higher mania showed poorer executive functioning at older ages.

Conclusions: Findings indicate neurocognitive development may be altered in adolescents with mood pathology and suggest directions for longitudinal studies.

Keywords: Neurocognition, Adolescence, Mania, Anhedonia

Disclosure: Nothing to disclose.

P342. Adaptive Versus Maladaptive Forms of Regret After Chronic Social Stress

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Background: Stress-related disorders such as depression are debilitating illnesses in which individuals struggle with severe emotional dysregulation. Regret describes the phenomenon in which an individual recognizes that an alternative action could have led to a better outcome. It is widely accepted that regret contributes to disease burden. Yet, little is known about the neurobiology of what might make this process maladaptive and what aspects of regret, if any, carry positive utility that is worth preserving in order to restore healthy emotional processing and adaptive coping. Animal models used for the study of depression and related disorders have made significant contributions to the field, including identifying key molecular mediators, such as CREB, which regulates the transcription of stress-sensitive genes that control responses to rewarding and stressful stimuli in a brain-region-specific manner. However, the role of CREB in decision-making is far less understood. Furthermore, animal models have been limited in their ability to capture the complexity of affective processes in decision making observed in human patients with stress-related disorders. To shed light on this translational gap, we combined the well-established chronic social defeat stress model in mice that effectively distinguishes between stress-susceptible and stress-resilient animals with molecular manipulations and novel approaches in neuroeconomics that have only recently demonstrated the ability to extract the behavioral and neurophysiological correlates of regret in rodents.

Methods: By characterizing mice exposed to chronic social defeat stress (CSDS) on a novel neuroeconomic decision-making paradigm termed "Restaurant Row" (RRow), we demonstrate fundamentally distinct forms of regret between stress-resilient (RES) versus stress-susceptible (SUS) phenotypes, and establish a region-specific role for CREB, a key transcription factor implicated in chronic stress action. We subjected C57BL/6J male mice to CSDS and identified SUS and RES mice based on a rapid social interaction screen. We then characterized SUS, RES, and non-stressed control (CON) mice longitudinally on RRow. Mice have a limited time period each day to forage for their sole source of food by navigating a maze with four uniquely flavored and contextualized feeding sites, or "restaurants." Each restaurant has a separate offer zone (OZ) and wait zone (WZ). Upon entry into the OZ, a tone sounds whose pitch indicates how long of a delay mice will have to wait in a cued countdown should they choose to enter the WZ. A key feature of the RRow task is the ability to behaviorally segregate aspects of information processing that are directly linked to maximizing reward apart from other motivators. On this task, choice history can carry added weight capable of influencing subsequent decisions but only in certain economic situations defined by the subjective value of a specific sequence of offers and the combination of choices made. From this rich economic dataset across multiple

dimensions of value, fundamentally distinct types of regret-inducing scenarios can be operationalized each with their respective counterfactual control decisions that could have been selected as an alternative and economically more advantageous option.

Results: We found that SUS mice were uniquely sensitive to regret type I: missed opportunities when rejecting high-value offers followed by encountering low-value offers. These scenarios capture risky decisions with poor outcomes to which neither RES nor CON mice were sensitive. Conversely, RES mice were more sensitive to regret type II: placing more weight on change-of-mind quit decisions in the WZ when correcting previous mistakes that followed accepting low-value offers in the OZ. SUS mice lost sensitivity to regret type II. Furthermore, RES mice were more sensitive to valuing past investments in the form of time already waited, or sunk costs, during these change-of-mind decisions. Overexpressing a dominant-negative form of CREB in the medial prefrontal cortex versus nucleus accumbens, a molecular manipulation known to promote SUS or RES respectively, differentially perturbed these distinct forms of regret.

Conclusions: These data reveal new insights into how adaptive versus maladaptive stress responses are related to distinct forms of counterfactual thinking. We found that one type of regret is absent as a loss-of-function effect in stress-susceptible animals and conversely enhanced as a gain-of-function effect in stress-resilient animals. Another type of regret is uniquely present as a gain-of-function effect in stress-susceptible animals only. The study provides a novel framework for understanding pathological versus healthy forms of regret that hinge on the framing of mistakes. This work can guide not only the development of new diagnostic tools or interventions for depression but can also steer psychotherapy toward unveiling distinct computations through decision narratives by directly inspiring new lines of psychodynamic questioning in patients in a way that may tap into circuit-computation specific processes.

Keywords: Depression, Neuroeconomics, Stress Resilience and Susceptibility, Prefrontal Cortex, Nucleus Accumbens

Disclosure: Nothing to disclose.

P343. Increased Expression of Excitatory Amino Acid Transporter EAAT3 in Forebrain Neurons Confers Resilience to Unpredictable Chronic Stress in Mice

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Background: The pathophysiology of depression is associated with dysregulation of the glutamate system and its clearance mechanisms in brain regions mediating cognitive-emotional behaviors. Chronic stress results in an increase in extracellular glutamate and dysregulation of the glutamatergic system in cortical and limbic brain areas both in patients with MDD and animal models of depression. However, the mechanisms underlying the abnormal glutamatergic transmission in depression are incompletely understood. The excitatory amino acid transporter 3 (EAAT3) - a member of the high-affinity glutamate transporters, which plays an essential role in transporting glutamate across plasma membranes in neurons, and in maintaining extracellular glutamate concentrations below neurotoxic levels, may have a pivotal role in the dysregulation of glutamatergic signaling associated to depression. This study seeks to evaluate the consequences of the unpredictable chronic mild stress (UCMS) model on the expression of glutamate transporters and receptors in the cortical-limbic brain areas in wild type (WT) mice and in EAAT3glo/CMKII mice, a novel transgenic mouse model with increased EAAT3 expression in forebrain neurons.

Methods: Animals: WT mice, control (EAAT3glo, no Cre) and CaMKIIα -driven EAAT3 overexpressing (EAAT3glo/CMKII) mice from both sexes were subjected to UCMS and compared to non-stressed mice for each genotype. 7-14 mice from both sexes were used per genotype, per condition.

UCMS: Mice were daily challenged to one or more stressors according to a semi-random schedule for 5 weeks. The stressors used were: restraint stress, modifying the light and dark cycle, wet bedding, substitution of sawdust with water, removal of sawdust, tilting the cage by 45°, repeated changes of bedding, replace home cage bedding by the bedding of a CF-1 mouse and stroboscope light.

Behavior: Mice were assessed for anxiety-like (open field test) and depressive-like behaviors (sucrose preference test, tail suspension test and social interaction test) in baseline (no stress) and UCMS conditions. Weight gain and coat status was also scored over the experimental timeline.

Molecular: Protein levels of AMPA and NMDA receptors subunits and glutamate transporters were analyzed by western blot.

Results: UCMS increased EAAT1 (t-test = -2.61, df = 4, $p = 0.05$), NMDA receptor GluN2A (t-test = -2.99, df = 8, $p < 0.05$) and GluN2B (t-test = -2.57, df = 10, $p < 0.05$) subunits, and AMPA receptors GluA1 (t-test = -5.21, df = 4, $p < 0.01$) subunits protein levels in the hippocampus in WT mice. WT mice and EAAT3glo (no Cre) control mice challenged to UCMS displayed increased anxiety like-behavior in the open field test and depressive-like behavior in all paradigms tested. As previously reported, in baseline conditions (no stress) EAAT3glo/CMKII mice displayed increased anxiety-like behavior. Remarkably, EAAT3glo/CMKII mice challenged to UCMS did not display any depressive-like behavior in the sucrose preference test, tails suspension test or social interaction test.

Conclusions: Hippocampal glutamatergic system alterations may underlie depressive-like behaviors. Our findings strongly suggest that forebrain EAAT3 gain-of-function may be linked to a resilient phenotype to chronic stress.

Keywords: Chronic Unpredictable Mild Stress, Glutamate Transporter (EAAT3), Depression, Glutamatergic Transmission

Disclosure: Nothing to disclose.

P344. Cortical Cell Cycle Abnormalities in Chronic Stress

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Background: Stress is characterized as a state in which intrinsic or extrinsic stimuli evoke a dynamic and complex repertoire of biological, physiological, and behavioral adaptive responses of the organism. Stress can be either a triggering or aggravating factor for many pathological conditions, including cancer, coronary heart disease, major depressive disorder and neurodegenerative disorders. Several lines of evidence have indicated that the prefrontal cortex (PFC) is highly sensitive to stress, where stress induces volumetric, cytoarchitectural, and morphological changes. Consequences of chronic exposure to stress in the PFC include learning and memory impairments, and behavioral changes.

Little is known about chronic stress-induced changes in the cell cycle regulators in the brain, especially in the PFC. The p16 and p21 cyclin-dependent kinase inhibitors are involved in cell cycle pathways associated with the cellular stress response. In order to provide a better understanding of the impact of variable durations of CRS on the cell cycle regulation in PFC, we evaluated mRNA and

protein levels of both p16 and p21 in mice. We also investigated potential links between p16 and p21 expression changes and anxiety- and depressive-like behaviors using correlational analysis.

Methods: Independent groups of C57BL/6J mice (6 groups, 8 mice/group, 50% female) were exposed to chronic restraint stress (CRS) from 3 to 35 days, and were assessed on behavioral tests measuring anxiety and anhedonia. In addition, PFC mRNA and protein levels of p16 and p21 were quantified using qPCR and Western Blot, respectively, at each time point. Correlation analyses were used to investigate the impact of chronic stress on the mRNA and protein expression of p16 and p21, and anxiety- and depression-like behaviors.

Results: Analysis of variance showed that p16 mRNA expression was significantly different across the stress groups ($p = 0.006$). When sex was added as a co-factor, there was no main effect of sex ($p = 0.213$) and the difference in mRNA expression of p16 across groups remained statistically significant ($p = 0.006$). Pairwise comparison showed that p16 mRNA expression was significantly reduced on days 3 ($p = 0.031$), 21 ($p = 0.007$), and 35 ($p = 0.029$) compared to the control group. p21 mRNA expression was significantly different among groups ($p = 0.024$). When sex was added as a co-factor, we found no main effect of sex on gene expression ($p = 0.803$), and the difference in the mRNA expression of p21 between groups remained statistically significant ($p = 0.027$). Pairwise analysis showed that p21 mRNA expression was significantly reduced only on day 28 ($p = 0.037$) compared to the control group.

p16 protein expression was significantly different among groups ($p = 0.009$). After including sex as a co-factor, there was no difference in the protein expression of p16 between groups ($p = 0.129$). We also found no main effect of sex on p16 expression ($p = 0.899$). Pairwise analysis showed that p16 protein expression was significantly increased on day 14 ($p = 0.025$) compared to the control group. We found a marginally significant difference on p21 protein expression among groups ($p = 0.056$). When sex was added as a co-factor, there was no difference in the protein expression of p21 between groups was found ($p = 0.352$), but we found a significant main effect of sex on p21 protein expression ($p = 0.017$). Pairwise contrast analysis showed that p21 protein expression was significantly increased on days 3 ($p = 0.016$), 14 ($p = 0.005$), 21 ($p = 0.005$), 28 ($p = 0.045$), and 35 ($p = 0.028$) compared to the control group.

Correlation analyses were performed to determine the potential relationship between p16 and p21 expression levels and behavioral outcomes. p16 mRNA expression was significantly negatively correlated with residual avoidance in the shelter zone ($r = -.318$, $p = 0.034$), positively with weight gain ($r = .337$, $p = 0.024$), and negatively with sucrose consumption ($r = .355$, $p = 0.018$). p21 mRNA levels showed a significant correlation with worsened coat state ($r = -.400$; $p = 0.006$). Splitting the samples by sex, there were no significant correlations between p16/p21 mRNA levels and behavior outcomes. p16 protein expression was significantly negatively correlated with worsened coat state ($r = -.378$, $p = 0.011$). p21 protein expression was also significantly correlated with reduced sucrose consumption ($r = -.342$, $p = 0.036$). Splitting by sex, correlations analysis revealed a positive correlation between p21 protein expression and residual avoidance in shelter zone ($r = .547$, $p = 0.012$) and reduced sucrose consumption ($r = -.609$, $p = 0.004$) in males, while the females showed no significant correlation with behavioral outcomes. Finally, we did not find a significant correlation between mRNA and protein expression levels of p16 ($r = -.046$, $p = 0.768$) and p21 ($r = -.184$, $p = 0.262$)

Conclusions: Our present study extends the existing literature providing evidence that postmitotic cells have a complex stress response that involves senescence markers. We confirmed that mice subjected to CRS showed dysregulation of cell cycle response systems, suggesting an abnormal response of the cellular defense system to stress linked to depressive-like behavior. In summary, these findings provide strong justification for future studies to investigate which brain region and cells display abnormal p16 and p21 mRNA and protein expression.

Keywords: Chronic Stress, Prefrontal Cortex, Kinase Inhibitor, Stress and Anxiety Behavior

Disclosure: Nothing to disclose.

P345. Maternal High-Fat Diet Causes Male Offspring-Specific Serotonin Deficiency

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Background: Over 50% of women in the United States are overweight when they become pregnant. Maternal high-fat diet (mHFD) creates an environment of chronic inflammation and is associated with detrimental outcomes for offspring, including increased susceptibility to psychiatric disorders. Despite these observations, many studies are correlative and do not propose a mechanism through which mHFD contributes to offspring psychiatric disorder development. The placenta is a critical interface between maternal environment and the developing fetal brain, and as such is poised to translate maternal insults into offspring neural outcomes. Further, the placenta is the primary source of fetal forebrain serotonin, and decreased serotonin signaling is correlated with increased vulnerability to stress in rodents. Serotonin levels are decreased in the context of inflammation; thus, we hypothesize mHFD-induced inflammation reduces prenatal serotonin levels, altering the development of the central serotonin system and causing anxiety-/depressive-like behaviors in mice.

Methods: We used a mouse model of maternal high-fat diet (mHFD) to determine placenta and brain macrophage density, placenta Tlr4 expression, and serotonin-dependent behavioral outcomes in male and female offspring ($n >= 3$ dams per diet per experiment; 2-way ANOVAs). We also assessed placental and brain serotonin levels in both mouse mHFD offspring and human fetal tissue in which triglyceride levels from maternal placenta were used as a proxy for maternal fat distribution (39 matched maternal decidua, fetal brain, and fetal placenta sets; linear regression analyses).

Results: Maternal high-fat diet increases macrophage density and toll-like receptor 4 (Tlr4) expression in male and female placenta, and Iba1 and CD68 immunoreactivity, gross measures of microglia activation state, in male and female fetal brain tissue ($p < 0.05$; main effect of diet 2-way ANOVA). Female mHFD offspring display diminished social behavior, and preliminary results suggests that abrogation of Tlr4-dependent signaling in macrophages improves behavioral outcomes in females. Male mHFD offspring display normal social behavior, but show increased depressive-like behavior, which is associated with decreased serotonin (5-HT). Maternal dietary tryptophan enrichment restores mHFD-induced serotonin deficiency in male offspring and rescues male behavioral deficits. Finally, in a set of 39 matched human fetal brain and placenta tissues, we observed that maternal triglyceride accumulation significantly negatively correlated with fetal brain serotonin levels in male fetuses only ($r = -0.4909$, $p < 0.05$ for males, $r = 0.2286$, $p = 0.3062$ for females).

Conclusions: Maternal high-fat diet and resulting high maternal weight negatively impact the development of the serotonin system specifically in male offspring, which may contribute to sex-biased psychiatric disorder susceptibility.

Keywords: Immunity and Neurodevelopment by Sex, Mood Disorders, Serotonin

Disclosure: Nothing to disclose.

P346. Ventral Hippocampal Projections to the Orbitofrontal Cortex Regulate Reversal Learning and Stress Resilience

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Background: Cognitive flexibility is impaired in many stress-induced psychiatric disorders, and higher levels of cognitive flexibility can promote stress resilience, possibly by enabling flexible adjustment of coping strategies. Which neural circuits regulate cognitive flexibility, and how these circuits are implicated in stress resilience, is largely unknown. An important aspect of cognitive flexibility is reversal learning, which is the ability to adjust behavior when previously learned action-outcome associations change. At the neural circuit level, studies have implicated the hippocampus and orbitofrontal cortex (OFC) in reversal learning. Our own previous work has highlighted the ventral part of the hippocampus as a crucial mediator of stress resilience. How the ventral hippocampus and OFC interact to facilitate reversal learning, stress coping, and resilience has never been investigated.

Methods: We bilaterally injected retrograde Cre virus (AAVrg.hSyn.HI.eGFP-Cre.WPRE.SV40) in OFC (mOFC), and Cre-dependent inhibitory hM4Di DREADD virus (AAV9.hSyn.DIO.hM4Di.mCherry; experimental mice) or mCherry virus (AAV9.hSyn.DIO.mCherry; control mice) in vCA1. To test vCA1-mOFC inhibition effects on reversal learning, male mice ($n = 10$ control, $n = 10$ inhibition) were food restricted to 85% body weight, trained to dig in two baited bowls, and learned that the rewarded bowl was marked with cinnamon scent and the unbaited bowl with Garlic. On the reversal day, the reward location was switched to Garlic scent and all mice were injected with CNO (5mg/kg, i.*p*.) 30min before the first trial. Mice were considered to have learned the association after 8 correct out of 10 consecutive trials. The number of trials to criterion was compared between groups using unpaired student's t-test. For stress experiments, male mice ($n = 6$ control, $n = 6$ inhibition) were exposed to sub-chronic social defeat by aggressive male CD1 retired breeders for 5 min each day over a 5-day period. Distance travelled was recorded on each day of defeat as a measure of active escape/coping behavior and compared by 2-Way repeated measures ANOVA. Mice were tested in a social interaction (SI) test 24 hr after the defeat. The ratio of time spent with a novel mouse over time spent with an empty cup was analyzed using EthovisionXT (Noldus) and compared using unpaired student's t-test. Overall mobility was tested in a 10 min open field test, and corticosterone (CORT) levels were measured in plasma using EIA assay (Arbor Assays) 30 min after exposure to acute stress. Both tests were analyzed by unpaired t-test. All data are mean±s.e.m.

Results: Chronic inhibition of vCA1-OFC projections impaired reversal learning in the Digging Task (Control: 21.2 ± 3.8 trials to criterion; Inhibition: 31.1 ± 4.48 trials to criterion; $n = 10$; unpaired student's t-test: $p = 0.0014$). Projection inhibition reduced distance travelled during social defeat (Control: Day 1: 487 ± 16.5 cm; Day 2: 361 ± 13.2 cm; Day 3: 274 ± 14 cm; Day 4: 357 ± 15 cm; Day 5: 246 ± 10.9 cm; Inhibition: Day 1: 199 ± 6.4 cm; Day 2: 102 ± 9.2 cm; Day 3: 63 ± 8 cm; Day 4: 75.4 ± 5.9 cm; Day 5: 95.4 ± 6.3 cm; $n = 6$; Two-Way repeated measures ANOVA shows main effect of Inhibition: $p = 0.0002$. No effect of Day: $p = 0.1014$; no interaction effect: $p = 0.8454$).

without affecting distance travelled in an open field arena (Control: 2533 ± 304.1 cm; Inhibition: 2282 ± 156.4 cm; $n = 6$; unpaired student's t-test: $p = 0.48$), indicating less active escape behavior (i.e., a more passive stress coping strategy) during defeat without effects on overall mobility. Mice with vCA1-OFC inhibition during defeat showed lower SI ratios than control mice (Control: 1.2 ± 0.1; Inhibition: 0.69 ± 0.07; $n = 6$; unpaired t-test: $p = 0.007$) and higher CORT levels in response to an acute defeat (Control: 268.9 ± 59.4ng/ml; Inhibition: 486.7 ± 43.5ng/ml; $n = 6$, unpaired t-test: $p = 0.014$), indicating higher levels of stress-induced social avoidance and stress reactivity.

Conclusions: Our results show that vCA1-OFC projections are important for reversal learning and for employing an active stress coping strategy during social defeat, which is important for stress resilience. Targeting vCA1-OFC projections may thus promote stress resilience and rescue cognitive flexibility deficits in psychiatric disorders.

Keywords: Cognitive Flexibility, Neural Circuits, Stress Resilience

Disclosure: Nothing to disclose.

P347. Sex Differences in Neural Representation of Threat in Ventral Hippocampal and Prefrontal Cortical Projections to Nucleus Accumbens

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Background: Learning about and responding to emotionally significant events is dysregulated in many neuropsychiatric disorders including depression and anxiety disorders. The nucleus accumbens (NAc) plays a role in neural encoding and behavioral responding to emotionally significant stimuli. To guide behavior, the NAc integrates dopaminergic input from the ventral tegmental area with information from several glutamatergic inputs, including the ventral hippocampus (vHIP) and prefrontal cortex (PFC). Previous work from our lab suggests that neural activity in the vHIP-NAc is increased by chronic variable stress and may also encode individual differences in vulnerability to future stress in both male and female mice. To understand more about how neural activity in this pathway, and another glutamatergic input, the PFC-NAc, is shaped by aversive experiences we examined neural activity during encoding of aversive stimuli (foot-shock) or threat predicting cues in male and female mice.

Methods: All experiments were conducted in accordance with guidelines of McGill University's Comparative Medicine and Animal Resources Center and approved by the McGill Animal Care Committee. Using frame-projected independent fiber photometry (FIP) and GCaMP7f to image in vivo calcium activity across multiple brain regions in male ($n = 8$) and female mice ($n = 9$), we recorded NAc-projecting neurons in the PFC and vHIP during a Pavlovian fear conditioning paradigm in which mice encounter both threat cues (CS+) predicting shock (0.5sec, 0.5mA) and neutral cues (CS-) with no outcome.

Results: Neural activity in both the vHIP-NAc and PFC-NAc encodes aversive foot-shock and differentiates a threat-predictive cue from a neutral cue, with pathway-specific temporal patterns and sex differences. Both pathways show large elevations in activity in response to foot-shock (PFC: M: $n = 8$, $p = 0.005$; *F*: $n = 9$, $p = 0.0004$; vHIP: M; $n = 8$, $p = 0.008$ *F*; $n = 9$, $p = 0.004$), and in the PFC-NAc, this neural response was greater in females than males ($p = 0.02$). We observe that, upon robust behavioral discrimination of threat-predicting and neutral cues, neural activity in vHIP-NAc is significantly elevated at cue onset to the CS + compared to the CS- in males ($p = 0.01$) but not females ($p > 0.92$). In the PFC-NAc, neural activity during the CS + was suppressed immediately prior to cue termination and shock delivery in females ($p = 0.04$) but not males ($p = 0.1$).

Conclusions: Our findings show that both the vHIP and PFC relay information about threat prediction to the NAc that may

underlie appropriate fear responding, with significant sex differences. The elevated PFC-NAc response to foot-shock in females may be relevant to understanding mechanisms of increased vulnerability to chronic stress. Similarly, differences in neural activity during aversive cues in both PFC-NAc and vHIP-NAc suggest pathway-specific sex differences in neural encoding of threat that may relate to sex differences in responding to threat.

Keywords: Fear Conditioning, Stress Resilience and Susceptibility, Fiber Photometry

Disclosure: Nothing to disclose.

P348. Behavioral and Molecular Aftermath of a Single Psychedelic Drug Exposure in Mice

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Background: Psychedelics, traditionally best-known for their mind-altering properties, are emerging as a potential new paradigm in the treatment of certain mood disorders. The most distinct characteristic of their potential therapeutic effect is its lingering nature, as improvements persist long after the acute action dissipates. We aimed to characterize behavioral manifestations and molecular correlates in mice 24h after exposure to a psychedelic drug (DOI).

Methods: Utilizing wild-type and 5-HT2A receptor knockout (KO) male mice we characterized the involvement of 5-HT2AR in learned helplessness and the development of synaptic plasticity. Inclusion of females is part of our current work in progress. Mice (C57BL/6) were administered with the potent serotonin 5-HT2A/2CR-selective agonist DOI (2 mg/kg). We also included a battery of molecular (frontal cortex 5-HT2AR binding) and behavioral assays (expression of head-twitch responses, or HTR) to determine the development of tolerance to the drug action in wild-type and Beta-arrestin 2 (Barr2)-KO animals.

Experiments involving behavior assays and molecular readouts employed 5-15 male mice per treatment and genotype group depending on the paradigm employed. Statistical significance of experiments involving two groups (i.e., drug vs. vehicle) was assessed by Student's t test. Experiments that involved measures during a time-course (i.e., drug vs vehicle during different epochs) and/or involved different variables (i.e. treatment and genotype) were assessed by two-way ANOVA followed by Sidak's multiple comparison test. The level of significance was set at $p = 0.05$.

Results: We observed increases in activity in the forced swimming test and faster development of contextual fear extinction upon cessation of the noxious stimulus in wildtype, but not in 5-HT2A-KO mice, 24h after exposure to DOI. Concurrently, we observed increases synaptic plasticity and downregulation of 5-HT2AR density in the frontal lobe. Mice also developed tolerance to the acute effects of the drug on HTR upon re-exposure to DOI. This effect was blocked by a 5-HT2AR antagonist (M100907), but it was unaffected by the absence of Barr2.

Conclusions: Adaptive responses and synaptic plasticity phenomena observed in mice following administration of DOI 24h prior involved 5-HT2AR on its action. We also observed that such exposure resulted in a decrease of frontal cortex 5-HT2AR density and manifestation of DOI-induced HTR at that same timepoint through a Barr2-independent mechanism. Our results further support the idea that the effect of psychedelics is not limited to their acute pharmacological interactions; conversely a lingering complex behavioral and molecular landscape unfolds. How these different domains are intertwined will be crucial to understand the substrates of psychedelics potential as therapeutics.

Keywords: Psychedelics, Adaptive Behavior, Tolerance

Disclosure: Consultancy fees: Consultant (Self)
Share holder: Stock / Equity (Self)

P349. Multi-Omic Characterizations of Suicide Attempts in Adults With Major Depressive Disorder Reveals Associations of Circadian Genes and Plasma Metabolites

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Background: Suicidality is a global public health challenge. In the absence of predictive biomarkers, identifying individuals at risk of attempting suicide is challenging, as standard clinical and risk assessments are inherently subjective. Twin and adoption studies estimate that the heritability of suicide attempts may be as high as 50%. Predictive biomarkers that identify individuals at risk for suicide attempts would assist with enhanced risk assessments and the development of targeted therapeutics.

Methods: We developed a search strategy to identify single nucleotide variants (SNVs) associated with suicide attempts, with the following criteria: ((SNV) OR (SNVs) OR (SNP) OR (SNPs) OR (single nucleotide variant) OR (single nucleotide variants) OR (single nucleotide polymorphisms) OR (single nucleotide polymorphisms)) AND ((suicide attempt) OR (suicide attempts)). Significant (by study definition) SNVs were extracted. Additionally, the GWAS Catalog was searched for suicide-attempt traits, and SNVs of suggestive significance ($p < 10\text{-}5$) were extracted. Genotypes for these SNVs were obtained from 245 individuals with Major Depressive Disorder (MDD) from the Pharmacogenomics Research Network Antidepressant Medication Study (PGRN-AMPS) and 111 individuals with MDD from the Combining Medications to Enhance Depression Outcomes (CO-MED) study. Patients ($n = 46$) from PGRN-AMPS and CO-MED with prior suicide attempts were identified. Chi-Square tests assessed genetic associations with suicide attempt history. Additionally, 153 metabolites (glycerophospholipids, carnitines, sphingomyelins, amino acids, and biogenic amines) were assayed at baseline (before initiation of antidepressant therapies) with the Biocrates p180 platform in PGRN-AMPS and CO-MED individuals. To better understand the relationships between SNVs associated with suicide attempts and metabolomic profiles of suicide attempters, metabolomic and genomic integration analysis was conducted. The resulting networks, comprising correlated SNVs and metabolites, were compared in individuals with and without a history of suicide attempts to identify potential biomarkers of interest.

Results: The literature search produced 227 studies, 113 of which met our study inclusion criteria, yielding 187 unique SNVs. The GWAS Catalog search produced 31 studies, 28 of which met inclusion criteria, yielding an additional 176 unique SNVs. There were significant differences among 9 SNVs in PGRN-AMPS and CO-MED individuals with and without a history of suicide attempt according to chi-square tests ($p < 0.05$). rs1982350, an intronic SNV in ARNTL (Aryl Hydrocarbon Receptor Nuclear Translocator Like, which encodes the BMAL1 protein), was the most significant ($p =$ 8.1E-05). Metabolomic and genomic integration demonstrated that rs1982350 along with rs3805148 (an intronic SNV in CLOCK) were associated with several phosphatidylcholines, amino acids, and kynurenine in suicide non-attempters ($p < 0.05$; $r > 0.1$). However, these SNV and metabolite associations were absent from the network of suicide attempters. The ARNTL (BMAL1) and

CLOCK SNVs and their associated metabolites most significantly differentiate the networks of suicide attempters and non-attempters. This statistical result suggests a potential functional relationship between the CLOCK and ARNTL (BMAL1) SNVs, their associated metabolites, and suicidality. In the literature, BMAL1 and CLOCK heterodimerize to initiate transcription of circadian rhythm genes. Additionally, circadian rhythm dysfunction (e.g., disrupted sleep patterns) has been associated with suicide attempts.

Conclusions: The relationship of circulating phosphatidylcholines, amino acids, and kynurenine with BMAL1:CLOCK regulated circadian processes may be disturbed in suicide attempters. This aligns with disrupted circadian processes (e.g., sleep) in suicide attempters. This relationship should be further investigated to better understand the joint influence of circadian signaling and phosphatidylcholine, amino acid, and kynurenine variation on risk for suicide attempts. An improved understanding may ultimately yield clinically actionable laboratory tests to identify individuals at an increased risk of attempting suicide and advance drug discovery.

Keywords: Multi-omics, Suicide Attempt, Genomics, Metabolomics

Disclosure: Nothing to disclose.

P350. Methodological Challenges in Psychedelic Drug Trials: Efficacy and Safety of Psilocybin in Treatment-Resistant Major Depression (EPIsoDE) – Rationale, Study Design and Current Status

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Background: Psychedelic drugs represent one of the most promising treatment approaches in contemporary psychiatry (Gründer, 2021; Gründer and Jungaberle, 2021). Specifically, psilocybin, a partial serotonin (5-HT) 2A receptor agonist, has shown promising safety and efficacy results in the treatment of major depression and other psychiatric disorders (e.g. reviewed in Rucker et al., 2018; Mertens and Preller, 2021). While results from modern pilot studies on psilocybin in the treatment of major depression and other psychiatric disorders are promising, most of those trials lack randomization, double-blinding, and a sufficient sample size. The use of double-blinding and an appropriate placebo is particularly difficult in psychedelic trials, facing ethical issues as well as the problem of "expectation bias" and the risk of nocebo effects in patients in the comparator groups (Gründer and Mertens, in press). Accordingly, there is an immense need for additional, larger, well-designed randomized-controlled studies.

Methods: Objectives: The phase 2 EPIsoDE-Trial (NCT04670081) aims to investigate the efficacy and safety of a high psilocybin dose (25 mg, p.o.) administered in a psychotherapeutic context in treatment-resistant major depression in a randomized-controlled, bi-centric, parallel-group, double-blind design. We expect significant and stable treatment responses after a high (25 mg) dose of psilocybin in comparison to placebo (100 mg nicotinamide) and a low/supposedly inactive control dose (5 mg psilocybin), while provoking only mild and transient adverse events (AE). As a secondary objective the effect of a second high dose six weeks after the first dose will be assessed. Exploratory objectives are aimed at identifying potential neurobiological and psychological therapeutic mechanisms of psilocybin treatment.

Methods: 144 patients (25 – 65 years of age) diagnosed with treatment-resistant major depression of moderate to severe degree will be enrolled in the study, all of which will receive two dosing sessions six weeks apart. Treatment-resistance is defined as no improvement in depression despite two adequate courses of antidepressant treatment. After informed consent and potentially down-titration of their antidepressant medication, patients will be randomized to one of four treatment arms: 1) receiving placebo (100 mg nicotinamide) first, 25 mg psilocybin second; 2) receiving the presumably sub-effective psilocybin dose (5 mg) first, the high dose (25 mg) second; 3a) receiving 25 mg psilocybin first, 5 mg psilocybin second; 3b) receiving the high psilocybin dose (25 mg) at both sessions. Dosing sessions will be accompanied by multiple psychotherapeutic preparation and integration sessions. The second dose takes place after assessment of the primary endpoint; the primary endpoint is treatment response, defined as a minimum of 50% reduction in symptoms as measured with the Hamilton Rating Scale for Depression (HAM-D), six weeks after the first dose.

Results: -

Conclusions: The trial is currently being conducted at the Central Institute of Mental Health (sponsor) in Mannheim as well as the Charité Universitätsmedizin Berlin, Campus Charité Mitte. The trial design, including the randomization, double blinding and comparator conditions, will allow valid conclusions on the efficacy and safety of psilocybin treatment in major depression.

The trial is funded by the German Federal Ministry of Education and Research (BMBF 01EN2006A and 01EN2006B); it has been approved by the Federal Institute for Drugs and Medical Devices (BfArM) and the responsible Ethics Committees.

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Keywords: Psilocybin, Major Depression Disorder, Randomized-Controlled Trial, Treatment-Resistance

Disclosure: Nothing to disclose.

P351. Enduring Downregulation of Critical Transcriptional and Translational Cellular Stress Signaling Gene Networks in Postpartum Depression

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Background: Postpartum depression (PPD) affects 15% of women and results in numerous negative health outcomes for both mother and child. Given the burden of this illness, there is immense value in defining molecular markers that could confer PPD risk, symptomology, and heritability in women. During pregnancy, circulating levels of estradiol and progesterone (E2

+ P) rise >10-fold above those normally present across the menstrual cycle, then fall rapidly at birth. Clinically, the administration and withdrawal of these supraphysiologic levels of E2 + P induces symptoms of depression in asymptomatic, ovarian-suppressed women with a history of PPD, but not in control women participating in an identical hormone manipulation protocol (Bloch et al., 2000). Furthermore, previous studies suggest both genetic and epigenetic differences in women at risk for PPD.

Methods: To better understand the cellular role of E2 + P in precipitating mood symptoms in PPD, we derived lymphoblastoid cell lines (LCLs) from women with past PPD ($n = 9$) and matched asymptomatic control women ($n = 11$) with no history of PPD or Axis 1 psychiatric illness. All LCLs were cultured in three treatment conditions to mimic the hormonal states that occur during pregnancy and parturition: no added E2 + P (Baseline); supraphysiologic E2 + P (Addback); and supraphysiologic E2 + P followed by E2 + P removal (Withdrawal). LCLs were then subjected to whole-transcriptome RNA sequencing. Quality control, unsupervised clustering, analysis of differential gene expression (edgeR), and weighted gene correlation network analysis (WGCNA) were all performed in R.

Results: EdgeR detected differentially expressed genes (DEGs) between PPD and control LCLs in all three treatments but saw the most profound statistical difference (>30 FDR corrected $p < 0.05$ DEGs) with E2 + P Addback. There was significant overlap in DEGs across experimental conditions, suggesting a pronounced role of past PPD on gene expression. The most statistically significant of these DEGs were IMPACT and WWTR1, highly evolutionarily conserved genes that were decreased in PPD. IMPACT ($F(1,54) = 45.96$, $p < 0.0001$) is a translational regulator that maintains homeostasis during circumstances of cellular stress. WWTR1, aka TAZ ($F(1,54) = 52.06$, $p < 0.0001$), is a transcriptional co-activator that mediates cell proliferation and apoptosis in the stress shunting 'Hippo' signaling pathway. IMPACT and WWTR1 were significantly correlated ($F(1,18) = 18.86$, $p = 0.0004$), and their protein expression was significantly decreased in PPD ($p < 0.01$). Further analysis of E2 + P Addback and Withdrawal specific DEGs revealed that GATA3 (nominal $p < 3.56$x10-5) and MYC (nominal $p < 2.91$x10-4), hormone-mediated transcription factors that can alter IMPACT and WWTR1 expression, were significantly increased in PPD. Although downstream WWTR1-mediated genes are not well expressed in LCLs, the downstream targets of IMPACT, like eiF2α, showed significant changes in phosphorylation in a E2 + P-dependent fashion. Finally, WGCNA revealed eight diagnosis-significant modules, most of which were significantly linked to the GATA3 and MYC-associated E2F transcriptional networks.

Conclusions: These findings implicate both E2 + P-independent and -dependent differential cellular responses in PPD. The magnitude of overlap in DEGs between treatment groups suggests that past PPD is strongly reflected in significantly altered gene expression. The enduring dysregulation of evolutionarily conserved transcription (WWTR1, E2F, GATA3, MYC) and translation (IMPACT) networks may suggest women with PPD could have an impaired ability to maintain typical cellular homeostasis with the stress of pregnancy. The differential effects of hormone Addback and Withdrawal in PPD were present, but more mechanistically nuanced. For instance, although eiF2a was not a DEG in any treatment condition, eiF2α-Ser51 phosphorylation, of which IMPACT is a primary mediator, was significantly changed in PPD compared to control LCLs only after E2 + P withdrawal. Furthermore, the finding that the most extreme gene expression differences were during E2 + P Addback (and not during Withdrawal) potentially provides molecular support for recent work suggesting PPD may begin before parturition. Future studies will investigate the E2 + P-sensitive mechanisms underlying the roles of IMPACT, WWTR1, and E2F gene networks in other models for PPD, to better understand how an altered ability to regulate transcriptional and translational cellular response may influence mood or PPD risk.

Keywords: Postpartum Depression, Estradiol, Progesterone, Ovarian Hormones, RNA-seq

Disclosure: Nothing to disclose.

P352. Interactions Between Peripheral Myeloid Cells and the Brain in Stress-Impaired Social Behaviors

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Background: Chronic psychosocial stress, an important risk factor for major depressive disorder (MDD), induces profound changes in the immune system associated with behavioral alterations relevant to MDD. However, the mechanisms linking peripheral immune system activation and neuronal dysfunction in the Nucleus accumbens (NAc) and as a consequence, deficits in social reward processing, are still poorly understood.

Methods: In a murine model of chronic social defeat stress (CSDS), we applied mass cytometry, bulk and single-cell RNA-sequencing to characterize immune cells in circulation and the central nervous system. Using pharmacological and genetic strategies, we investigated the causal effects of the identified murine targets on stress-induced behavioral and electrophysiological changes. Lastly, using a translational approach, we validated the murine findings in patients with MDD and non-depressed healthy controls (HC).

Results: In circulation, CSDS led to a dysregulation of several leukocyte subpopulation frequencies of both the myeloid and lymphoid lineage. In brain, however, we observed an increased accumulation of Ly6chigh monocytes in brain-border regions specifically in susceptible mice. Single-cell RNA-sequencing of brain infiltrating monocytes identified increased expression of the endopeptidase matrix metalloproteinase 8 (Mmp8) in stress-susceptible versus resilient or control mice. Plasma levels of MMP8 correlated positively with social avoidance. Combination of intraperitoneal administration of recombinant MMPs and a subthreshold social defeat was sufficient to induce social avoidance and reduced social reward. Bone marrow chimeric mice that lack Mmp8 only in peripheral immune cells also showed decreased CSDS-induced social avoidance compared to wildtype chimeras. These behavioral changes went along with blunted stress-induced increased spontaneous excitatory postsynaptic currents (sEPSCs) and neuronal excitability in the NAc. Finally, we showed that plasma levels of MMP8 were increased in patients with MDD compared to HC.

Conclusions: Our findings provide mechanistic evidence that neuro-immune interactions are relevant to the etiopathophysiology of stress-induced social behavior deficits. Targeting specific inflammatory molecules such as matrix metalloproteinases could constitute interesting novel therapeutic targets for stress-related neuropsychiatric disorders.

Keywords: Depression, Inflammation, Nucleus Accumbens, Immune System, Social Defeat Stress

Disclosure: Nothing to disclose.

P353. Psilocybin Therapy Increases Cognitive and Neural Flexibility in Patients With Major Depressive Disorder

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Background: Psilocybin has shown promise for the treatment of major depressive disorder, a disorder often accompanied by cognitive rigidity. One mechanism by which psilocybin could reduce depressive symptomology is by increasing cognitive flexibility and neural flexibility, particularly of the anterior cingulate cortex (ACC), a region implicated in depression, psychedelic drug action, and cognitive flexibility.

Methods: In an open-label, waitlist-controlled study of 24 patients with major depressive disorder, we tested the enduring effects of psilocybin therapy on depression, cognitive flexibility, as measured by perseverative errors on a set-shifting task, neural flexibility, as measured by dynamics of functional connectivity (dFC) via functional magnetic resonance imaging, and metabolite concentrations in the ACC, as measured via magnetic resonance spectroscopy.

Results: Psilocybin therapy reduced depression and increased cognitive flexibility for at least four weeks post-treatment, though these improvements were not correlated. One-week after psilocybin therapy, glutamate and N-acetylaspartate concentrations were decreased in the ACC, and dFC was increased between the ACC and the posterior cingulate cortex (PCC). Surprisingly, these increases in dFC between the ACC and PCC were associated with less improvements in cognitive flexibility after psilocybin therapy. Connectome-based predictive modeling demonstrated that baseline dFC emanating from the ACC predicted improvements in cognitive flexibility. In these models, greater baseline dFC was associated with better baseline cognitive flexibility but less improvements in cognitive flexibility.

Conclusions: These findings show that psilocybin therapy in patients with depression can enhance cognitive flexibility and neural flexibility, especially of the ACC, but suggest a nuanced relationship between neural flexibility and depression symptomology. Whereas some enduring increases in neural dynamics may allow for shifting out of a maladaptively rigid state, larger persisting increases in neural dynamics may be detrimental to the efficacy of psilocybin therapy.

Keywords: Psilocybin, Major Depressive Disorder (MDD), Cognitive Flexibility, Functional MRI (fMRI), Dynamic Functional Connectivity

Disclosure: Nothing to disclose.

P354. Late-Life Depression is Associated With Increased Levels of the Pro-Aging Marker GDF-15

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Background: In the past few decades, there is growing evidence that the growth differentiation factor-15 (GDF-15) plays a significant role in aging and multi-system disease development. The GDF-15 has a largely pleotropic effect and has been associated with both the development of cancers, metabolic, inflammatory, and cardiovascular diseases. Emerging evidence suggest that the GDF-15 is a progeroid protein and that strongly correlates with chronological age, decreases telomerase activity, and increases mortality risk in older adults. Few studies have investigated the association between GDF-15 and neuropsychiatric disorders, with higher levels found in bipolar disorder, MDD, and dementia. However, these studies had small sample sizes and no studies evaluated GDF-15 levels in older adults with major depression.

This study aimed to evaluate the circulating levels of GDF-15 in a large cohort of adults with major depressive episode. Our working hypothesis was that older adults with major depressive episode would have higher GDF-15 levels than non-depressed comparison individuals.

Methods: We included 430 older adults in this analysis, 308 with acute major depressive episode (MDE) and 108 controls (both groups contained male and female participants). These individuals were recruited in 4 different sites (St Louis, San Diego, Pittsburgh, and Toronto, CA). All individuals with MDE were not under antidepressant treatment at the time of psychiatric assessment and blood draw. GDF-15 was measured in the serum using a multiplex immunoassay.

Results: LLD subjects were older, had a higher proportion of females, higher BMI values, and higher medical comorbidity burden. There were no significant differences between groups in other demographic and clinical data. Subjects with LLD had a significantly higher GDF-15 plasma levels compared to the HC subjects (t-test = -6.43, d.f.= 437, $p < 0.001$). After controlling for potential confounding variables, the association between LLD diagnosis and plasma GDF-15 levels remained statistically significant after controlling for the effect of these potential confounding variables ($z = 2.15$, $p = 0.031$).

GDF-15 plasma levels were significantly correlated with age ($r = .13$, $p = .017$), BMI ($r = .13$, $p = .009$), and CIRS-G score ($r = .13$, $p = <.001$). We did not find a significant correlation between GDF-15 and the severity of depressive symptoms measured by the MADRS in the LLD subjects only ($r = .13$, $p = .18$). Other characteristics of the depressive episode were also not significantly associated with GDF-15 levels, including recurrence (t-test = -1.3, d.f. =306, $p = .194$), duration of the depressive episode ($r = -.03$, $p = .67$, $n = 306$), or history of previous suicide attempt (t-test = 1.79, d.f. =428, $p = .07$). The presence of comorbid anxiety disorders (all and specific anxiety disorder diagnosis) during the depressive episode did not significantly influence the levels of GDF-15.

Conclusions: This study has demonstrated that individuals with LLD have higher circulating levels of GDF-15 and worse medical co-morbidity burden compared to those without LLD. Additionally, GDF-15 levels were not significantly associated with severe-depressive symptoms. Our findings provide additional evidence that major depressive episode in older adults is associated with age-related biological changes and support the hypothesis of an accelerated biological aging in these individuals.

Keywords: Late-Life Depression, GDF-15, Accelerated Aging

Disclosure: Nothing to disclose.

P355. Charting the Proteome Landscape in Major Psychiatric Disorders: From Biomarkers to Biological Pathways to Precision Psychiatry

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Background: Precision Psychiatry is a recognized priority. Schizophrenia (SZ), Bipolar Disorder (BD), and Major Depressive Disorder (MDD) share many of their underlying biologies, and there is a lack of biological validity in their diagnosis, which is a hindrance to developing diagnostic tests and discovering and developing new drugs that would be a perfect molecular fit to a given person. Proteomic studies have evolved the field by identifying several proteins that could be biomarkers in those disorders; however, their results have varied widely, and individual biomarkers have failed to advance diagnostics and therapeutics.

Our goal is to leverage the broad variability among different studies and strengthen the knowledge provided by individual proteins by systematically interrogating the literature to uncover biological pathways with stronger biological meaning.

Methods: This study is a systematic review of all proteomics studies in BD, MDD, and SZ compared to controls in serum or plasma. We extracted all differentially expressed proteins in BD, MDD, or SZ. Protein identification was made according to the UniProt Database. We mapped each protein to its corresponding gene. We then conducted over-representative analysis (ORA) and gene set enrichment analysis (GSEA) in WebGestalt to unveil which biological pathways were shared or unique to each disorder. Analyses were adjusted for multiple testing corrections.

Results: We included 51 studies with 9,423 participants. 486 proteins were found altered in cases compared to controls (192 in SZ; 190 in BD; and 365 in MDD). The majority of the enriched pathways were shared among SZ, BD, and MDD. The top pathways in all three disorders were associated with the immune system and complement cascade. Other pathways shared among SZ, BD, and MDD were interleukin-12 signaling, MAPK1/MAPK3 signaling, PI3K-Akt Signaling, Toll-like Receptor Signaling, Activation of Matrix Metalloproteinases, Class A/1 (Rhodopsin-like receptors), GPCR downstream signaling, Advanced glycosylation end-product receptor signaling, JAK-STAT signaling, and Regulation of Insulin-like Growth Factor (IGF) transport. Pathways shared between SZ and BD were integrin cell-surface interactions and syndecan interactions. Shared between BD and MDD were the NRF2 pathway, signaling by EGFR, and the Ras signaling pathway. Unique to SZ were interleukin receptor SHC signaling, and TFAP2 (AP-2) family regulation of growth factors; unique to MDD were oncostatin M signaling, ECM-receptor interaction, plasminogen activating cascade, and PPAR signaling pathway.

Conclusions: We curated an ensemble knowledge of 486 altered serum proteins in BD, MDD, and SZ and uncovered their biological pathways. Alterations in pathways related to immune-inflammation were pervasive and transdiagnostic. The immunoinflammatory response, as assessed in peripheral blood, is a shared mechanism across SZ, BD, and MDD, which might imply that the periphery is an unspecific representation of a mechanism placed in the brain and probably a secondary phenomenon to a primarily central origin; it also implies that the biological boundaries among SZ, BD, and MDD mostly do not resemble current nosological categories and need to be completely redefined by the identification of new sub-types. Future drug development for SZ, BD, and MDD could target those identified pathways and be tested in transdiagnostic clinical trials guided by the specific biological pathways altered in a particular person.

Keywords: Precision Medicine for Mood Disorders, Bioinformatics, Peripheral Biomarker, Proteomics, Drug Discovery - New Approaches

Disclosure: Nothing to disclose.

P356. A Female-Specific Role for the EphA2 Receptor Within the Prefrontal Cortex in Mediating Depression-Like Behaviours in Rats

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Background: Ephrins (Eph) and their associated ephrin receptors are predominantly expressed at excitatory synapses in various brain regions implicated in depression, including the frontal cortex and hippocampus (HIP), and are involved in the regulation of synaptic transmission, dendritic spine morphology and synaptic plasticity. Animal models used for the study of depression suggest that Eph-ephrin signaling in the rodent prefrontal cortex (PFC), particularly through the EphA4 and EphB2 receptors, may be involved in mediating depression-like behaviour. However, the lack of selective pharmacological agents for the other ephrin receptor subtypes has limited our understanding of their role in neuropsychiatric disorders. In this study, using a selective activating peptide for the EphA2 receptor, the functional role of the receptor in regulating anxiety and depression-like behaviour in male and female rats was evaluated. As we have previously shown that neuronal oscillatory activity is coupled to depression-like behaviours in a sex-specific manner, the sex-specific impact of EphA2 receptor activation on neuronal system function was also determined.

Methods: Male and female Wistar rats were bilaterally implanted with cannulae into the PFC, concomitant with stainless steel electrode implantation in the cingulate cortex (Cg), nucleus accumbens (NAc), and dorsal HIP. To target the EphA2 receptor, a selective EphA2 nanomolar peptide ligand agonist (5 nM solution, 1µl/side) was used. Following recovery, animals underwent a 7-day protocol to evaluate the impacts of EphA2 receptor activation on behaviour and neuronal oscillatory activity. Rats were administered an infusion of the EphA2 peptide agonist or vehicle 15 minutes prior to each behavioural test (no more than one infusion/test per day) that included the forced swim test (FST), sucrose preference test (SPT), or elevated plus maze (EPM). On a different day, local field potential recordings were performed in awake animals in clear plexiglass boxes. Recordings were collected for 15-minutes prior to the intra-PFC infusion with the assigned treatment (baseline) and for 45-minutes immediately post-infusion. At the end of the study, brains were removed for subsequent protein expression analysis in the PFC by Western Blot.

Results: Only female animals displayed significantly increased immobility time ($P < 0.001$) and reduced latency to immobility ($P = 0.002$) in the FST following EphA2 receptor peptide administration. Similarly, in the EPM, only females exhibited a significant reduction in time spent in the open arms ($P = 0.02$) and the number of open arm entries ($P = 0.028$) post-fusion. Behaviour in the SPT was not altered in either sex. Female rats that received an intra-PFC infusion of the EphA2 receptor peptide exhibited significantly greater HIP delta power ($P = 0.01$) and lower HIP theta power ($P = 0.004$) compared to vehicle infused females. This same pattern was observed within the NAc of male rats, with those that received an EphA2 receptor peptide infusion having displayed greater delta ($P = 0.015$) and lower theta power ($P = 0.003$), compared to vehicle infused males. No significant effects of treatment on low frequency band power in the Cg were observed in either sex. In the high frequency bands, the only observed difference was lower high gamma power within the NAc of EphA2 receptor peptide infused female rats compared to control females. When coherence was examined, female rats that received an infusion of the EphA2 receptor peptide exhibited greater theta coherence than vehicle infused females between the Cg-NAc ($P < 0.001$) and Cg-dHIP ($P = 0.005$). Between the NAc-dHIP, both male ($P = 0.017$) and female ($P = 0.006$) animals infused with the EphA2 receptor peptide had greater delta coherence compared to the sex-matched vehicle infused animals. Across the high frequency bands, no significant effects of treatment in either sex were observed between the Cg-NAc or Cg-dHIP. However, between the NAc-dHIP, EphA2 receptor peptide infusion in males induced greater beta coherence ($P = 0.007$) than vehicle infusion. Total PFC EphA2 receptor expression was not altered by drug treatment. However, the level of phosphorylated ephexin-1, a downstream substrate of the EphA2 receptor, was significantly elevated in the PFC of females only upon EphA2 receptor activation. No drug-induced differences in total PFC ephexin-1 or RhoA expression were found in either sex or treatment group. $N = 8$-10/group.

Conclusions: These preclinical findings identify key sex differences in the molecular, neurophysiological and behavioural

outcomes of PFC EphA2 receptor activation. Specifically, whereas PFC EphA2 receptor activation increased ephexin-1 activity and induced depressive- and anxiety-like behaviour in the female rats, no such effects were evident male rats. The effects of PFC EphA2 receptor activation on spectral power and coherence were predominantly focused within the low frequency range in both males and females, but showed distinct regional specificity depending on the sex. Further, the increased theta coherence involving the Cg were observed selectively in the female animals. Overall, these findings implicate EphA2 receptor-ephexin-1 signaling in the PFC as potentially having a unique female-specific role in regulating behaviours that may have relevance to depression, and further, highlight the critical need for the inclusion of sex as an experimental variable in research.

Keywords: Anxiety and Depression, EphA2 Receptor, Neuronal Oscillations, Ephrin

Disclosure: Nothing to disclose.

P357. Stress-Induced Alterations in Neural Responsivity to Food Reward Cues in Unmedicated Individuals With Variable Appetite/Weight Phenotypes of Major Depressive Disorder

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Background: Divergent phenotypes in major depressive disorder (MDD) related to appetite and weight change are largely understudied from a mechanistic perspective, despite evidence of differences in clinical outcomes and morbidity. Compared to those with hypophagic depression (HypoMDD; decreased appetite/weight loss during an episode), individuals with hyperphagic depression (HyperMDD; increased appetite/weight gain) exhibit greater suicidality and medical comorbidities, with recent data suggesting that hyperphagic behaviors stem in part from disruption in mesolimbic regions governing reward. In rodents, chronic social defeat stress (a paradigm resulting in depressive-like behaviors) can result in the emergence of a phenotype similar to HyperMDD, with elevated food intake and weight gain in response to stress. These data provide preclinical evidence of interactions between stress and reward that might be implicated in the pathophysiology underlying endophenotypes of MDD. The current study examined the effects of a robust psychosocial stressor and a matched, non-stressful protocol on mesolimbic reward and stress circuitry responsivity to a food incentive delay (FID) task (measuring reward anticipation and feedback) in individuals with HyperMDD, HypoMDD, and healthy controls.

Methods: We studied 90 adults [40 healthy control (HC; 20F/20M), 29 unmedicated Hypophagic MDD (HypoMDD; 15F/14M); 21 unmedicated Hyperphagic MDD (HyperMDD; 9F/12M)], each of whom completed 2 study visits (~1 week apart) involving serial blood sampling (for cortisol), completion of the Maastricht Acute Stress Task (MAST; an acute psychosocial stressor; Stress Visit) or a non-stressful version of the MAST (Control Visit; visit order counterbalanced), MRI scanning (FID task) on a Siemens 3T Skyra, and questionnaires (Perceived Stress Scale to assess current perceived life stress and the Snaith-Hamilton Pleasure Scale to measure hedonic capacity). Changes in cortisol, reaction time (RT) during the FID task, brain activity, and brain-behavior relationships were examined in the context of response to psychosocial stress. fMRI data were analyzed using SPM12 using a full factorial design to examine main effects of Group and Visit, and Group x Visit interaction effects for two contrasts of interest: food reward anticipation vs. neutral anticipation; food reward receipt vs. neutral success. ROI analyses, with an a priori threshold of p (FWE-corrected)<0.05, examined effects in the hypothalamus, nucleus accumbens, caudate, amygdala, hippocampus, and ventromedial prefrontal cortex (VMPFC).

Results: For cortisol responsivity, a repeated measures ANOVA showed a Time x Visit interaction ($F = 54.72$, $p < 0.001$), driven by higher cortisol response to the MAST at Time 20 (immediately following the MAST) vs. Time 0 (immediately preceding the MAST) during the Stress Visit, but not at the Control Visit. For RTs during the FID, analyses indicated a main effect of Group ($F = 3.41$, $p = 0.02$), driven by faster RT in HyperMDD vs. HC ($p = 0.002$), and a trend for faster RT in HyperMDD vs. HypoMDD ($p = 0.07$). We also found a main effect of Cue ($F = 5.08$, $p = 0.03$), driven by faster RT when subjects responded on Food vs. Neutral cues. During the FID, for anticipation of food reward vs. neutral, there a main effect of Group in the VMPFC ($F = 12.26$, $p = 0.007$), driven by greater activation in HC vs. HypoMDD ($p = 0.001$) and in HC vs. HyperMDD ($p = 0.0009$); HypoMDD and HyperMDD groups did not differ ($p = 0.31$). VMPFC activation was negatively associated with perceived stress scores across groups (r = -0.35, $p = 0.001$). There was also a main effect of Visit in the caudate ($F = 16.03$, $p = 0.047$), driven by attenuated activation during the Stress Visit vs. Control Visit ($p = 0.002$). Caudate activation during the Stress Visit (but not the Control Visit) was positively associated with hedonic capacity across groups (r = 0.3, $p = 0.008$). There was also a Group x Visit interaction in the amygdala ($F = 8.14$, $p = 0.032$) and at a trend level in the hypothalamus ($F = 6.28$, $p = 0.06$). In the amygdala, the interaction resulted from hypoactivation in the HyperMDD group (relative to HC and HypoMDD) during the Stress Visit vs. hyperactivation (relative to HC and HypoMDD) during the Control Visit. In the amygdala, the HC and HypoMDD groups did not show differences in activation between Visits. In the hypothalamus, the HC group exhibited hyperactivation (relative to both MDD groups) during the Stress Visit vs. hypoactivation (relative to both MDD groups) during the Control Visit. For receipt of food reward vs. neutral, there was main effect of Visit in the hypothalamus ($F = 9.35$, $p = 0.05$), resulting from attenuated activation during Stress vs. Control Visit.

Conclusions: These data indicate an effect of psychosocial stress on behavioral reward sensitivity across groups, and in particular in response to food cues in the HyperMDD. In addition, findings elucidate stress-induced alterations of brain activity in mesolimbic circuitry in response to psychosocial stress in appetite phenotypes of MDD, particularly in the amygdala. Stress-induced amygdala hypoactivation during anticipation of food reward in HyperMDD provides evidence in support of a reward deficit model of aberrant neural response to food rewards, a potential mechanism for stress-induced overeating in this group. These findings provide insight into novel neuroanatomical targets in the development of treatments for HyperMDD and related conditions, such as anorexia nervosa and obesity.

Keywords: Acute Stress, Reward, Major Depressive Disorder (MDD), Appetite, Cortisol Response to Stress

Disclosure: Nothing to disclose.

P358. Genetics and Brain Imaging Show a Role for NFIA in Astrocytes in Suicide Attempt

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Background: Although genetics and human brain imaging are collecting data at tremendous speed, the accumulation of this information does not always result in a much better mechanistic understanding of the studied phenotypes. One reason is that genetics and human brain imaging tend to work in isolation. We developed ProcessGenesList (PGL), a strategy to use the

aggregated mRNA expression levels of groups of genes known or suspected to be associated with a certain disorder, symptom, or phenotype to generate human brain imaging study hypotheses. We applied PGL to suicide to study a) which brain regions are altered in humans with past suicide attempt, b) genetic interactions (single nucleotide polymorphisms, SNPs) with brain imaging characteristics in the setting of past attempt (ATT), c) ex-vivo human tissue to verify those interactions using immunohistochemistry, and d) mouse mutant models to study possible mechanisms and to drive additional human studies.

Methods: PGL: We used all SNPs with $p = 10\text{-}3$ or less from a suicide attempt GWAS (Willour, 2012) and averaged the mRNA expression of the associated genes in all brain regions studied in the Allen Atlas. We obtained 8 brain regions for further study. Brain imaging: We used a sample of 423 inpatients at The Menninger Clinic in Houston, TX (133 with past attempt). We used resting state functional connectivity (Conn analysis tool) and morphometry (FreeSurfer). Human genetics: SNPs were studied by PCR. Ex-vivo: We used amygdala tissue punches from 10 suicide decedent brains and 10 controls. Mouse: We used NFIA loss of function in the amygdala for immunohistochemistry, electrophysiology, and genetic studies.

Results: Using PGL we found 8 brain regions for further study, including the subiculum and the corpus callosum. When studied using resting state functional connectivity, ATT patients showed increased subiculum/habenula and subiculum/dorsolateral prefrontal cortex (which interacted with APAK7 genotype). We studied the corpus callosum with FreeSurfer and found that the anterior region was larger in ATT patients, but only if the rs2474388 in NFIA SNP was GT/GG. In ex-vivo amygdala tissue, NFIA was shown to significantly be overexpressed in suicide decedent brains ($P < 0.001$). In mice, NFIA amygdala loss of function showed lower MAO-B and lower GABA, abnormal excitation/inhibition patterns in astrocytes, and reduced fear conditioning and anxiety-like behaviors. We then studied MAO-B in ex-vivo tissue and found increased MAO-B.

Conclusions: We showed that a) PGL resulted in hypotheses that when studied uncovered brain features altered in ATT (subicular connectivity and callosal volume), b) Subicular connectivity interacted with AKAP7 genotype, and callosal volume interacted with NFIA genotype, c) NFIA (important in astrocyte function in the amygdala) expression was increased in suicide decedent brain amydgala, d) mice with amygdalar NFIA loss-of-function had astrocyte abnormalities, decreases in MAO-B and GABA, and low fear and anxiety-like behaviors, finally, e) the mouse study informed an additional ex-vivo study and we foun increased MAO-B in decedent brain amygdala tissue.

Keywords: Suicide Attempt, Astrocytes, Resting State Functional Connectivity, NFIA

Disclosure: Nothing to disclose.

P359. Are Symptoms and Function Distinct Depression Treatment Targets in the Real World?

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Background: Treatment for depression aims to minimize disease severity and optimize daily function, while mitigating relapse, self-harm, and secondary complications (e. g. substance use disorders or general medical conditions). Yet, efficacy trials focus almost exclusively on symptoms, which are insufficient outcomes for the roughly 1 in 3 depressed patients who cannot achieve sustained symptom remission. This analysis examines the relationship between overall function and a measure of overall disease severity in a large real-world sample of depressed outpatients.

Methods: Data were derived from a large Electronic Health Record (EHR) collected between 2007-2020 that encompassed 24 mental health care systems in which 422,731 patients were treated in both in- and outpatient treatment settings. The analytic sample consisted of adult outpatients (> 18 years) with a recorded ICD-9/10 diagnosis of non-psychotic Major Depressive Disorder with at least 1 baseline and 2 post-baseline outpatient visits at which both the Global Assessment of Function (GAF; Range:1-100 with higher numbers reflecting better function) and the Clinical Global Index of Severity (CGI-S; Range:1-7 with higher numbers reflecting worse disease severity) were collected. Given the nature of the data source, this analysis was exempt from IRB review.

For predictive and discriminative analyses, we divided patients into those with improved (I) (lower) or worsened (W) (higher) CGI-S scores between baseline and the 2nd post-baseline visit, eliminating those without change in the CGI-S. Spearman's rho correlations and decision tree regression analyses defined the relationship between the CGI-S and GAF cross-sectionally and as change measures between visits for both cohorts. Bivariate, multivariable and mediation analyses were conducted to understand the effect of clinical and socio-demographic variables (e. g. gender, age, race, employment status, gender and comorbid diagnosis) on the relation between GAF and CGI-S.

Ordinary least-squares models (OLS) were built to determine whether using either CGI-S, 10-point GAF groups (defined a priori as a meaningful change) or the combination could discriminate between those with at least 2 baseline comorbid psychiatric illnesses (the median for both cohorts) from those with fewer or predict time between the baseline and the next post-baseline visit.

Results: Altogether 1,265 patients formed the analytic sample for the I cohort; and 2,941 formed the W cohort. Cross-sectional correlations between CGI-S and GAF at baseline, 2nd and 3rd visits were -0.38, -0.39, -0.38; and -0.53, -0.53 and -0.52 for the I and W cohorts, respectively. Further, the correlation between change in scores from baseline to 3rd visit were -0.04 and -0.24 for the I and W cohort respectively.

No changes in CGI-S scores were able to predict a meaningful (at least 10-points) change in the GAF based on the decision-tree regression models for either cohort. In the I cohort, a > 3-point decrease in CGI-S predicted a 3.6-point increase in GAF, while a 2-point decrease in CGI-S predicted a 6.8-point increase in GAF. Decision rules obtained from the W cohort predicted a 6.6-point decrease in GAF from a 2-point increase in CGI-S but predicted an 8.6-point decrease in GAF from a > 3-point increase in CGI-S.

Several factors (e. g. race, employment status, psychiatric comorbidities, neuro-degenerative and substance use disorder) had statistically significant indirect effects on GAF and CGI-S for both worsened and improved CGI-S cohorts. However, the magnitude of change in CGI-S from these variables in relation to the GAF was very small (<=0.31-point change in CGI-S).

Discriminant analysis on both cohorts showed weak discriminant ability of CGI-S, GAF or the combination to discriminate between patients with high comorbid illness load. AUC scores using CGI-S, 10-point GAF group and its combination were 0.57, 0.57 and 0.58 for the I cohort and 0.55, 0.57 and 0.58 for the W cohort.

For predictive analysis, using both CGI-S and 10-point GAF groups within the model improved predictive ability when compared to using either CGI-S or GAF alone for both cohorts, ($p < 0.001$) though overall predictive ability was low ($R2 = 0.014$, 0.063, 0.065 for CGI-S, GAF, combination in the improved cohort and 0.016, 0.017 and 0.022 for the worsened cohort).

Conclusions: For MDD patients that improved and for those that worsened, the CGI-S and GAF total scores were only modestly correlated at each visit and as between-visits change measures. A non-linear and asymmetrical relation was found between CGI-S and GAF. Socio-demographic variables did not meaningfully affect the relationship between changes in these two measures. The

combination of both measures performed only modestly better than either alone in predicting between visit times and in discriminating between those with more or fewer psychiatric comorbidities. The CGI-S and GAF measure distinct constructs, suggesting that they may be useful as composite or compound outcome measures, especially in persons with difficult to treat depressions.

Keywords: Major Depressive Disorder (MDD), Functioning, Depressive Symptoms

Disclosure: Holmusk: Employee (Self)

P360. Sex and Brain Region Differences in Microglia-Specific Gene Expression

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Background: Microglia are the resident macrophages of the brain, performing many roles related to brain homeostasis, including modulation of synapses. In the healthy adult brain, microglia exhibit a ramified morphology, sampling the local environment by extending and retracting processes. Various stimuli, including infection, trauma, or altered neuronal activity, induce changes in microglia shape, gene expression, and function; these microglia take on an amoeboid shape and are called "reactive". In this reactive state, microglia may engulf and eliminate synaptic material. Although this process occurs during development to sculp circuits and in an activity-based manner in adults to modulate neuronal activity and synaptic plasticity, excess reactive microglia may contribute to pathological elimination of synapses/dendritic spines in disease. Studies assessing morphological features of microglia found regional differences as well as sex differences in some brain regions. Transcriptomics studies of isolated microglia also suggest differences based on brain region and sex. However, markers used to isolated microglia in these studies (e.g., CD11b, Cx3cr1) are not expressed exclusively by microglia, but are expressed by resident and infiltrating macrophages. Here, we assessed the transcriptional profile of microglia after sorting with the microglia-specific marker TMEM119, with a specific focus on whether the transcriptional profile varied by brain region and/or sex.

Methods: Adult mice ($n = 6$/sex; 10-15 weeks old), heterozygous for Tmem119-2A-CreERT2 and Ai14(RCL-tdT)-D were used. Mice were group-housed in 12-hour light/dark with food and water ad libitum. Mice were administered 75mg tamoxifen/kg body weight via intraperitoneal injection once every 24 hours, for five days. Mice were sacrificed 10-21 days post final injection. Prefrontal cortex (PFC) was isolated from sections at Bregma +2.96 to +1.42 mm, striatum from sections at +1.42 to -0.46 mm, and midbrain from sections at -2.06 to -3.88 mm. Individual cell suspensions were generated of harvested tissue using Miltenyl Biotec Adult Brain Dissociation Kit. Fluorescent activated cell sorting was used to isolate tdTomato labeled microglia. RNA was extracted from isolated microglia using Nextera XT DNA Library Preparation Kit and RNA was sequenced using SMARTer-seq v4 Ultra-low Input Kit. Data was analyzed using DESeq2, with $p < 0.05$ and fold change>1.2 considered differentially expressed (DE). Pathway overrepresentation was assessed using Metascape and IPA with expressed transcripts as background.

Results: We found striking differences in microglia-specific gene expression between brain regions. Genes that were more highly expressed in microglia isolated from midbrain compared to PFC (886 genes) and striatum (728 genes) were enriched for pathways related to immune function (e.g., adaptive immune response, inflammatory response, response to interferon beta). Notably, midbrain isolated microglia had a transcriptional profile consistent with disease associated microglia, with higher expression of Cd68, Timp2, Clec7a, for instance, and lower expression of P2ry12 and P2ry13. Genes more highly expressed in microglia isolated from PFC vs midbrain (865 genes) and vs striatum (500 genes) were enriched for pathways related to synapses (e.g., postsynapse, synapse organization). Genes more highly expressed in microglia isolated from the striatum vs midbrain (949 genes) were enriched for pathways related to neurons and synapses (e.g., axon, modulation of chemical synapse) and genes more highly expressed in microglia isolated from the striatum compared to the PFC (818 genes) were enriched for pathways related to mitosis and the extracellular matrix (e.g., microtubule spindle mitotic, extracellular matrix structure).

We also found sex differences in expression of microglia-specific genes in all 3 brain regions. In the midbrain, DE genes (366) were enriched for pathways related to cell cycle regulation, long-term synaptic depression, and response to selenium ion, with RORα and RORc as top predicted upstream regulators. In the PFC, DE genes (517) were enriched for estrogen-mediated S-phase entry and altered T and B cell signaling, with Jak1/2 as predicted upstream regulator. In the striatum, DE genes (547 genes) were enriched for IL17 signaling, VEGF signaling, and response to selenium ion pathways, with DLG1 as a top predicted upstream regulator. Interestingly, in the striatum, several genes related to MAP kinase phosphatase activity (e.g., several Dusp genes) were more highly expressed in male-isolated microglia.

Conclusions: These results suggests both brain region and sex differences in the transcriptional profile of isolated microglia. Notably, microglia isolated from the midbrain exhibit a transcriptional profile consistent with being disease-associated microglia compared to microglia isolated from the PFC or striatum. Sex differences in the transcriptional profile of microglia may contribute to reported sex differences in microglia morphology, and our studies suggest potential upstream regulators. Future studies will assess the effects of stress on microglia gene expression, with a specific focus on whether stress elicits brain region- or sex-specific effects.

Keywords: Microglia, Sex Difference, Transcriptomics

Disclosure: Nothing to disclose.

P361. Sex Differences in Omega-3 Fatty Acids for MDD With High Inflammation

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Background: Epidemiological, biomarker, and some clinical trials suggest that eicosapentaenoic acid (EPA)-enriched n-3 fatty acids ameliorate depressive symptoms for some patients with major depressive disorder (MDD). A preliminary study demonstrated that overweight-obese patients with depression and high inflammatory markers were the most likely to benefit from n-3 therapy. A secondary analysis of these data also indicated that there were sex differences in the association between body mass index (BMI) and inflammatory markers, with high inflammation being associated with high BMI in women, but not men. In a follow-up study, we evaluated the dose response effects of n-3 fatty acids on depression in subjects with MDD, high BMI, and high inflammation. Subjects randomized to 4 g EPA daily were more likely than placebo to achieve sustained response on the IDS-C. As an exploratory study, we evaluated sex differences in baseline measures of inflammation, change in n-3 levels, and antidepressant response.

Methods: This 2-site (MGH-Emory), UG3-funded study recruited subjects with MDD (IDS-C scores ≥ 25), BMI > 25 and hs-CRP levels > 3 mg/L. Sixty-one subjects (46 women and 15 men) were randomized to oral placebo, 1 g, 2 g, or 4 g of EPA daily for 12 weeks. Capsules contained approximately 1000 mg n-3 fatty acids, with an EPA:DHA ratio of 4:1. The primary endpoints were a sustained (weeks 8 and 12) decrease in level of plasma IL-6 or LPS-stimulated peripheral blood mononuclear cell TNF-a levels (effect size [ES] > 0.4) for n-3 vs. placebo. Secondary endpoints were a decrease in mean IDS-C scores with ES > 0.35 for any dose of EPA vs. placebo and a sustained response with ES > 0.35 for any dose of EPA vs. placebo. Exploratory measures included changes in hs-CRP.

Results: As previously demonstrated, there were sex differences in the association between BMI and inflammatory markers, with high inflammation being associated with high BMI in women, but not men. In contrast, other than hs-CRP which was significantly higher at baseline in females ($p = 0.006$), there were no sex differences in baseline levels of other plasma cytokines or n-3 levels. For the overall study, subjects randomized to 2 g and 4 g EPA daily were more likely than placebo to achieve sustained response (at both week 8 and 12) on the IDS-C, with odds ratios (OR) of 2.3 and 3.4 respectively (exceeding the hypothesized ES > 0.35). Response in the 4 g EPA group was primarily driven by females with 75% responding vs 25% for males. Exploratory analysis of hs-CRP levels demonstrated a statistically significant decrease over time with the 4 g dose. The increased response in females vs males was associated with a significantly greater change in plasma EPA ($p = 0.008$), a greater decrease in plasma hsCRP, and greater change in 18-HEPE, a pro-resolving lipid metabolite of EPA.

Conclusions: These data extend our previous findings that a subset of women with MDD are more likely to have elevated biomarkers of inflammation than men and suggest that there may be sex differences in the response to high dose n-3 in subjects with MDD. Larger confirmatory studies are needed to discern if women manifesting MDD and elevated biomarkers of inflammation represent a subset of patients who are uniquely responsive to high dose n-3 therapy.

Keywords: Depression Inflammation Cytokine, Lipids, Sex

Disclosure: Nothing to disclose.

P362. Epigenetic GrimAge Acceleration in Bipolar Disorder

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Background: Bipolar Disorder (BD) has been previously associated with functional impairment and accelerated epigenetic aging, although the link between these two variables is still unknown. We aimed to investigate the relationship between epigenetic aging acceleration measures with functioning and clinical outcomes in patients with BD and controls.

Methods: Blood genome-wide DNA methylation (DNAm) levels were measured in BD patients ($N = 90$) and matched healthy controls ($N = 40$) with the Infinium EPIC BeadChip (Illumina). Epigenetic age estimates were calculated using an online tool, including Horvath DNAm age, Hannum DNAm age, PhenoAge, apparent methylomic aging rate (AMAR), and the recently developed lifespan predictor GrimAge. Measures of aging acceleration were estimated by regressing the predicted epigenetic ages on chronological ages and using the residuals as 'acceleration indexes' (intrinsic (IEAA) and extrinsic epigenetic age acceleration (EEAA), PhenoAgeAccel, and GrimAgeAccel). The association between multiple epigenetic aging acceleration indexes with clinical variables and functioning status was tested by linear models while controlling for chronological age, sex, race/ethnicity, smoking status, and blood cell count estimates.

Results: All predicted DNAm age clocks were significantly correlated with chronological age. BD was not significantly associated with the Horvath Age acceleration residual, Horvath. IEAA, Hannum acceleration residual, Hannum AMAR, Hannum. IEAA, Hannum.EEAA, or PhenoAgeAccel. However, BD was significantly associated with greater GrimAge acceleration (GrimAgeAccel) in unadjusted ($\beta = 0.18$, $p = 0.015$) and minimally-adjusted models ($\beta = 2.574$, $p = 0.013$, controlled for age, sex, and race), but not after controlling for use of tobacco and blood cell counts ($p = 0.345$). Greater length of illness ($\beta = 0.205$, $p = 0.03$), any comorbid substance abuse or dependence ($\beta = 2.484$, $p = 0.011$), and medication status ($\beta = -4.454$, $p = 0.016$) significantly predicted GrimAgeAccel in all models. Finally, a greater GrimAgeAccel predicted poorer functional outcomes measured by the Functioning Assessment Short Test and Global Assessment of Functioning ($p < 0.05$) in both groups.

Conclusions: Epigenetic aging, as measured by the lifespan predictor GrimAge, is accelerated by comorbid substance use and longer length of illness in BD, with a protective effect of medication. Moreover, this acceleration may contribute to functional decline in patients with BD.

Keywords: Bipolar Disorder, Accelerated Aging, Epigenetic Age Acceleration, DNA Methylation, Functioning

Disclosure: Nothing to disclose.

P363. Polygenic Risk as a Predictor of Bipolar Related Mood Disorder in At-Risk Offspring

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Background: Bipolar Disorder (BD) is highly heritable, but mechanisms of this heritability are still unknown. Polygenic risk scores (PRS) are quantitative markers of risk constructed from large genome-wide association studies (GWAS) that can be calculated on the individual level. We previously built a risk calculator using clinical and demographic variables to predict new-onset BD within offspring of parents with BD. Here, we assessed whether PRS for BD (BD-PRS) predicted new-onset BD and mood disorder in at-risk youth, after accounting for other predictors, and whether adding BD-PRS to a risk calculator improved prediction capability. These findings build on a recent analysis of these data (submitted for publication) showing that BD-PRS was specifically elevated in the parents with BD and at-risk offspring, particularly those with mood disorders. In contrast to the previous analysis, the current work focuses only on effects of BD-PRS within at-risk offspring.

Methods: We calculated BD-PRS scores on a subset of offspring at familial risk in the Pittsburgh Bipolar Offspring Study (BIOS), a longitudinal study of offspring of parents with bipolar disorder I/II and community controls that have been assessed approximately every two years for over twelve years. Since large-scale genome-wide association studies used to derive BD-PRS scoring have been limited to white individuals, we limited our sample to 256 white offspring of parents with bipolar disorder with sufficient genetic data. We first assessed the degree to which, in this at-risk population, BD-PRS predicted bipolar spectrum disorder (BSD; including BD-I, BD-II, and Other Specified BD) in offspring, and whether there were any interactions with other predictors of

interest. Next, we assessed whether BD-PRS predicted any mood disorder, since depression may also be related to BD in at-risk samples. Finally, in previous work, we have developed a risk calculator to predict BSD based on subthreshold clinical symptoms, parental age of onset, global functioning, and age; here, we assessed the degree to which BD-PRS improved this risk calculator to predict BD and mood disorder onset.

Results: Within the 256 offspring of parents with BD, 16 had BSD at intake and 42 developed BSD over the course of follow-up; 36 had mood disorders at intake and 82 developed mood disorders over follow-up. Within the at-risk offspring, the BD-PRS was marginally associated with lifetime BSD (HR = 1.22, $p = 0.1$) and new-onset BSD (HR = 1.28, $p = 0.07$). There was a significant interaction ($p = 0.01$), whereby BD-PRS was a significant predictor of new-onset BD in offspring of parents with early-onset BD (age < 18; HR = 1.49, $p = 0.007$), but not later onset BD (HR = 0.6, $p = 0.1$). A similar pattern was observed with lifetime BD (interaction $p = 0.09$). Higher BD-PRS was associated with a marginally increased rate of developing mood disorders, regardless of parental age of onset (HR = 1.23, $p = 0.06$). However, this increase was not observed across follow-up, but rather limited to age >18 (interaction $p = .01$; offspring <18 years old: HR = 1.00, $p = 0.98$; offspring >18 years old: HR = 1.87, $p = 0.003$). BD-PRS alone had AUCs of 0.57 (0.48-0.67) and 0.59 (0.55-0.62) for new-onset BSD and mood disorder, respectively. In these youth, the original risk calculator for BSD had an AUC of .77 (0.70-0.84); adding BD-PRS, allowing interactions with parental age of onset and age, yielded an AUC of .80 (0.73-0.87). Regarding prediction of mood disorder, the original risk calculator (developed for BSD) had an AUC of 0.78 (0.73-0.82); adding BD-PRS, allowing interactions with parental age of onset and age, did not improve the AUC.

Conclusions: We find that BD-PRS is marginally predictive across the sample of at-risk youth, but that it interacts with other predictors (i.e., age, parent age of onset) to predict more strongly in certain subgroups. Specifically, BD-PRS predicts new-onset BSD only in offspring of parents with early-onset BD; we hypothesize that this may either be because (1) we have not followed the sample long enough to observe the effect of BD-PRS in the offspring of parents with late-onset BD (who may be more likely to have later-onset BSD themselves) or (2) late-onset BD is not as likely to be transmitted via polygenic risk. In contrast, no such interaction is observed with mood disorders (including BSD and depression), where BD-PRS is associated with mood disorder across the sample, but not until adulthood. While BD-PRS generally has poor predictive power for both BSD and mood disorders, combined with other clinical factors, BD-PRS marginally adds to the predictive power of the risk calculator for BSD.

Keywords: Bipolar Disorder, Polygenic Risk Score, Familial Risk, Risk Calculator

Disclosure: Nothing to disclose.

P364. Brain Structure Associated With Future Suicide Thoughts and Behaviors in Female Adolescents With Bipolar Disorder

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Background: Suicide thoughts and behaviors (STBs) often emerge during adolescence. Rates are higher in adolescent females and the majority of adolescents who die by suicide have a mood disorder. While neuroimaging studies of adolescents with mood disorders are emerging, these are primarily investigations of associations to prior STBs which could reflect consequences of the prior STBs rather than represent risk for future STBs. In a collaboration of the international neuroimaging HOPES (Help Overcome and Prevent the Emergence of Suicide) consortium, grey and white matter structure was investigated in female adolescents with and without future STBs.

Methods: The study sample included 92 female adolescents with bipolar disorder or major depressive disorder. Structural and diffusion-weighted magnetic resonance imaging was performed and data processed using Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) consortium pipelines. STBs were assessed on average two years later to provide subject assignment to three groups: a group with future suicide behavior (fSB, $n = 40$), a group with future suicidal thoughts (fST, $n = 33$) and a group without future STBs (fnonSTB, $n = 19$). One-way analyses of covariance comparing baseline grey matter cortical surface area, thickness, subcortical grey volumes, or white matter tensor-based fractional anisotropy across the three groups were performed and were followed by pairwise comparisons in significant regions ($p < 0.05$).

Results: Compared to fnonSTBs, fSTs and fSBs showed significant decreases in cortical thickness of right inferior frontal gyrus pars orbitalis and middle temporal gyrus and lower fractional anisotropy in left uncinate fasciculus and corona radiata. The fSBs additionally showed lower fractional anisotropy in right uncinate and superior fronto-occipital fasciculi.

Conclusions: Results of this HOPES consortium study support alterations in grey and white matter in brain systems that subserve emotion and other behavioral regulation in risk for future STBs in female adolescents with mood disorders. Differences that extend bilaterally in white matter may contribute to risk for the transition to future suicide behavior. These findings suggest these brain regions and the behaviors they subserve as neurobehavioral targets to understand the developmental pathophysiology of STBs and for the generation of strategies to prevent suicide in female adolescents with mood disorders.

Keywords: Suicide Prediction, Suicide Attempt, Suicidal Ideation, Mood Disorders, MRI

Disclosure: Nothing to disclose.

P365. Incilius Alvarius Cell-Based Synthesis of 5-MEO-DMT

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Background: Incilius alvarius (also known as the Colorado River or Sonoran Desert toad) is a source of natural, psychoactive tryptamines including 5-MeO-DMT (5-methoxy-N,N-dimethyltryptamine) and bufotenin. There is growing interest in the psychiatric therapeutic potential of 5-MeO-DMT. Although an optimized chemical synthetic pathway exists for the production of 5-MeO-DMT, there is a need for naturally sourced 5-MEO-DMT to advance research. The parotoid gland of Incilius alvarius also produces a range poorly characterized molecules including bufagenins, bufotoxins and indolealkylamines, possible "entourage molecules" that may have clinical utility. Incilius alvarius is currently under severe ecological pressure due to consumer demand for natural 5-MeO-DMT and habitat loss.

Methods: We anesthetized 2 Incilius alvarius toads and conducted a wedge biopsy under aseptic conditions in conformity with current best practice in the care of laboratory animals. Glandular tissue was identified in the explants, prepared, dissociated and seeded using standard amphibian cell culture protocols in a basal media preparation supplemented with 10% FBS, antibiotics, sugars, hormones and ionic compounds in 24-well cell culture plates. Culture was undertaken in an incubator at 25°C

and 5% CO2. Upon confluence, the cells were disassociated and passaged into additional well plates. Media exchange was done every 3-4 days and the culture was maintained for 45 days. Media was analyzed at 12, 14 and 36 days for the presence of 5-MeO-DMT using a Xevo TQ-S tandem mass spectrometry and UPLC system (Waters Corporation, Milford MA, USA).

Results: Dried media from the cell culture had a light tan appearance that was similar to the known appearance of dried Incilius alvarius parotoid secretion. HRMS/MS fragmentation showed conformity in structure with 5-MEO-DMT. UPLC showed a purity of 16-22% (within the reported range of 5-MeO-DMT concentration in Incilius alvarius parotoid secretion).

Conclusions: To our knowledge, this is the first report of successful production of 5-MeO-DMT from Incilius alvarius parotoid cell culture. These findings constitute preliminary evidence of the feasibility of cell-based 5-MeO-DMT production as a potential source of research and clinical material. The potential availability of "natural" 5-MeO-DMT produced through cellular agriculture, as opposed to the cruel and destructive practice of "milking" Incilius alvarius, also supports efforts to ensure the protection of our planet's entheogen heritage.

Keywords: Psychedelics, Natural Compound, Dimethyltryptamine

Disclosure: Taliaz, Back of the Yard Algae Sciences: Advisory Board (Self) Hadasit Medical Research Corporation: Patent (Self)

P366. Analgesic and Antinociceptive Effects of the Ketamine Metabolite (2R,6R)-Hydroxynorketamine (HNK) in Mice

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Background: An estimated 20% of adults in the U.S. suffer from an ongoing pain condition, and their pain may cause significant limitations in their work capability, social life, and ability to provide self-care. Severe chronic pain is challenging to treat and increases the risk for developing psychological disorders such as depression. Identifying novel treatment modalities that are effective in alleviating pain is essential to improve clinical treatment and rehabilitation for patients with ongoing pain conditions.

(2R,6R)-hydroxynorketamine (HNK) is a ketamine metabolite that has emerged from laboratory studies as possessing antidepressant effects while lacking the severe side effects of the parent drug. This study examined whether (2R,6R)-HNK may also exert another therapeutic effect of ketamine, analgesia. The results show that (2R,6R)-HNK has the potential to be a safer pain treatment alternative to ketamine that could be made widely available to patients.

Methods: Male and female C57BL/6J mice, age 8-12 weeks, were group-housed (4-5 per cage) in 12-hour light/dark cycles with standard food and water available ad libitum. All drugs were administered via the intraperitoneal route. All experimental protocols were approved by the Uniformed Services University IACUC and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Two measures of pain sensitivity were used. Sensitivity to a noxious thermal stimulus was assessed by measuring the latency to respond once placed upon a hot plate set to 50°C. Localized inflammatory pain was induced in male mice via subcutaneous λ-carrageenan injection into the animal's hind paw. Mechanosensitivity before and after induction of inflammatory pain was measured by the paw withdrawal threshold using manual von Frey filaments. Carrageenan produced mechanical allodynia within 2 hours that lasted for more than 48 hours. Mice were treated with (2R,6R)-HNK, carprofen, or saline 2 hours following the induction of inflammation.

Results: (2R,6R)-HNK produced antinociceptive effects on the hot plate in male and female mice. The antinociceptive effect was similar for both sexes with onset approximately 10 hours and duration lasting between 24 to 48 hours. Doses of 10-16 mg/kg produced significant antinociception in males, while doses of 10-30 mg/kg were significant in females. The pain reducing effects in both sexes were diminished at doses above 16 and 30 mg/kg in males and females, respectively.

Mice were pretreated with different receptor antagonists to examine the potential mechanism for (2R,6R)-HNK mediated antinociception. The opioid receptor antagonist naltrexone (1 mg/kg) administered 45 minutes before (2R,6R)-HNK had no impact on its antinociceptive effects, but did block morphine-induced antinociception. In contrast, the AMPA receptor blocker NBQX (10 mg/kg) administered 30 minutes before (2R,6R)-HNK prevented its antinociceptive effects, suggesting a glutamatergic mechanism for (2R,6R)-HNK antinociception.

In the inflammatory pain condition, (2R,6R)-HNK produced a reversal of mechanical allodynia in male and female mice at doses of 10 and 30 mg/kg. The onset for inflammatory analgesia was less than 1 hour with a duration greater than 24 hours following a single injection. In a separate experiment with male animals only, the pain reduction effects of (2R,6R)-HNK (10 mg/kg) were compared with carprofen (5 mg/kg), a NSAID typically used to treat inflammatory pain. The effect of (2R,6R)-HNK was comparable with the effects of carprofen on the reversal of mechanical allodynia.

Conclusions: These results demonstrate that (2R,6R)-HNK produces pain reduction under two conditions, antinociception in healthy mice and reversal of allodynia in mice with an inflammatory pain condition. These findings agree with a previous report showing that (2R,6R)-HNK produced a reversal of hyperalgesia present in preclinical models of postoperative type pain, neuropathic pain, and CRPS type pain (Kroin et al., 2019). The mechanism involves glutamatergic AMPA receptors but not opioid receptors. The characteristics of (2R,6R)-HNK reversal of allodynia shown here demonstrate great promise for its potential in treating inflammatory pain in addition to other types of pain.

Keywords: Pain sensitivity, Analgesia, (2R,6R)-hydroxynorketamine, Anti-inflammatory

Disclosure: Nothing to disclose.

P367. Levomilnacipran but Not Duloxetine Inhibits Norepinephrine Reuptake Throughout its Therapeutic Dose Range in Healthy Male Volunteers

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Background: Several medications that potently inhibit norepinephrine (NE) reuptake are effective in the treatment of major depressive disorder (MDD). Norepinephrine reuptake inhibition can also exert therapeutic benefits in attention deficit disorder. Dual serotonin (5-HT)/NE reuptake inhibitors are also first-line treatment for MDD, but for venlafaxine and duloxetine doses higher than those necessary to potently inhibit 5-HT reuptake are necessary to inhibit the NE reuptake process [1-3]. While occupancy of 5-HT reuptake transporters has been reliably assessed using positron emission tomography in humans, there remain specificity issues with the main ligand used to label NE transporters [4]. An alternative approach to assessing NE reuptake is the tyramine pressor response. Despite being a marker of peripheral NE reuptake activity, the latter test correlates well with the capacity of drugs like desipramine,

clomipramine, and venlafaxine to inhibit NE reuptake [1-3]. The objectives were to assess: 1) whether levomilnacipran and duloxetine significantly inhibit NE at their minimal effective doses for MDD (40 and 60 mg/day, respectively), and 2) at what dose these two medications begin to significantly inhibit NE reuptake.

Methods: Healthy male participants (18-40 years of age) were initially randomized to take either placebo for 21 days, levomilnacipran (40 mg/day for 7 days, increased to 80 mg/day for 7 days, and then 120 mg/day for 7 days), or duloxetine (60 mg/day for 7 days, 90 mg/day for 7 days, and 120 mg/day for 7 days). Participants could prolong administration periods to allow for adaptation to side effects. The activity of NE transporters was evaluated by assessing the increase in systolic blood pressure (SBP) using iv bolus of 4, 6, and 8 mg of tyramine.

Results: : There were 10 completers in the placebo group, 10 for levomilnacipran, and 9 for duloxetine. Two participants withdrew due to side effects, one in each of the active treatment arms. Tyramine injections produced significant dose-dependent increases in SBP at baseline in all three groups ($p < 0.001$) and the pressor responses were not modified after 7, 14 or 21 days in the placebo group ($p > 0.05$). Using post-hoc two-way repeated measures MANOVA, levomilnacipran separated from placebo at 40 mg/day ($F1,15 = 32.0$, $p < 0.0001$), whereas duloxetine separated from placebo only at 120 mg/day ($F1,11 = 7.5$, $p = 0.02$).

Conclusions: These results confirm that both levomilnacipran and duloxetine have the capacity to block NE reuptake within their therapeutic ranges. However, they differ in their doses necessary to interfere significantly with the NE reuptake process: levomilnacipran acts as a robust NE reuptake blocker from its minimal effective dose for MDD, whereas duloxetine needs to reach its maximal recommended therapeutic dose. Taken together with prior results [1,2], duloxetine can only be considered a dual reuptake blocker when titrated to its maximal recommended dose. Ongoing analyses from this data set will determine the dose at which levomilnacipran begins to engage the 5-HT reuptake process to a physiologically relevant degree.

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Keywords: Selective Serotonin Reuptake Inhibitors (SSRIs), Serotonin and Norepinephrine Reuptake Inhibitor, Levomilnacipran, Duloxetine

Disclosure: Allergan: Grant (Self)
Allergan: Honoraria, Consultant (Self)

P368. Functional Connectivity Predictors of Antidepressant Treatment Outcomes in Late-Life Depression

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Background: Late-life depression is associated with a poor response to conventional antidepressant medications and high recurrence risk. Past work in late-life depression examining predictors of antidepressant outcomes has primarily focused on cognitive performance deficits and structural markers of brain aging. There has been less work examining how measures of intrinsic network functional connectivity (FC) may influence antidepressant treatment outcomes in depressed elders.

Methods: 95 individuals aged 60 years or older with Major Depressive Disorder were enrolled in a single-site, randomized 8-week trial of escitalopram or placebo in a two-to-one ratio. MRI was obtained after a two-week washout of pre-existing antidepressant medications but prior to starting blinded study drug. Resting-state fMRI was analyzed by measuring seed-to-seed functional connectivity in predetermined a priori regions focusing on key intrinsic functional networks including the default mode network (DMN), cognitive control network, and limbic network. Primary analyses utilized mixed models examining depression severity (by MADRS) as the repeated outcome measure. Covariates included time, treatment assignment, age, gender, white matter hyperintensity volume, and seed-to-seed FC. Initial models examined a three-way interaction between time, treatment arm, and FC. If this did not achieve statistical significance, the three-way interaction was removed, and two-way interactions were considered.

Results: Analyses of network FC exhibited the strongest relationships with treatment outcome with DMN regions. In mixed models, a poorer response to treatment was associated with FC in the DMN, including the posterior cingulate cortex (PCC) and left ($t = -2.09$, $p = 0.0372$) and right ($t = -2.13$, $p = 0.0364$) hippocampi. We further observed relationships with treatment outcome in anterior DMN regions including FC between the medial PFC and left ($t = -2.38$, $p = 0.0179$) and right ($t = -2.85$, $p = 0.0055$) rostral anterior cingulate cortex (ACC). We similarly observed a relationship between response and FC between the left hippocampus and subgenual ACC ($t = 2.19$, $p = 0.0312$). Subsequent exploratory analyses extended these findings, associating treatment response with FC in the cognitive control network, limbic network, and additional DMN regions.

Conclusions: DMN and hippocampal connectivity measures obtained pre-treatment are associated with subsequent treatment response. Further work is needed to determine how brain aging influences these key intrinsic networks in order to decrease the likelihood of responding to treatment.

Clinical trials.gov registry: NCT02332291

Keywords: Depression, rsfMRI Functional Connectivity, Clinical Trial, Geriatric

Disclosure: Nothing to disclose.

P369. A Novel Peripheral Biomarker for Depression and Antidepressant Response

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Background: Previous data suggest that the heterotrimeric G protein, Gsalpha (Gsα) is ensconced predominantly in lipid rafts in subjects with major depressive disorder (MDD), resulting in impaired stimulation of adenylyl cyclase. Both diminished Gsα-adenylyl cyclase coupling and an increase in the proportion of Gsα in lipid rafts have been observed in MDD. Antidepressants accumulate slowly in lipid rafts and evoke translocation of Gsα out of lipid rafts toward a more productive association with adenylyl cyclase, resulting in sustained cAMP elevation and sequelae such as increased brain-derived neurotrophic factor.

"Rapid-acting" antidepressant compounds, such as ketamine, have the same effect, but on an accelerated timescale. Thus, we hypothesize that heightened accumulation of Gsα in lipid rafts is a biomarker for depression, and that the translocation of Gsα from those rafts is a biomarker for clinical response to antidepressants. We examined the potential of this biomarker in a small proof-of-concept study of subjects with MDD where it may be harnessed for diagnostic and treatment-prognostic purposes.

Methods: Subjects meeting DSM5 criteria for acute major depressive disorder (MDD) with a minimum HamD-17 score ≥15 and healthy controls were recruited at Emory University school of medicine and consented to collection of blood samples to measure PGE1 activation in platelets (as an indicator of Gsα-adenylyl cyclase coupling) with the AlphaScreen (Perhin Elmer) assay. Subsequently, consenting MDD subjects participated in a 6-week open label antidepressant therapy and were evaluated by HAM-D17 (primary), HAM-D6, MADRS, and subject rated IDS-SR30 at screen and 6 weeks. Blood draws were scheduled at screening for all participants and 6 weeks after antidepressants for the consenting MDD subjects. Comparison of the extent of PGE1 activation of adenylyl cyclase (over "basal") was used to indicate the extent of coupling of Gsα with adenylyl cyclase. Change in the extent of biomarker activation was compared in antidepressant responders (improvement of HAM-D17 ≥ 50% from screen) and non-responders in this small study.

Results: There were 20 subjects with MDD and 45 healthy controls completing the study. At screen, platelet samples examining PGE1 from MDD subjects showed a lower response to PGE1 activation than healthy controls. Eleven of 20 participating MDD subjects were antidepressant responders at 6 weeks. Antidepressant responders showed a marked increase in PGE1 activated adenylyl cyclase at 6 weeks compared to non-responders. Responders revealed a 62.0% improvement of PGE1 activation from the screen assessment in contrast to a -14.1% decrement in the non-responder cohort (ANOVA: $F = 5.25$; $p = 0.03$). The calculated effect size was 0.93 for the PGE/Gsα lipid-raft biomarker. Eight of the 11 responders (72.7%) on contrast to 2 of the 9 non-responders (22.2%) improved by at least 30% from the screen assessment in the PGE1 activation analysis (Fisher exact = 0.07), and the positive predictive value of PGE1 activation for response/non-response was 75.0%.

Conclusions: In this small sample, the translocation of Gsα from lipids rafts as reflected by an increase in PGE1 activated adenylyl cyclase was used as a biomarker reflecting cAMP signaling/lipid-raft status of Gsα and was associated with antidepressant response in MDD subjects. These data suggest that a simple, high-throughput capable assay for antidepressant response can be developed and used to create a platform for personalized medicine for subjects with MDD. Future studies will determine whether this biomarker can anticipate antidepressant response early in treatment and prior to the clinical metrics.

Keywords: Cyclic AMP, Lipid Rafts, GPCR, Antidepressant, Biomarker

Disclosure: Pax Neuroscience: Stock / Equity (Self)

P370. Backtranslating Sex Differences in Peripheral Immune Dysregulation From Patients With Major Depressive Disorder to Mice

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Background: Women account for 2/3rds of the 300 million people world-wide who have depression and are more likely to be treatment resistant. Women may present with fundamentally different symptoms of depression supporting the possibility that men are being under diagnosed. In an age of personalized medicine, no current treatments for depression take sex into consideration. Here we examined the relationship between sex, treatment resistant status, symptomatology and circulating cytokines in patients with depression. We back translate our findings into mice and identified key cytokines linked to behavioral endophenotypes that differ within the context of sex.

Methods: We used multiplex ELISA to quantify plasma cytokine protein levels regulated by treatment resistant ($n = 27$) and non-treatment resistant depression ($n = 23$) in men and women compared to age matched (21-55 years) healthy controls ($n = 28$). To test the ability of mouse stress models to recapitulate the cytokine changes found in depressed individuals we examined circulating levels of cytokines for male and female mice exposed to 6 ($n = 40$) or 28 day ($n = 40$) variable stress. Cytokines from patients with depression were correlated to subtypes of behavior indicated by the QIDS index. Cytokines from mice exposed to variable stress were correlated with behavioral responses across a test battery that included novelty induced hypophagia, splash test, forced swim test and social interaction.

Results: We found a number of cytokines that were significantly altered by depression vs. healthy controls across depression subtype/ sex including IL10, MCP-1 VEGF, IL-17 and IFN-y . However, most cytokines differed by a combination of treatment resistant status and sex. Women with treatment resistant depression had the largest number of cytokine changes, followed by non-treatment resistant men. Most cytokine changes negatively correlated with concentration in women with treatment resistant depression (p values < 0.05). IL-1a positively correlated with over-eating and weight gain. MCP-1 positively correlated with oversleeping. In contrast, for women who were not treatment resistant, most cytokine changes related to oversleeping/over-eating, loss of interest and restlessness (p values < 0.05). In men with treatment resistant depression most cytokine changes related to feelings of restlessness (p values <0.05) and insomnia (GSF/IL12). Many of the cytokines altered in humans were not detectable in mice. However, of those we could detect IL-12p40, GM-CSF, MCP-1 and IL10 were altered by 6-day variable stress. Whereas only IL12p40 was altered by 28 days of stress. Correlations between cytokines and behaviors in mice following stress matched more closely with relationships between cytokines and endophenotypes in humans with depression.

Conclusions: Cytokines changes in blood of patients with depression were driven by the sex and treatment resistant status of the individual. Overall, cytokine levels are predicative of different specific symptomatology of patients with depression and that relationship differed by treatment resistant status and sex. While only a few of the cytokine changes in depressed patients could be altered by stress in rodents, the relationship between cytokines and behavioral endophenotypes could be recapitulated. These data suggest examining cytokine levels within the context of symptomatology and individual differences when identifying accurate biomarkers and treatment targets.

Keywords: Depression Inflammation Cytokine, Stress, Sex Differences

Disclosure: Nothing to disclose.

P371. A Combinatorial Pharmacogenomic Algorithm is Predictive of Sertraline Metabolism in Patients With Major Depressive Disorder

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Background: Pharmacogenomic testing can aid in treatment selection for patients with Major Depressive Disorder (MDD) by identifying gene-drug interactions that may impact medication metabolism. Although there have been rapid advancements in this field, there is not a consensus about the approach to pharmacogenomic testing or even what genes are relevant for many antidepressants. Here we assessed the ability of a combinatorial pharmacogenomic test (weighted assessment of multiple genes) to predict meaningful variations in sertraline blood levels relative to the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for CYP2C19.

Methods: All patients were enrolled in the Genomics Used to Improve DEpression Decisions (GUIDED) trial – a large, patient- and rater-blinded, randomized, controlled trial that included patients diagnosed with MDD who had an inadequate response to ≥1 psychotropic medication ($N = 1{,}167$). A subset of 124 patients reported taking sertraline within 2 weeks of the screening blood draw and had sertraline blood concentrations quantified using LC-MS/MS. The combinatorial pharmacogenomic test reported a weighted assessment of individual phenotypes based on multiple pharmacokinetic genes relevant for medications included on the test. For sertraline, this included CYP2C19, CYP2B6, and CYP3A4. Medications were categorized according to the predicted level of gene-drug interactions (GDI) and change in metabolism (increase, decrease). Sertraline log-transformed concentration/dose ratios were compared between combinatorial pharmacogenomic test categories and CPIC CYP2C19 phenotypes. Tests were linear trend tests.

Results: Sertraline concentration/dose ratios were significantly different between CY2C19 phenotypes ($p = 0.0003$) and gene-drug interaction categories from the combinatorial pharmacogenomic test ($p = 5.8e\text{-}06$). Sertraline blood levels were 71% lower when the combinatorial pharmacogenomic test predicted significant GDI with increased metabolism compared to no GDI ($p = 0.001$). Similarly, sertraline blood levels were 134% higher when the combinatorial pharmacogenomic test predicted significant GDI with decreased metabolism compared to no GDI ($p = 2.7x10\text{-}5$). In a multivariate analysis that included CYP2C19 and the combinatorial pharmacogenomic algorithm, only the combinatorial pharmacogenomic algorithm remained significant ($p = 3.8x10\text{-}5$).

Conclusions: Combinatorial pharmacogenomic testing was a significant predictor of sertraline blood levels for patients within the GUIDED trial, accounting for all variance predicted by CYP2C19 alone and adding significant, independent information. This suggests that the combinatorial pharmacogenomic test may provide more clinically relevant information to inform medication decisions regarding sertraline compared to phenotypes based on CYP2C19 alone.

Keywords: Pharmacogenomics, Sertraline, Major Depressive Disorder (MDD)

Disclosure: Janssen, Otsuka: Contracted Research (Self)
Sage Therapeutics: Advisory Board (Self)
Xenon Pharmaceuticals: Consultant (Self)
American Psychiatric Press, Wolters Kluwer, University of Massachusetts Medical School: Royalties (Self)

P373. Metabolic Profile of Lumateperone (ITI-007) Monotherapy in Bipolar Depression: A Post Hoc Analysis of 2 Randomized, Placebo-Controlled Trials

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Background: Treatments for bipolar depression are limited and can be associated with a spectrum of undesirable side effects, including metabolic disturbances. Lumateperone (lumateperone tosylate, ITI-007), a mechanistically novel antipsychotic that simultaneously modulates serotonin, dopamine, and glutamate neurotransmission, is FDA-approved for the treatment of schizophrenia and is being investigated in bipolar depression. This novel mechanism of action could confer improved tolerability compared with available treatment options for patients with bipolar I or bipolar II disorder experiencing a major depressive episode (MDE, bipolar depression).

Lumateperone 42 mg monotherapy was evaluated in 2 Phase 3 randomized, double-blind, placebo-controlled, 6-week trials (Study 401 [NCT02600494], Study 404 [NCT03249376]) in patients with bipolar depression. A pooled safety analysis of these studies assessed the metabolic profile of lumateperone 42 mg monotherapy in the treatment of bipolar depression.

Methods: The 2 studies investigated lumateperone 42 mg treatment in patients (18–75 years) with a confirmed diagnosis of bipolar I or bipolar II disorder experiencing an MDE (Montgomery-Åsberg Depression Rating Scale [MADRS] Total score ≥ 20 and Clinical Global Impression Scale-Bipolar Version-Severity [CGI-BP-S] score ≥ 4). Lumateperone 42 mg was administered orally once-daily for 6 weeks. Safety data were pooled from studies 401 and 404 including adverse events (AEs) that were spontaneously reported or discovered during physical examination, laboratory parameters, and vital signs.

Results: The safety population comprised 746 patients (lumateperone, 372; placebo, 374). Compared with placebo, mean changes from baseline to the last on-treatment value in weight were similar for lumateperone 42 mg (+0.06 kg) and for placebo (+0.19 kg). There was no potentially clinically significant weight gain (≥7% increase) during the treatment period for lumateperone 42 mg (lumateperone, 0%; placebo, 1.4%). Mean increase from baseline in body mass index for lumateperone (+0.02 kg/m2) was similar to placebo (+0.07 kg/m2). Changes in mean waist circumference were not clinically significant between groups (lumateperone, 0.18 cm; placebo, 0.03 cm).

Mean change from baseline to the last on-treatment measure in cholesterol were similar between treatment groups for total cholesterol (lumateperone, −0.6 mg/dL; placebo, −1.1 mg/dL), LDL-cholesterol (lumateperone, −0.7 mg/dL; placebo, −0.6 mg/dL), and HDL-cholesterol (lumateperone, +0.4 mg/dL; placebo, 0.0 mg/dL). Mean changes from baseline to the last on-treatment measure were similar between groups for triglycerides (lumateperone, −1.4 mg/dL; placebo, −4.0 mg/dL) and glucose (lumateperone, +0.1 mg/dL; placebo, 0.0 mg/dL).

Conclusions: In patients with bipolar I or bipolar II disorder with an MDE, lumateperone 42 mg monotherapy was generally well-tolerated and had a metabolic profile that was similar to placebo. These results suggest indirectly that lumateperone may have a more benign metabolic profile than currently approved treatments for bipolar depression.

Keywords: Bipolar Depression, Mood Disorders, Bipolar I And II Disorder, Antipsychotic Treatment, Psychotic Disorders

Disclosure: IntraCellular Therapies, Inc.: Employee, Stock/Equity (Self)

P374. Preliminary Results for ECT Amplitude-Determined Seizure Titration to Maximize Clinical and Cognitive Outcomes

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Background: Electroconvulsive therapy (ECT) stimulation parameter selection reflects a balance between antidepressant efficacy and cognitive adverse effects. ECT stimulation parameters

associated with more antidepressant efficacy (non-focal electrode placement, longer pulse width) are associated with increased risk of cognitive adverse effects. Amplitude is currently fixed at 800 or 900 milliamperes (mA) in standard clinical practice with no clinical or scientific basis. Amplitude determines the intensity of the spatial distribution of the electric field (E-field). With a fixed extracranial amplitude, the ECT "dose" as represented by the E-field is highly variable due to anatomic differences in skin, skull, fluid, and brain tissue. This anatomic variability is prominent in older (age 50+) patients with depression and can compromise both antidepressant efficacy (insufficient stimulation of mood-related circuitry) and safety (inducing cognitive impairment due to excessive stimulation of cognitive related circuitry). Amplitude titration can reduce the variability related to fixed amplitude dosing and optimize clinical and cognitive outcomes. Here, we performed amplitude-determined seizure titration during the first treatment with subsequent treatments at fixed amplitude (800 mA). We assessed the relationship between amplitude-determined seizure titration and antidepressant and cognitive outcomes with fixed amplitude ECT.

Methods: Subjects with major depressive disorder or bipolar II diagnoses and age between 50 and 80 years received antidepressant ratings (Inventory of Depressive Symptomatology – Clinician Rated (IDS-C)) and neuropsychological assessments pre-, mid-, and post-ECT. For cognitive outcomes, we focused on the Delis Kaplan Executive Function Verbal Fluency Test (Letter Fluency Scaled Score; DKVFLFSS), a frontal-temporal cognitive task sensitive to cognitive impairment with right unilateral electrode placement (RUL) and amplitude strength. The first treatment determined amplitude-determined seizure threshold with RUL. Amplitude titration requires an interface (Soterix Medical 4x1 High Definition – ECT Multi-channel Stimulation Interface, Investigational Device Exemption #G200123) to attenuate the pulse amplitude from an FDA-cleared ECT device. Subsequent treatments were completed with RUL at 800 mA amplitude. Pulse width (1.0 milliseconds), pulse train duration (8 seconds), and frequency (20 hertz) were fixed during amplitude titration and subsequent treatments. Subjects had to demonstrate antidepressant response (defined as > 25% reduction in the IDS-C) to remain in the experimental protocol. If subjects did not demonstrate sufficient antidepressant response, subjects received bitemporal electrode placement (BT) for the remainder of the ECT series. For these preliminary results, we used descriptive and non-parametric statistics (spearman rank correlation coefficient, Wilcoxon rank-sum test) to assess the relationship between amplitude determined seizure threshold and antidepressant and cognitive results with RUL.

Results: Eight subjects (64.6 years +/- 10.5, 3 females) completed the study protocol to date. Amplitude-determined seizure thresholds demonstrated a wide range (361.6 mA +/- 160.1, range: 189 – 686 mA). Sex differences were not evident with amplitude determined seizure threshold ($z = 1.0$, $p = 0.30$). The relationship between amplitude-determined seizure threshold and antidepressant response (percent change in IDS-C) showed an inverse relationship (rho = -0.71, $p = 0.04$). Females had improved antidepressant response relative to males ($z = -2.24$, $p = 0.03$). The relationship between amplitude-determined seizure threshold and cognitive outcome (difference in DKVFLFSS) was not significant (rho = -0.49, $p = 0.21$). Sex differences were not evident with cognitive outcomes ($z = -1.81$, $p = 0.07$).

Conclusions: Our data demonstrated a 3.5x range of amplitude-determined seizure thresholds providing early support for individualized and empirically determined amplitudes. The inverse relationship between amplitude-determined seizure threshold suggested that lower amplitudes for seizure titration were consistent with subsequent antidepressant response with 800 mA. While amplitude titration will standardize the ECT dose across individuals with anatomic differences, the RUL amplitude necessary for seizure initiation will be insufficient to induce hippocampal neuroplasticity and antidepressant response. Analogous to the "six-times seizure threshold" multiplier for RUL ECT, amplitude-determined seizure threshold and the individualized amplitude sufficient for antidepressant response will require a multiplier that will bridge amplitude titration to the hippocampal "neuroplasticity threshold", which is defined as E-field sufficient for neuroplasticity. The implications of this relationship suggest that individuals with a higher amplitude-determined seizure threshold may need higher amplitudes with RUL (> 800 mA) or BT electrode placement to achieve an adequate ECT dose. Our goal is to further delineate the relationships between amplitude titration, electric field strength, and hippocampal neuroplasticity to individualize ECT-amplitude and maximize both clinical and cognitive outcomes.

Keywords: Electroconvulsive Therapy, Major Depressive Disorder (MDD), Cognition

Disclosure: Nothing to disclose.

P375. Plasma Cell-Free DNA Methylomics of Bipolar Disorder With and Without Rapid Cycling

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Background: Rapid cycling (RC) burdens bipolar disorder (BD) patients further by causing more severe disability and increased suicidality. Because diagnosing RC can be challenging, RC patients are at risk of rapid decline due to delayed suitable treatment. Circulating cell-free DNA (cfDNA) present in plasma, urine, cerebrospinal fluid, and other bodily fluids are short DNA fragments believed to be derived from cells undergoing apoptosis and necrosis and, possibly, released by active secretion thus allowing cfDNA to become potential biomarker of various conditions and diseases. Here, we aimed to identify the differences in the circulating cfDNA methylome between BD patients with and without RC. The cfDNA methylome could potentially be developed as a diagnostic test for BD RC.

Methods: This study included 93 (46 RC and 47 non-RC) participants enrolled in the Mayo Clinic Bipolar Disorder Biobank. The biobank was approved by the Mayo Clinic Institutional Review Board. Clinical phenotypes were confirmed by the Structured Clinical Interview for DSM-IV. About 25 ng of cfDNA was used for bisulfite conversion and methylome microarray analysis performed at the University of Minnesota Genomics Center in a single batch. Genome-wide methylomic profiling was performed on the 850K Infinium MethylationEPIC BeadChip platform (Illumina Inc., San Diego, CA, USA).

Principal component analysis (PCA) on the top 2000 most variable CpG probes considering all samples was conducted to assess global differences in methylome. Levels of cfDNA methylation were compared between RC groups using a linear model adjusted for age and sex. Association between the clusters and clinical factors were tested by chi-square and Fisher's exact tests for categorical variables and t-tests for continuous variables.

We used the cell-type methylomic deconvolution algorithm to estimate the cell types of origin in each subject's plasma cfDNA sample. Differentially methylated positions (DMPs; methylation status at individual sites) were identified by the R Bioconductor package limma. Differentially methylated regions (DMRs; methylation status across a genomic region) were identified by the R Bioconductor package DMRcate. Gene set enrichment analysis to determine if methylation levels of any gene sets were significantly

associated with rapid cycling was performed using the missMethyl Bioconductor R package.

Results: Rapid cyclers and non-rapid cyclers were not significantly different in terms of age, sex, substance abuse and dependence history, and the use of lithium, mood stabilizers, and antidepressants. A significantly higher percentage of subjects were on antipsychotics in the rapid cycling group than the non-rapid cycling group (65.2% vs 40.4%).

PCA suggested differences in methylation profiles between RC groups ($p = 0.039$) although no significant differentially methylated probes (DMPs; $q > 0.15$) were found. The top four CpG sites which differed between groups at $p < 1E\text{-}05$ were located in CGGPB1 which encodes for CGG triplet repeat-binding protein 1, a nuclear protein that selectively binds to unmethylated CGG trinucleotide repeats; PEX10 which encodes peroxisomal biogenesis factor 10, a ubiquitously expressed peroxisomal matrix protein; NR0B2 which encodes nuclear receptor subfamily 0 group B member 2, an orphan nuclear receptor that interacts with receptors of estrogen, retinol, bile acid, and thyroid hormone to suppress the transcriptional activity of these nuclear receptors as well as with peroxisome proliferator-activated receptor α and γ; and TP53I11 (tumor protein P53-inducible protein 11), a target of TP53 with important role in conserving stability by preventing genome mutation.

Gene set enrichment analysis on top DMPs ($p < 0.05$) showed significant enrichment of gene sets related to nervous system tissues, such as neurons, synapse, and glutamate neurotransmission. Other top notable gene sets were related to parathyroid regulation and calcium signaling.

Between rapid cyclers and non-rapid cyclers, no significant differences in estimated cfDNA cell/tissue types of origin were found ($p >$ Bonferroni-adjusted $\alpha = 0.05/26 = 0.001923$), hence no adjustment in tissue/cell type origin distribution on the methylome analysis was conducted.

Conclusions: In this study, we used a microarray method to detect plasma cfDNA methylation levels to examine the cfDNA methylomic differences that were associated with rapid cycling in BD. We found global differences in methylation profiles between BD rapid cyclers and non-rapid cyclers, four CpG sites with differential methylation levels between BD rapid cyclers and non-rapid cyclers at $p < 1E\text{-}05$ (not genome-wide significant), and significant enrichment in pathways related to neurons and synaptic functions among the top CpG sites that differed between the groups. To our knowledge, this is the first investigation of cfDNA methylomics as potential biomarkers for BD rapid cycling, thereby marking an example of cfDNA application in psychiatric diagnosis and providing insights into amelioration of rapid mood cycling in BD.

Keywords: Cell-free DNA, Epigenetics, Bipolar Disorder, Rapid Cycling, Biomarker

Disclosure: Nothing to disclose.

P376. Examining White Matter Microstructure in Suicide Attempt and Treatment-Resistant Depression Using Tract-Based Spatial Statistics and Free Water Imaging

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Background: Clinical prediction of suicide attempt is limited, and the neurobiological factors distinguishing suicide ideators from suicide attempters are understudied. Preliminary investigations have indicated the use of spatially and temporally specific neuroimaging modalities to illustrate circuit-level dysfunction in psychiatric disease, including depression and suicide. Diffusion Tensor Imaging (DTI) provides the opportunity to identify white matter microstructural correlates associated with suicide attempt history. The source of white matter alterations may be further elucidated using free water imaging correction to isolate signal specific to the fibre tract and quantify the fractional volume of the free water compartment.

Methods: DTI data were acquired from $N = 36$ male and female patients with treatment-resistant major depressive disorder ($n = 20$ suicide ideators, $n = 16$ suicide attempters, ages 18-65 years). Tract-based spatial statistics (TBSS) was performed in FMRIB Software Library (FSL) using an unpaired two-samples t-test including age and sex as covariates. Free water imaging correction was applied through estimation of a constrained bi-tensor model via an in house MatLab-based script developed at Harvard University. Between-group differences of suicide ideators versus attempters were identified at a family-wise error (FWE) corrected significance threshold of $p \leq 0.05$. Subsequent exploratory analyses were performed at an uncorrected significance threshold of $p \leq 0.01$.

Results: Suicide ideator and attempter groups were well matched with no significant between-group differences in age, sex, handedness, depression severity, length of current major depressive episode, number of previous depressive episodes, or age of onset of major depressive disorder (all $p > 0.05$). TBSS revealed significantly elevated mean and axial diffusivity in suicide attempters relative to suicide ideators, as well as elevated extracellular free water in several fronto-thalamo-limbic tracts (FWE $p \leq 0.05$). Exploratory analyses revealed reduced fractional anisotropy and elevated radial diffusivity in focal white matter areas (uncorrected $p \leq 0.01$). Alterations in several overlapping tracts across diffusion metrics included the inferior and superior longitudinal fasciculi, uncinate fasciculus, inferior fronto-occipital fasciculus, anterior thalamic radiation, anterior limb of the internal capsule, and cingulum bundle adjacent to the hippocampus. Free water correction appeared to increase detection of fractional anisotropy changes and suppress spurious differences in axial and radial diffusivity.

Conclusions: In this preliminary investigation, the identification of significantly altered diffusion metrics in suicide attempters compared to suicide ideators suggests white matter pathology in treatment-resistant depression and suicide attempt. The effect of free water correction on all diffusion metrics and the elevation of free water itself provide evidence toward the source of anisotropic changes. To our knowledge, these results provide the first evidence of extracellular free water alterations associated with history of suicide attempt. Future investigations are recommended to explore the combined impact of these measures in suicide and depression.

Keywords: Diffusion Tensor Imaging (DTI), Suicide Attempt, Treatment-Resistant Depression, Free Water Imaging

Disclosure: Nothing to disclose.

P377. Depression Epigenome Wide Association Study Meta-Analysis Reveals Differential Methylation Positions and Regions Annotated to TNNT3

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Background: Epigenetic mechanism has been hypothesized to play a role in MDD etiology. In this study, we performed meta-analysis between two case-control MDD cohorts to identify differentially methylated positions (DMPs) and differentially methylated regions (DMRs).

Methods: Whole blood samples were collected from two cohorts and DNA methylation was measured using Illumina MethylationEPIC array at 850 000 CpG sites throughout the genome. For the first

cohort, the controls were self-reported not to have MDD collected by BioIVT ($N = 32$) and the MDD cases ($N = 191$) were drawn from OBSERVEMDD0001 study (NCT02489305), where a patient must have met DSM-V criteria for nonpsychotic, recurrent MDD within the past 24 months (ie, the start of the most recent major depressive episode (MDE) must be ≤ 24 months before screening); have a Montgomery Asberg Depression Rating Scale (MADRS) total score ≤14 at screening and baseline visits; have evidence of recent response (within the past 3 months) to an oral antidepressant treatment regimen (taken at an optimal dosage and for an adequate duration); and be currently taking and responding to an oral antidepressant treatment regimen. The samples from the participants with MDD could have been obtained from either a baseline visit or a follow up visit. For the second cohort, the MDD cases ($N = 359$) were drawn from the Molecular Biomarkers of Antidepressant Response study, where a patient must have had a diagnosis of current MDE, as per the SCID-I and HAMD-21 ≥ 20, while the controls ($N = 68$) were recruited through advertisement. Two or more samples from the same patient could have been collected. EWAS association analysis was conducted using limma while correcting for age, gender, estimated cell composition, five surrogate variables aiming to capture systematic technical variations (sample relatedness were corrected for using duplicateCorrelation) for each cohort. Inflation of test statistics was also corrected using BACON. This was followed by a meta-analysis between the 2 cohorts. DMPs with association *p*-values less than 6x10-8 were considered as study wide significant. Methylglm based pathway enrichment analysis was performed using DMPs with association *p*-value less than 0.0001. Lastly, comb-*p* analysis was performed to identify DMRs in the genome consisting of ≥ 3 probes. For DMR analysis, a Sidak-corrected *p*-value less than 0.05 was considered as study wide significant.

Results: Multiple cytosine-phosphate-guanine (CpG) sites annotated to TNNT3 were associated with MDD status reaching study wide significance, including cg08337959 ($p = 2.3$ x 10-11). Among DMPs with association *p*-values less than 0.0001, pathways from Reactome such as ras activation upon Ca2+ influx through NMDA receptor ($p = 0.0001$, *p*-adjusted = 0.05) and long-term potentiation ($p = 0.0002$, *p*-adjusted = 0.05) were enriched in the depressed samples. A total of 127 DMRs with Sidak-corrected *p*-value < 0.05 were identified from the meta-analysis, including DMRs annotated to TNNT3 (chr11: 1,948,933 to 1,949,130 [6 probes], corrected $p = 4.32$ x 10-41), NRXN1 (corrected $p = 1.19$ x 10-11), IL17RA (corrected $p = 9.31$ x 10-8), and neuropeptide FF receptor 2 (NPFFR2) (corrected $p = 8.19$ x 10-7).

Conclusions: Using 2 cohorts of depression case control samples, we identified DMPs and DMRs associated with depression case status. Future meta-analysis with other MDD case control cohorts in the scientific community under the leadership of Psychiatric Genomic Consortium will further elucidate the epigenetic mechanisms associated with depression.

Keywords: EWAS, Depression, DNA Methylation

Disclosure: Johnson and Johnson: Employee (Self)

P379. Kappa-Opioid Receptors (KORs) on Microglia: Expression and Regulation of Pro-Inflammatory Signaling

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Background: There is growing evidence that neuropeptide receptors expressed on microglia have important roles in the regulation of microglia function. Previous studies suggest that Oprk1, the mRNA that encodes kappa-opioid receptors (KORs), is expressed in microglia, and that activation of KOR receptors on microglia in vitro suppresses pro-inflammatory signaling. Beyond this small number of studies, however, the potential roles for microglia KORs in inflammatory and stress-related conditions have not been thoroughly investigated. Here, we examined KOR expression and function within nucleus accumbens (NAc) cell subtypes under baseline conditions and in response to immune system activation.

Methods: We used single-cell RNA sequencing (scRNAseq) of NAc in male C57Bl/6J mice to quantify Oprk1 expression in neurons and microglia. To examine if KORs play a functional role in regulation of inflammatory signaling, we administered the long-acting and selective KOR antagonist JDTic (30 mg/kg, IP) 24 hours prior to lipopolysaccharide ([LPS], 0.5 mg/kg, IP), which produces robust but transient immune activation. In Experiment 1, three hours after LPS, we isolated microglia for rt-qPCR analysis using magnetic cell sorting to separate NAc homogenates into microglia/brain macrophage (CD11b+) and non-microglia (CD11b-) fractions. We then extracted RNA and performed rt-qPCR to determine changes in proinflammatory gene expression. Simultaneously, we collected plasma for systemic cytokine analysis. In Experiment 2, we implanted mice with intraperitoneal wireless telemetry devices (TA-F10, DSI) to enable continuous measurement of core body temperature and locomotor activity. After one week of recovery, we collected 3 days of baseline data before administering JDTic (30 mg/kg, IP) followed 24 hr later by LPS (0.5 mg/kg, IP). After 24 hr of data collection, we perfused the mice for immunohistochemistry analysis of microglia markers. Group differences were examined using two-way ANOVAs and Sidak's multiple comparisons tests.

Results: In our NAc scRNAseq dataset, we found expression of Oprk1 in both neurons and microglia. These findings are consistent with previous reports, although our data suggest that the mRNA is expressed in some but not all NAc microglia. In Experiment 1, we found that LPS significantly downregulated Oprk1 expression in microglia ($p < 0.05$). In microglia isolation experiments, LPS substantially upregulated the proinflammatory cytokines Il-1ß, Tnf, and Il-6, in both CD11B + (p's < 0.01) and CD11B- fractions (p's < 0.01), although expression levels were substantially higher in the CD11B + fraction. Pretreatment with KOR antagonist JDTic significantly enhanced LPS-induced upregulation of inflammatory cytokines Il-1ß ($p < 0.05$), Tnf ($p < 0.05$), and Il-6 ($p = 0.05$) in the CD11B + fraction and Tnf ($p < 0.05$), Il-6 ($p < 0.01$), but not Il-1ß ($p > 0.05$) in the CD11B- fraction (n's = 6 per group). JDTic also affected LPS-induced peripheral inflammatory responses, producing enhanced levels of Il-1ß and IL-6 in blood (p's < 0.01). In Experiment 2, LPS suppressed locomotor activity ($p < 0.01$) and produced hyperthermia ($p < 0.05$) for several hours following administration (n's = 4-5 per group). While JDTic pretreatment enhanced LPS-induced hyperthermia, it did not affect the altered levels of locomotor activity.

Conclusions: These studies suggests that KORs play an important role in the regulation of inflammatory signaling in the brain. We demonstrate that KOR mRNA is expressed in microglia and that its expression is regulated by LPS-induced immune activation. We also demonstrate that KOR signaling in microglia appears functional and regulates inflammation, as administration of a KOR antagonist amplifies LPS-induced central inflammatory processes (upregulation of proinflammatory genes in microglia), peripheral inflammatory processes (elevation of peripheral cytokines), and physiological responses (hyperthermia). Further work is needed to more thoroughly characterize the ways in which KORs expressed on microglia versus other immune cells contribute to regulation of these endpoints. Likewise, future studies are needed to determine if there are sex differences in these effects. Regardless, these studies may have important implications for the use of KOR antagonists in treatment of depressive disorders, which have been previously associated with elevated pro-inflammatory signaling.

Keywords: Kappa Opioid Receptor, Immune Responses, Depression and Anxiety, Microglia

Disclosure: Nothing to disclose.

P380. Proliferation of Putative GABA/Glutamate Co-Releasing Terminals in the Primate Habenula

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Background: The lateral habenula (LHb) responds to aversive stimuli, and its hyperactivity is hypothesized to contribute to depression. Most of the central nervous system uses a balance of excitatory and inhibitory transmission from separate inputs to control neural activity. However, multiple inputs to the lateral habenula co-release GABA with glutamate in rodents; the balance of GABA and glutamate at these synapses may regulate LHb responses to aversive stimuli and mood. Here, we investigated whether the magnitude and topography of co-release of GABA and glutamate in the LHb is conserved in primates.

Methods: We performed immunohistochemistry on brain slices for GAD and Vglut2 ($N = 34$ slices, 6 mice; $N = 23$ slices, 5 monkeys; $N = 11$ slices, 6 humans) and VGAT and Vglut2 ($N = 34$ slices, 6 mice; $N = 23$ slices, 5 monkeys; $N = 8$ slices, 4 humans) at the level of the habenula and quantified the amount and topography of GAD/Vglut2 and VGAT/Vglut2 co-labeling within tiled confocal images (63X) using custom Matlab code. ANOVA and Student's t-tests were used to determine statistical significance. Both sexes were included.

Results: We found increased GAD/Vglut2 and VGAT/Vglut2 co-labeling within the LHb compared to the adjacent thalamus in all species (all $P < .0001$). We also found increased GAD/Vglut2 and VGAT/Vglut2 co-labeling in the monkey and human LHb compared to the mouse LHb ($P < .0001$ and $P < .005$, respectively), and increased GAD/Vglut2 co-labeling in the human LHb compared to the monkey LHb ($P < .05$). The weighted center-of-mass of GAD/Vglut2 co-labeling was shifted dorsally in the LHb of monkeys and humans compared to mice (both $P < .002$).

Conclusions: Our data indicate substantially increased co-labeling of GAD (the synthesizing enzyme for GABA) and VGLUT2 (vesicular glutamate transporter), as well as VGAT (vesicular GABA transporter) and VGLUT2, in synaptic terminals in the monkey and human LHb, consistent with increased co-release of GABA and glutamate from individual terminals onto primate LHb neurons. We also found an expanded topography of co-release in the primate LHb, perhaps partly due to expansion of the LHb in primates. Thus, co-release of GABA with glutamate may be a mechanism for regulation of LHb activity and mood that proliferated during primate evolution.

Keywords: Neurotransmitter Co-Release, Habenula, Monkey

Disclosure: Nothing to disclose.

P381. Base Excision Repair Mechanism (BER) as a Potential Target for Treatment of Bipolar Disorder: A Case-Control Candidate Gene Study

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Background: Background:

Bipolar disorder (BD) is associated with early aging, increased mortality and morbidity due to various medical conditions which are known to be associated with oxidatively generated DNA damage. Recent evidence shows increased cancer risk in BD, particularly breast cancer in women, and lithium may have a potential protective effect on cancer. BD presents increased levels of oxidatively induced DNA damage. Base excision repair (BER) is the major repair mechanism of oxidative DNA damage. It is a stepwise process operated by multiple enzymes. We have previously shown increased protein and down regulated gene expression levels of genes encoding for BER enzymes in peripheral blood samples of BD patients compared to controls. Genes encoding BER enzymes were either hypomethylated or hyper methylated in post-mortem brain tissue samples of BD patients in comparison to controls. In this study we aimed to explore the association of 24 genes encoding for BER enzymes with BD and with lithium response. We hypothesized that genes encoding for BER enzymes were going to show significant association to BD and lithium response.

Methods: The previously published GWAS summary statistics of the Psychiatric Genomics Consortium (PGC)-Bipolar Disorder, including patients with BD ($n = 20{,}352$) and controls ($n = 31{,}358$), were used to perform gene-level analysis of candidate BER genes using the MAGMA approach implemented in the GWAS functional mapping and annotation platform FUMA. The genes APEX1, FEN1, LIG1, LIG3, MBD4, MUTYH, MPG, OGG1, NLTH1, NEIL1, NEIL2, NEIL3, PARP1, PNKP, PCNA, POLB, POLD1, POLE, POLG, SMUG1, TDG, UNG1, UNG2, and XRCC1, which encode for their corresponding BER enzymes, were included in the analysis.

Likewise, previously published GWAS summary statistics of 2500 patients with BD from the International Consortium on Lithium Genetics (ConLiGen) were used to perform gene level analysis for the association between lithium response and BER genes. Lithium response was analyzed as binary variable based on a score ≥7 on Alda scale after subtracting B subscale scores from A subscale scores.

Results: The gene encoding for the BER enzyme FEN1 met genome-wide significance criteria for association with BD ($p = 4.5E\text{-}09$), whereas genes encoding for MBD4 ($p = 0.0063$), TDG ($p = 0.0073$), MUTYH ($p = 0.042$), and PARP1 ($p = 0.043$) showed nominally statistically significant association to BD. PARP1 was the only gene that showed nominally statistically significant ($p = 0.011$) association with lithium response.

Conclusions: Our findings provide further evidence for the involvement of the BER mechanism in the pathogenesis of BD and response to lithium. The genes showing significant association to BD function at different steps of the BER pathway, suggesting a large-scale operational challenge in the DNA repair mechanism in BD. We do not yet know whether the altered DNA repair mechanism is the primary source or result of increased oxidative DNA damage in BD.

FEN1 is known to have a significant role in maintaining integrity of the mitochondrial DNA. MBD4, TDG and MUTYH are also known to be involved in mitochondrial DNA repair. Present data allows BD to be conceptualized as a mitochondrial disease. Recent research is exploring significance of FEN1 inhibitors in cancer treatment. PARP-1 is a key enzyme in regulating the balance between DNA repair and cell death in the case of severe DNA damage. Lithium was shown to reduce PARP-1 activity in response to oxidative stress in HeLa cells. As a non- competitive inhibitor of GSK-3, lithium induces apoptosis, and suppresses carcinoid cancer cell growth in vitro via increasing the PARP1 cleavage. Both PARP1 and lithium are active homeostatic regulators, meaning their effects are most pronounced in the presence of pathology. The interaction between lithium and PARP1 may be one of the mechanisms enabling lithium's mood stabilizing effect. Taken together, the findings point at a future direction where BER mechanism can be explored for understanding the pathogenesis of BD and as a target for novel treatment options of illness. Data and experience from the cancer field can contribute significantly to our understanding of BER enzymes as novel targets for treatment of BD.

Keywords: Bipolar Disorder, Oxidative DNA Damage, Base Excision DNA Repair, Lithium, Gene Level Analysis

Disclosure: Nothing to disclose.

P382. Psychedelics for the Treatment of Mental Disorders: A Systematic Review and Network Meta-Analysis

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Background: Psychedelics, like MDMA, LSD, ayahuasca, and psilocybin, may have antidepressant and anxiolytic properties. Comparative performance of psychedelics for treating symptoms of mental disorders is unclear due to few head-to-head trials. We aimed to compare the efficacy and safety of psychedelics for depression, anxiety, and posttraumatic stress disorder symptoms in adults. Secondary outcome was dichotomized all-cause treatment discontinuation

Methods: Four major bibliographic databases (PubMed, MEDLINE, PsycINFO, and Embase) were searched for trials comparing psychedelic (with or without concomitant therapies) to February 2021. Randomized trials comparing psychedelics for mood, anxiety, or posttraumatic stress disorder symptoms in adults were included after blind review by three independent reviewers. We followed the PRISMA guidelines for abstracting data and applied the Cochrane risk of bias tool for evaluating the study-level risk of bias. Data abstraction was done by one reviewer and confirmed by two independent blind reviewers. Primary outcomes were scores on instruments for depression, anxiety, and posttraumatic stress disorder symptoms and treatment discontinuation due to adverse effects. Secondary outcomes were dichotomized all-cause treatment discontinuation. We pooled effect sizes using frequentist random-effects network meta-analysis models to generate summary rate ratios (RRs) and Cohen's d standardized mean differences (SMDs). Negative Cohen's d (SMDs) or RRs<1 indicate that the treatment reduced the parameter of interest relative to the control (e.g., signifying a beneficial effect for depression severity).

Results: The systematic search provided a total of 1368 citations, from these, we selected 67 studies for eligibility assessment ($n = 562$, 54% female) and chose 17 studies for full text review. None of the investigated psychedelics caused more dropouts or dropouts due to adverse events than placebo, indicating high tolerability of psychedelic-assisted therapy. Psilocybin (d = -2.71, 95% CI: -3.46, -1.96) and ayahuasca (d = -1.57; 95% CI: -2.90, -0.23) had large effect sizes for depressive symptoms, while MDMA (d = -0.95, 95% CI: -1.28, -0.62) showed efficacy for PTSD symptoms. Psilocybin also demonstrated a large anxiolytic effect (d = -1.88; 95% CI: -2.70, -1.06). In parallel, psilocybin, ayahuasca, and MDMA demonstrated large effect sizes for response and remission rates, ranging from RRs of 2.65 to 5.44. In head-to-head comparisons between psychedelics, psilocybin outperformed MDMA and LSD for depression symptoms. We downgraded the strength of evidence due to heterogeneity, publication bias, and imprecision in some outcomes.

Conclusions: While some psychedelics appear well-tolerated and demonstrate some efficacy for symptoms of depression, anxiety, and PTSD, the strength of evidence in our estimates was low to very low for most agents given the small sample sizes and few RCTs. A lack of consistent evidence precludes a definitive hierarchy of treatments and points to a need for additional, high-quality RCTs.

Keywords: Psychedelics, Depression and Anxiety, PTSD

Disclosure: Nothing to disclose.

P383. The Genetic Liability of Severe Mental Illness and Chronotype

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Background: Chronotype disruptions, often in the form of circadian misalignment, are known to be correlated among patients with bipolar disorder (BD), schizophrenia (SCZ) and other forms of severe mental illness. These circadian disturbances include misalignment in sleep and daily activity patterns. It remains unclear if genetic liability towards chronotype corresponds with an individual's risk of developing a psychiatric disorder. Polygenic risk scores (PRS) are a well-established methodology to estimate the genetic liability associated with a particular disease or trait using SNP association derived from genome-wide association (GWA) data. There has been increasing evidence of shared genetic risk between forms of severe mental illness, including SCZ and BD, through the collective sum of common risk alleles, though little is known about the genetic degree of overlap with chronotype predisposition.

Methods: In this study we used genotype data derived from approximately 420,000 single nucleotide polymorphisms (SNP) of 415 subjects with BD I (with clinically defined levels of lithium responsiveness) derived from the Pharmacogenetics of Bipolar Disorder (PGBD) trial. PRS for BD and SCZ were calculated using summary statistics from the most recent PGC analyses. At the first study visit of the PGBD trial, 385 subjects completed the Basic Language Morningness scale (BALM), a validated, 13-item self-reported measure of chronotype (36, 37). Higher BALM score corresponds to a greater level of morningness. Chronotype and morningness were calculated from summary statistics of the UK Biobank. Data were examined at five thresholds for statistical significance ($\alpha < 0.01$, 0.05, 0.1, 0.2 and 1.0). Fibroblast cell lines derived from skin biopsies of 59 PGBD study participants with informative clinical outcomes ($N = 44$ Li-R/15Li-NR) were transduced with the Per2-luc lentiviral reporter gene to assess circadian rhythms. BD patient fibroblast rhythms were recorded using ~1.2 x 106 cells in 35 mm plates over 5 days with a luminometer.

Results: The PRS using $p < 0.01$ showed significant overlap in PRS for BD and SCZ, significant overlap in PRS for chronotype and morningness, and trend level overlap between chronotype and BD at $p = 0.06$. At a PRS $p < 0.05$, there was a very strong correlation with morningness and chronotype, BD and SCZ, and no correlation between BD and chronotype or morningness. There was a significant negative correlation between SCHZ, chronotype and morningness. Unadjusted BALM scores demonstrated a modest but significant correlation with morningness, but no correlation with chronotype. By adjusting for age, morningness PRS explains slightly more variance for BALM than age alone ($R2 = 0.12$ AGE ONLY, 0.14 PRS $P < 0.05$, 0.15 PRS < 0.01). In cellular rhythm assays, PRS for morningness was nominally correlated with period (PRS $p < 0.01 = 0.13$ and PRS $p < 0.05 = 0.16$) but these were not statistically significant. Correlations were nominally higher than those for BD and SCZ but the difference was not significant. Similar results for phase r = 0.07, 0.13, 0.15, 0.16, 0.16 for each *p*-value respectively. In cells with BALM scores the absolute r values had insufficient power due to limited sample size.

Conclusions: Collectively, these data suggest there is some evidence of shared genetic liability towards the development of severe mental illness, specifically SCZ, with chronotype (dimensional) and morningness (categorical) at the most stringent *p* value <0.01). While not meeting threshold for statistical significance, cellular rhythms assays of BD I subjects demonstrated some evidence of correlation between PRS for morningness and

different parameters of circadian rhythm (period, phase), demonstrating the translational utility of cellular models to provide informative data of chronotype and morningness in corresponding clinical subjects.

Keywords: Circadian Rhythm, Severe Mental Illness, Polygenetic Risk Score

Disclosure: Nothing to disclose.

P384. EEG Power Spectral Analysis: A Potential Mechanistic Strategy to Assess rTMS Neurophysiological Effects?

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Background: Treatment Resistant Depression (TRD) is associated with humanistic and economic burden, with an enormous impact on health, productivity, and social dynamics. For over a decade, repetitive Transcranial Magnetic Stimulation (rTMS) has demonstrated effectiveness in TRD. This technique has been widely studied, and different neurophysiological approaches have been used to investigate its effects on neural circuitry modulation. Nevertheless, the mechanism of action of rTMS and its effects over time still are not fully understood. Quantitative Electroencephalography (qEEG) power spectral analysis might function as a mechanistic strategy to clarify the neurophysiological effects of rTMS and to optimize future treatment protocols.

Methods: In this longitudinal pilot study, resting-EEG activity was measured in six subjects with TRD who received up to 30 sessions of rTMS (5Hz, 120%MT, 3,000 pulses/session, over the left dorsolateral prefrontal cortex). The EEG was recorded through 64 channels, during 10 minutes, with eyes closed, at baseline (T0), after 10 rTMS sessions (T1), after 20 sessions (T2), and immediately after the last stimulation treatment (T3). The main outcomes were differences in the FFT-based power spectral analysis over time as measured by qEEG using the EEGLAB/MATLAB software system (The Mathworks, Inc.).

Results: One-way repeated measures ANOVA showed a significant decrease in the absolute power in the beta band over the left dorsolateral prefrontal cortex (F (3, 15) = 3.58, $p = 0.039$), with effects observed at T1 and progressing over time. No other significant changes were observed in delta, theta, or alpha power following the stimulation sessions ($p \geq .05$).

Conclusions: Our findings suggest that qEGG might be a feasible strategy to monitor rTMS-induced changes in cortical networks. Prior studies have shown an association between depressive symptoms and increased beta power in frontal regions. Our results demonstrated that rTMS elicited a reduction in beta power over time, starting as early as after the first ten sessions. Future studies might consider EEG acquisition during rTMS, a larger sample, and the correlation of qEEG findings with clinical outcomes to assess whether qEEG power spectral analysis might function as a rTMS biomarker for TRD.

Keywords: Quantitative Electroencephalography (qEEG), Repetitive Transcranial Magnetic Stimulation (rTMS), Major Depression Disorder, Neurophysiology, Non-Invasive Neuromodulation

Disclosure: Nothing to disclose.

P385. Exploring Relationships of GABA and Glutamate With Negative Valence Systems

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Background: Negative valence systems are defined by the NIMH Research Domain Criteria (RDoC) framework as systems responsible for responses to aversive situations or context, and includes constructs such as fear, anxiety and loss. Dysregulation of negative valence systems is a characteristic of mood disorders and is observed in other mental disorders, as evidenced by difficulties in affective processing and emotion regulation, with corresponding alterations in emotion regulation neurocircuitry. Dysregulation in GABAergic (primary inhibitory) and glutamatergic (primary excitatory) systems within this neurocircuitry has been implicated in mood and other disorders. However, the relationship of neurometabolic dysregulation to negative valence systems more generally is not well understood. We aimed to examine brain markers of GABAergic and glutamatergic function in a brain region involved in emotion regulation (dorsolateral prefrontal cortex (DLPFC)) and their relationship to function in negative valence systems, measured at the self-report and behavioral levels.

Methods: Twenty participants (14 women and 6 men) were recruited at Brigham and Women's Hospital, including both healthy participants and participants with a mood disorder in order to enrich the sample for a range of function within negative valence systems. Participants were ages 35-61 ($M = 46.50$, $SD = 8.50$). Self-report negative affect summary score from NIH Toolbox was assessed, along with a behavioral measure using an affective go/no-go task with positive, neutral, and negative valenced stimuli. Attentional bias toward negative stimuli was measured by comparing number of commission and omission errors and reaction time to negative stimuli against responses to positive and neutral stimuli. MR spectroscopy, using STEAM at 7 Tesla (TE = 20ms, TM = 10ms, TR = 3000ms), was used to measure markers for GABA and glutamate in the DLPFC and LCModel was used for neurometabolite quantification. Spectroscopy results were filtered to exclude results with a CRLB > 20. We conducted separate correlation analyses of each neurometabolic marker with negative affect scores from NIH Toolbox and with measures of attentional bias from the affective go/no-go task.

Results: We observed a significant inverse correlation between DLPFC GABA and the negative affect summary score ($r = -0.670$, $p = 0.009$). For analyses involving negative attentional bias we observed that for the contrast comparing response time to negative and positive stimuli, DLPFC GABA was significantly inversely correlated with response time consistent with bias towards negative stimuli ($r = -0.632$, $p = .027$). Further, results showed that DLPFC GABA was significantly inversely correlated with omission errors for negative versus neutral stimuli, consistent with bias towards negative stimuli ($r = -0.634$, $p = .027$). We did not observe a significant association between DLPFC GABA and commission errors. No other significant results were observed in testing relationships of glutamate in the DLPFC with either negative affect scores or measures of negative affective bias.

Conclusions: These results suggest that GABAergic function, measured in the DLPFC, is inversely correlated with negative affect and negative attentional bias. Specifically, lower levels of GABA in the DLPFC were associated with higher negative affect scores and increased attentional bias toward negative stimuli. This finding is in line with studies implicating lower GABA in individuals with mood disorders. Accordingly, our findings suggest a role for DLPFC GABA in negative valence systems functioning more generally. Despite the promising nature of these findings, they are preliminary in nature and should be interpreted with caution given the small sample size. If confirmed, these findings have the potential to inform clinical care regarding the use of GABAergic medications to improve outcomes in disorders involving negative valence systems.

Keywords: Negative Valence System, MR Spectroscopy, Mood Disorder

Disclosure: Nothing to disclose.

P386. Baseline Intrinsic Functional Brain Connectomes Predict Treatment Outcome for Depression Comorbid With Obesity: A Report From the Engage Randomized Controlled Trial

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Background: Typically, only 30-50% of patients with depression respond to antidepressant treatment, and lack of response is exacerbated with co-occurring obesity; only 17% of patients with depression and obesity respond to antidepressant treatment (1). Identifying predictors of treatment response can inform a precision medicine approach for treatment. Both depression and obesity have been characterized by altered intrinsic functional connectivity (FC) within and between large-scale neural networks (2, 3); however, it is still unclear whether those connectome signatures could be utilized to predict treatment response for depression comorbid with obesity.

Methods: We analyzed baseline functional MRI data of adult participants with depression and obesity who were randomized to receive an integrative collaborative care intervention (I-CARE, $n = 59$) that included problem-solving therapy with as-needed antidepressant medication for depression and a video-based behavioral weight loss treatment or usual care ($n = 49$) (4, 5). Participants in both treatment arms with ≥ 50% reduction of depression severity scores (20-item depression severity scale) at 6 months relative to baseline were defined as responders. Detailed steps for preprocessing and FC calculation could be found in previous publications (6, 7). In brief, concatenated residual time courses of three task fMRI data after removing the task-related activity were used to estimate baseline intrinsic functional connectome, using the Brainnetome atlas consisting of 246 cortical and subcortical regions of interest (ROI) labeled with Yeo's 7 networks parcellation (8). ROI-to-ROI pairwise FCs (246 x 246) were evaluated and transformed into z-scores using Fisher's z transformation for each participant. To identify predictive markers of treatment response at 6 months, we used a linear regression model with each pair of the baseline FC as the dependent variable and the response group (responder or non-responder) as the independent variable, with age, gender, and baseline depression severity as covariates. To evaluate the predictive power of the identified baseline FC markers from the linear regression model, we used logistic regression models with the response group as the dependent variable, and the identified baseline FC markers combined with the baseline covariates as independent variables and compared it with only including the baseline covariates as dependent variables. A 5-fold nested cross-validation was conducted to test the generalizability of each model. The confusion matrix was generated for the calculation of accuracy, sensitivity, and specificity. To further investigate whether the baseline FC markers were differentially associated with treatment response depending on treatment arm, we conducted a response (responder or non-responder) by treatment arm (I-CARE or usual care) two-way analysis of variance (ANOVA) model on the identified FC markers and focused on the interaction effect of response by treatment. To help understand the findings, exploratory analyses included comparing two sub-items of baseline depression symptoms (item 4: feeling tired or having little energy and item 5: poor appetite or overeating from the patient health questionnaire-9), which might be key factors of response for patients with depression and obesity, between the responder and non-responder groups using a two-sample t-test and examining their associations with identified baseline FC markers using Spearman's rank correlation within each treatment arm.

All pairwise ROI-to-ROI analyses were corrected for multiple comparisons via the network-based statistic (NBS) at an edge level $p < 0.001$ and a component level $p < 0.05$ using 1000 permutations (9). Exploratory analyses were not corrected for multiple comparisons.

Results: I-CARE was more effective in improving mood and reducing weight (10), and the overall response rate of depression at 6 months was 32%. Responders were characterized by a unique functional connectome profile compared to non-responders: a significantly lower baseline FC within the somatomotor network (SMN), between the SMN and the default mode network (DMN), visual network, dorsal and ventral attention network, limbic network, and subcortical regions, and a significantly higher baseline FC between the SMN and frontal-parietal network. A combination of baseline FC markers and covariates predicted treatment response with high accuracy, sensitivity, and specificity (cross-validated 88%, 89%, and 86% respectively), baseline covariates alone did not accurately predict treatment outcome (cross-validated 50%, 53%, and 43% respectively), indicating the utility of FC markers. We did not observe differences between I-CARE and usual care groups on the FC predictors of response. Responders had a tendency toward a higher baseline energy level ($p = 0.065$); however, it was not correlated with identified baseline FC makers within each treatment arm, indicating that higher baseline energy and the unique functional connectome might be trait-like characteristics of responders.

Conclusions: Using data from a precision medicine biomarker trial, we found that baseline functional connectomes, especially the within and between SMN FCs, were predictive biomarkers of treatment response for depression comorbid with obesity, irrespective of treatment type. Our findings highlight the promise of baseline functional connectome for tailoring treatment choices for depression comorbid with obesity.

Keywords: Depression and Obesity, Neuroimaging Biomarkers, Clinical Trial

Disclosure: Nothing to disclose.

P387. Aberrant Functional Maturation of a Novel Cell-Type-Specific Brain Pathway Impacts Reward-Seeking Behaviors After Early-Life Stress

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Background: The seeking of pleasure is a fundamental human behavior, executed by coordinated activity of the brain's reward circuitry. Disrupted operation of this circuit is thought to underlie major emotional disorders including depression and drug-abuse, disorders commonly arising after early-life stresses. Yet, how early-life adversities (ELA) impact the functional maturation of reward circuitries to promote disease remains unclear. The nucleus accumbens (NAc) is a major component of the reward circuit and key structure mediating pleasure, motivation, and emotional processes. Multiple inputs converge onto the NAc to modulate reward-seeking behaviors, including the basolateral amygdala (BLA). The BLA mediates associate learning for both aversive and appetitive stimuli, and stimulation of glutamatergic projections from the BLA to NAc promotes appetitive behaviors. In this study, we employ viral-genetic technologies to identify a novel

GABAergic projection that co-expresses the stress-related neuropeptide corticotropin-releasing hormone (CRH) and connects the basolateral amygdala (BLA) and nucleus accumbens (NAc). In the NAc, CRH + axon terminals modulate reward and motivational behaviors. Here, we identify the role of this CRH + BLA-NAc projection during reward in naïve and stress-experiencing mice.

Methods: To identify CRH + projections to the NAc, we utilized viral-genetic approaches to map these pathways using Cre-dependent viruses injected into CRH-IRES-Cre mice. To determine the function of the novel CRH + BLA-NAc projection we employ chemogenetic and optogenetic strategies in both control and early-life adversity experiencing mice. In these mice, we injected excitatory or inhibitory Cre-dependent DREADD (hM3Dq and hM4Di) and optogenetic (ChR2) carrying viruses into BLA, followed by medial NAc shell targeted microinjections of CNO or light activation. We tested the function of this pathway using three reward (sucrose preference, sex-cue approach, and palatable food tasks), and non-reward tasks (object location memory and open field).

Results: Viral genetic tracing paired with fluorescence in situ hybridization and immunostaining identified a novel GABAergic projection that co-expresses the stress-reactive neuropeptide CRH + projection from the BLA to the medial NAc shell. Excitation of this projection using chemo- and optogenetic tools reduced preference for sucrose, palatable food consumption, and sex-cue approach, but did not alter non-reward specific tasks (object location memory and open field). Compared with control mice, male mice that experienced ELA had reduced preference for sucrose, palatable food consumption and sex-cue approach. In adult ELA mice, chemogenetic inhibition of the CRH + BLA-NAc projection rescued all three reward behaviors.

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

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

Keywords: CRH, Reward, Adversity, Nucleus Accumbens, Amygdala

Disclosure: Nothing to disclose.

P388. Reduced Striatal Dopamine Binding to Rewards is Associated With Anhedonia Severity and Recent Stress

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Background: Stress reduces goal-directed behavior and reward responsiveness and has been implicated as a significant contributor to the pathophysiology and manifestation of anhedonia. Anhedonia – deficits in motivation, effort, or pleasure – is a core feature of stress-related psychopathologies including major depression and posttraumatic stress disorder. A putative mechanism for the association between chronic stress and anhedonia is the downregulation of mesolimbic dopamine (DA) reward signaling. The purpose of this study was to use simultaneous PET-MR imaging to evaluate striatal dopaminergic functioning during reward processing in a transdiagnostic anhedonia sample, using the D2/D3 dopamine receptor antagonist [11C]raclopride. We hypothesized that, compared to a healthy control (HC) group, the anhedonic (ANH) group would demonstrate decreased striatal phasic DA release to rewards, indexed by the non-displaceable binding potential (BPND) of [11C]raclopride. Furthermore, we hypothesized that, within the ANH group, reduced phasic DA release in striatal regions would be associated with (a) greater anhedonia severity, and (b) elevated stress.

Methods: Adult participants (HC: $n = 12$, ANH: $n = 24$) underwent a simultaneous PET-MR scan session on a Siemens Biograph mMR scanner at the UNC Biomedical Research Imaging Center. Dynamic PET data were acquired for 70 minutes post-injection and reconstructed into 70 1-minute time frames. A bolus+infusion [11C]raclopride administration protocol was used to measure background dopaminergic tone and phasic dopaminergic release, via displacement of the tracer, in response to incentives. Participants completed a monetary incentive delay (MID) task, modified for use in PET-MR studies. The incentive task included a neutral run followed by two reward runs. During the two reward runs, concatenated into one reward block for PET analyses, money could be won; during the neutral run, money could not be won. Binding potential, the ratio of selectively bound ligand to nondisplaceable ligand in the tissue at equilibrium (BPND), was estimated from dynamic PET images for the neutral and reward states using the Simplified Reference Tissue Model (SRTM). BPND is negatively correlated with endogenous dopamine. In order to identify between-group differences in striatal BPND, a Z-score statistical map representing the difference between groups (ANH – CON) was created by comparing group-level voxel-wise BPND (reward – neutral) maps, thresholded at $Z > 2.58$. For clusters that showed group differences in striatal BPND in the reward relative to the neutral run, we examined associations between BPND values and anhedonia and stress measures within the ANH group. Anhedonia and stress measures were only collected in the ANH group (15 Females, 9 Males; aligned with 2:1 prevalence rates of major depression for females-to-males).

Results: A striatal cluster located in the left putamen demonstrated ANH > Control group differences for the contrast of (reward > neutral) BPND values, interpreted to mean decreased phasic DA release (increased BPND) in the reward relative to the neutral condition in the ANH group relative to the HC group. Within the ANH group, reduced phasic DA release to rewards in the left putamen was significantly correlated with recent stress, ($R = .45$, $p = .027$), assessed using the PTSD Symptom Checklist for DSM-5 (PCL-5). Recent stress was also assessed using the Perceived Stress Scale (PSS). Reduced phasic DA release to rewards in the left putamen was marginally associated with increased stress on the PSS, in the ANH group, demonstrating the same pattern as with stress measured by the PCL-5 ($R = .40$, $p = .054$). Moreover, there was a significant effect of recent stress ($b = .002$) on phasic DA release to rewards in the left putamen ($F(1,22) = 5.92$, $p = .024$, adjR2 = .17). Furthermore, within the ANH group, reduced phasic DA release to rewards in the left putamen was significantly correlated with anhedonia severity, ($R = .44$, $p = .032$), assessed using the anhedonia subscale of the Beck Depression Inventory (BDI-II) which comprised four items (loss of interest, loss of pleasure, loss of interest in sex and loss of energy). Finally, there was a significant effect of anhedonia severity ($b = .013$), assessed using the BDI-II, on phasic DA release to rewards in the left putamen ($F(1,22) = 5.20$, $p = .033$, adjR2 = .15). Anhedonia severity was also assessed using the Snaith-Hamilton Pleasure Scale (SHAPS); however, SHAPS scores were not significantly associated with reduced phasic DA release in the left putamen ($p > .05$).

Conclusions: In a transdiagnostic anhedonia sample, we found that recent stress and anhedonia severity are associated with reduced DA reward signaling in the left putamen. Relations between striatal DA release to reward and stress were evident

across two stress measures (the PCL-5 and the PSS), but relations between striatal DA release to reward and anhedonia were evident only using the anhedonia subscale of the BDI-II and not the SHAPS. The anhedonia subscale of the BDI-II assesses motivation toward rewards or anticipatory reward processing, whereas the SHAPS assesses aspects of consummatory reward, or pleasure, which may account for these contrasting results. Collectively, these findings highlight a molecular mechanism that may address, in part, the pathogenesis of impaired DA functioning in anhedonia and stress-related psychopathologies and provides support for the role of stress in the emergence of anhedonia.

Keywords: Simultaneous PET-MR, Anhedonia, Stress, Dopamine

Disclosure: Nothing to disclose.

P389. Resting Brain Dynamics in Childhood Sexual Abuse and Major Depressive Disorder: A Coactivation Pattern Analysis

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Background: Childhood maltreatment is a prominent risk factor for Major Depressive Disorder (MDD). Both childhood maltreatment and MDD putatively impact overlapping large-scale brain networks, including the default mode network (DMN), the frontoparietal network (FPN), and the salience network (SN). In addition to within-network functional abnormalities, childhood maltreatment and MDD have been separately linked to between-network dysfunction, including synchrony between the FPN and DMN. However, how child maltreatment and MDD may jointly or differentially influence these neural networks is unclear. Additionally, most the prior work has focused on examining static network properties, e.g., average network functional connectivity across the entire scan, a key gap given recent evidence that resting-state networks show dynamic changes in functioning over time. Distinct or overlapping dynamic network abnormalities related to MDD and childhood maltreatment remain unexplored.

Methods: Adult females, with and without a childhood sexual abuse (CSA) history, both healthy controls (HC; $n = 22$ without CSA, $n = 14$ with CSA) and MDD ($n = 18$ without CSA, $n = 17$ with CSA) participated in the study. Participants with CSA endorsed at least one incident of contact CSA between the ages of 5 to 14. All participants completed a resting state scan and filled out the Beck Depression Inventory II and the Response Styles Questionnaire (RSQ), a measure of rumination. Most participants ($n = 62$) also completed a third session, involving a psychosocial stressor. Cortisol samples and self-reported negative affect (NA) were collected at 5 times points, before, during, and after the stressor. For cortisol, area under the curve with respect to increase, a measure of stress-induced changes in cortisol levels, as well as the maximum level of NA reported during the stressor were calculated.

Neuroimaging data were subjected to whole-brain, voxel-wise coactivation pattern (CAP) analysis, which involves clustering the data to reveal transient network states of coordinated brain activity. The optimal number of CAPs was empirically determined using consensus clustering, which involves running k means clustering over several iterations, each time involving a randomly selected subsample of the data set and providing an index of clustering quality. Consensus clustering results indicated an optimal k of 8 and the 8 CAPs included an: 1) anterior DMN CAP, 2) SN CAP, 3) posterior DMN-FPN CAP, 4) visual system CAP, 5) prototypical DMN CAP, 6) dorsal attention network CAP, 7) somatosensory network CAP, and a 8) CAP involving prefrontal and posterior cingulate cortex regions. The following metrics were computed for each CAP: 1) time in CAP – the total number of volumes the participant spent in a CAP during the scan and 2) CAP transition frequencies – the number of times a specific CAP transitioned to another specific CAP divided by the total number of transitions.

A CSA (present/absent) x MDD (present/absent) MANCOVA was conducted on "time in CAP" across the 8 CAPs, controlling for fMRI motion metrics. Given that interactions between the DMN and FPN are implicated in MDD and CSA, CSA x MDD MANCOVAs were conducted on transitions involving DMN and FPN CAPs. Significant main effects and interactions were followed up with univariate ANCOVAs. CAPs showing group differences in "time in CAP" or "CAP transition frequencies" were correlated with depression and stress-related measures. Follow-up tests and correlations were corrected for multiple comparisons using FDR < 0.05.

Results: The MDD x CSA MANCOVA on "time in CAP" showed a significant main effect of MDD, Wilks Lambda $=0.63$, F (8, 58) $=$ 4.22, $p < 0.001$. There was no significant main effect of CSA or MDD x CSA interaction, all ps > 0.20. Follow-up ANCOVAs indicated that, relative to HCs, individuals with MDD spent more time in a posterior DMN-FPN CAP, F (1,65) $= 11.27$, uncorrected $p = 0.001$, FDR-corrected $p = 0.008$. With respect to transition frequencies, two MDD x CSA MANCOVA's were run, examining transitions between posterior DMN-FPN and anterior DMN CAPs as well as transitions between posterior DMN-FPN and prototypical DMN CAPs. Results from these analyses revealed a main effect of MDD, Wilk's Lambda $= 0.70$, F (2,64) $= 13.70$, $p < 0.001$, but no main effect of CSA or MDD x CSA interaction all ps > .0.30, on posterior DMN-FPN -prototypical DMN transition frequencies. Specifically, individuals with MDD transitioned more frequently between posterior DMN-FPN and prototypical DMN CAPs than HCs. More time spent in a posterior DMN-FPN CAP and transitioning more frequently between posterior DMN-FPN and prototypical DMN CAPS was associated with higher RSQ rumination scores, partial rs .26-.33, FDR-corrected ps < 0.05. There were no significant associations between CAP metrics and BDI-II scores or stress reactivity measures, all ps > 0.05.

Conclusions: These findings are consistent with prior work establishing MDD as a disorder characterized by DMN-FPN imbalances, and linking DMN-FPN functional abnormalities to MDD-related cognitive dysfunction, including rumination. The lack of CSA findings is consistent with a prior study that failed to find child maltreatment-related abnormalities in time spent in distinct network states or transitioning between different states. However, these findings need to be replicated in larger sample sizes incorporating longitudinal designs.

Keywords: Major Depression Disorder, Childhood Maltreatment, Resting State Brain Dynamics, Coactivation Pattern Analysis, Rumination

Disclosure: Nothing to disclose.

P390. Prescription of Unfavorable Combinations of Drugs for Mood Disorders and Physical Conditions: A Cross-Sectional National Database Survey

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Background: The aim of this cross-sectional study was to investigate prescriptions of unfavorable combinations of drugs for mood disorders and physical conditions.

Methods: We used the claims sampling data of 581,990 outpatients during January 2015 from the National Database of Health Insurance Claims and Specific Health Checkups of Japan provided by the Ministry of Health, Labor, and Welfare in Japan. Fisher's exact test was performed to compare the prescription rates of (1) non-steroidal anti-inflammatory drugs (NSAIDs), loop/thiazide diuretics, angiotensin-converting enzyme (ACE) inhibitors, and/or angiotensin II receptor blockers (ARBs) between lithium users and age- and sex-matched non-lithium users; (2) NSAIDs, antiplatelet drugs, and/or anticoagulants between selective serotonin reuptake inhibitor (SSRI)/serotonin-noradrenaline reuptake inhibitor (SNRI) users and age- and sex- matched non-users; and (3) warfarin between mirtazapine users and age- and sex-matched non-users. A Bonferroni corrected *p*-value of <0.05/3 was considered statistically significant.

Results: Prescriptions of NSAIDs, loop/thiazide diuretics, ACE inhibitors, and/or ARBs were less frequently administered to lithium users (18.3%, $n = 235/1,284$) than to non-users (31.9%, $n = 409/1,284$) ($p = 7.6\times10\text{-}10$). The prescription rates of NSAIDs, antiplatelet drugs, and/or anticoagulants were comparable between SSRI/SNRI users (23.1%, $n = 3,078/13,330$) and non-users (24.1%, $n = 3,219/13,330$) ($p = 0.044$). Warfarin was less frequently prescribed to mirtazapine users (0.78%, $n = 18/2,300$) than to non-users (1.65%, $n = 38/2,300$) ($p = 0.01$). Limitations: Actual treatment outcomes were not evaluated due to the cross-sectional study design.

Conclusions: A considerable proportion of patients receiving lithium, SSRIs/SNRIs or mirtazapine are treated with unfavorable polypharmacy. Further investigations are warranted to design interventions to reduce such pharmacotherapy for patients with mood disorders when necessary.

Keywords: Antidepressant, Bipolar Disorder, Depression, Lithium, Polypharmacy

Disclosure: Nothing to disclose.

P391. Nucleus Accumbens Mu Opioid Receptor Activation is Associated With Increase in Self-Esteem and Positive Mood After Experiencing Social Acceptance

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Background: Rejection Sensitivity is the heightened expectation or perception of social rejection and is a feature of many psychiatric disorders including major depressive disorder, bipolar disorder, and borderline personality disorder. Endogenous opioid pathways have been shown to play a role in the neurological response to social rejection and reward. Our group has previously demonstrated that both social rejection and acceptance induced mu opioid receptor (MOR) activation and this activation was related to mood alterations. However, the MOR related biological underpinnings of Rejection Sensitivity and how MOR activation relates to mood changes over time have not yet been examined. In the present investigation, we examine the relationship between Rejection Sensitivity, MOR activation and change in mood over time following social rejection and acceptance. We hypothesize that those with higher trait Rejection Sensitivity will have lower MOR activation in both rejection and acceptance stimuli and that MOR activation will be related to changes in mood and self-esteem over time.

Methods: A total of 75 healthy participants (39 female, mean age = 21.1 +/- 2.2 years) completed socially relevant rejection and acceptance tasks during two back-to-back positron emission tomography (PET) scans using the MOR radioligand [11C] carfentanil. During and after each block, self-reported levels of self-esteem, desire for social interaction, and mood ("happy and accepted" or "sad and rejected") were obtained. Trait Rejection Sensitivity was determined with the Adult Rejection Sensitivity Questionnaire at study initiation. Nondisplaceable binding potential (BPND) was calculated from bolus plus infusion delivery of tracer with occipital lobe reference-region based Logan graphical analysis. Task based MOR activation was determined by subtracting MOR BPND during acceptance or rejection from MOR BPND in the time-matched neutral block. MOR activation in the amygdala, midline thalamus, anterior insula, and nucleus accumbens were evaluated. Bonferroni correction for multiple comparisons was applied.

Results: MOR activation in the nucleus accumbens was positively associated with increase in ratings of self-esteem (*p*-value < 0.02 corrected, R = 0.35, 95% CI [0.10, 0.56]) and of feeling "happy and accepted" (*p*-value <0.03 corrected, R = 0.31, 95% CI [0.08, 0.51]) during the period between acceptance task administration and 5 minutes after the end of the acceptance task. MOR activation was not associated with change in desire for social interaction in relation to the acceptance task. Ratings of self-esteem or negative mood ("sad and rejected") taken during and after the rejection task had no association with MOR activation. Trait Rejection Sensitivity scores were not related to MOR activation in the amygdala, midline thalamus, anterior insula, or nucleus accumbens during either acceptance or rejection tasks.

Conclusions: The present investigation demonstrated that MOR activation in the nucleus accumbens was associated with increased self-esteem and positive mood after experiencing social acceptance. Contrary to our hypothesis, Trait Rejection Sensitivity was not related to MOR activation in the amygdala, midline thalamus, anterior insula, and nucleus accumbens as shown through region-based analysis. This suggests that in healthy individuals, the regional endogenous opioid response to social acceptance is independent of this trait.

Keywords: Mu-Opioid Receptor Binding Potential, Social Rejection, Self-Esteem, Mood, PET Imaging

Disclosure: Nothing to disclose.

P392. Intensive TMS in Treatment Refractory Patients with Major Depressive Disorder: A Pilot Replication

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Background: Approximately 30% - 40% of patients with major depressive disorder (MDD) have treatment resistant depression (TRD), defined as a failure to respond to two adequate medication trials or relapse during treatment. Patients with TRD experience severe depressive symptoms, including suicidal ideation. Transcranial magnetic stimulation (TMS) has proven efficacy for the treatment of TRD but is still relatively under-utilized, and, like most depression treatments, there is a delay in the onset of beneficial effects of TMS, generally administered as a 4 week course. Few evidence-based interventions exist for depression treatment with rapid onset. Thus, testing the efficacy of rapid-acting treatments could potentially help short-circuit the descent into suicidal states and could accelerate recovery. In this study we sought to replicate a recently developed protocol (Williams, Sudheimer et al. 2018) for concentrating the usual 4 weeks of TMS into 5 days of intensive treatment using the same treatment parameters in a treatment refractory cohort.

Methods: Participants ($n = 7$) were recruited from the outpatient clinics at the University of Pennsylvania. Participants (age 21-67; mean = 41.4; 4 males, 3 females) were required to meet criteria for unipolar TRD. They were excluded for symptoms of psychosis, other comorbid psychiatric illness including PTSD, OCD and substance abuse disorder, neurological syndromes including Parkinson's disease, seizure disorder, history of significant brain trauma, space occupying lesion, dementia, any serious medical illness (e.g. end stage renal disease), use of medications that could produce depression (e.g. beta-blockers) or MRI contraindications. All were assessed by SCID, Montgomery Asberg Depression Rating Scale (MADRS) and Scale for Suicidal Ideation (SSI) to determine if patients met inclusion and exclusion criteria. All underwent MRI scanning (Siemens 3T scanner) to obtain coordinates for neuronavigation guided TMS. Individualized targeting was conducted by selecting the dorsal lateral PFC coordinates corresponding to the gyral crest at the center of a meta-analytically derived DLPFC mask from Neurosynth (i.e. largest left hemisphere cluster using association test search term = dlPFC; z = 5.93 peak = -46, 38, 30) superimposed on each individual brain. The study was approved by the University of Pennsylvania Institutional Review Board. Participants were treated with open-label iTBS with 1800 pulses per session at 90% resting motor threshold and depth adjustment to the personalized anatomical target. Each session lasted 10min followed by a 50-min intersession interval. Our iTBS pulse parameters were identical to those used by Li et al. (Li, Chen et al. 2014) (3-pulse 50-Hz bursts at 5-Hz for 2-s trains, with trains every 10s). Ten sessions were applied per day (18000 pulses/day) for five consecutive days (90000 total pulses) using a Magventure Magpro X100 system. Depression severity was measured at baseline, immediately after the final session and at 2 weeks follow-up. To assess intensive TMS-induced changes in symptom severity from baseline, linear mixed effects regression models fit by restricted maximum likelihood (REML) were used with subject-level random intercepts. *P*-values for fixed-effects parameter estimates were estimated using Kenward-Roger's approximation and assessed individually at a 0.05 significance level.

Results: Participants had a history of a mean of 6.6 failed treatment trials with antidepressant monotherapy and mean of 2.4 augmentation trials. All participants were significantly impaired by illness resulting in either unemployment or disability. MADRS scores significantly decreased following treatment ($\beta = -12.7$, se = 2.8, $p < 0.001$) and remained significant 2-weeks post-treatment ($\beta = -12.4$, se = 3.0, $p = 0.002$). SSI scores significantly decreased following treatment ($\beta = -2.7$, se = 0.9, $p = 0.015$) and remained low but not statistically different from baseline at 2-weeks post-treatment ($\beta = -2.1$, se = 1.0, $p = 0.06$). All participants tolerated the therapy without adverse events.

Conclusions: This work confirms previously reported evidence for treatment of highly refractory depression with high-dose spaced iTBS. An important difference between this study and the Williams et al study (Stanford SAINT protocol) is the use of anatomical targeting rather than connectivity based targeting, which may have resulted in a less robust treatment response in the current study (37% vs 76% decrease). Nonetheless, we note that the patients who were treated in this study achieved an equivalent or better response in 5 days compared with traditional TMS lasting 4-6 weeks. We are planning a follow-up study with connectivity guided TMS to confirm the added benefit. A sham controlled study will be necessary to assess the treatment outcome in a larger sample of TRD patients.

Keywords: TMS, Major Depressive Disorder (MDD), MRI, Treatment Resistant Depression

Disclosure: Nothing to disclose.

P393. Thalamic Structure and Function in Youth With and At Familial Risk for Bipolar Disorder

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Background: Youth with and at familial risk for bipolar disorder have aberrant reward function in key prefrontal-striatal networks. However, thalamic structure, function, and connectivity associated with trait and state features of bipolar disorder before and around emergent mania is poorly understood.

Methods: Youth with ($n = 22$) versus without ($n = 23$) BD underwent structural neuroimaging during reward processing using the monetary incentive delay (MID) task at baseline and 12 months. Healthy Youth at high and low risk for mood disorders [BD-risk ($n = 40$), MDD-risk ($n = 41$), and HC ($n = 45$) [mean age 13.09 +/- 2.58, 56.3% female] also completed the MID task at baseline and were followed for behavioral and clinical outcomes over 4.37 +/- 2.29 years. Region of interest (ROI) analyses were conducted using an anatomically defined thalamus seed during reward anticipation and feedback. Psychophysiological interaction and whole-brain voxel-wise group differences were also conducted ($Z > 3.1$; FWE-cluster corrected $p < .05$). We also used machine learning (ML) techniques to evaluate whether neural correlates during reward processing with additional features could predict risk group membership. Based on previous studies that have shown success with classifying fMRI-based data with limited sample datasets, two machine learning techniques were evaluated: Support Vector Machine (SVM) and Random Forest.

Results: Relative to healthy controls, adolescents with BD showed greater reductions in thalamic volume from baseline to 12-month follow up (Treatment x time interaction, Left thalamus: $F(45) = 8.38$, $p = 0.006$; Right thalamus: $F(45) = 3.96$, $p = .05$). Among BD youth, reductions in both hemispheres were negatively associated with mania symptom improvement/maintenance ($p < 0.05$). Relative to MDD-risk and HC, BD-risk had decreased activation of the thalamus during anticipation of monetary gain $F(2,118) = 4.64$; $p = .01$ (FDR-corrected $p = .04$). BD-risk had less connectivity between the thalamus and left middle frontal gyrus ($Z > 3.1$; $p < .001$) and left superior temporal gyrus ($Z > 3.1$; $p < .05$) compared to MDD-risk. Voxel-wise, BD-risk had decreased activation in the cerebellum during anticipation and outcome of monetary gain relative to MDD-risk and HC ($Z > 3.1$; $p < .001$; $Z > 3.1$; $p < .01$, respectively). Decreased thalamic connectivity was associated with increased impulsivity at baseline and reduced prosocial behavior at follow-up. The best performing ML technique was a random forest with an average accuracy of 63.7% (SD 8%).

Conclusions: Compared to typically developing youth, BD youth showed statistically significant volumetric reductions in both the left and right thalamus over the course of 12 months, such that those with worsening scores had greater reductions in volume even after accounting for treatment, suggesting a possible disorder related phenomenon. Reduced thalamic activation and connectivity during reward processing may distinguish familial risk for BD from familial risk for MDD and represent early markers of vulnerability that precede symptom onset and may herald social dysfunction later in development. SVM and Random Forest ML models were able to accurately predict risk group membership based on thalamus activation and connectivity, impulsivity, and approach-withdrawal beyond chance. These findings suggest that BD-risk and MDD-risk youth may present with a combination of specific neural signatures and behaviors related to reward processing that are common and distinct. Differential activation

and connectivity of the thalamus could be specific to BD, which has been suggested in previous studies of youth with and at-risk for BD. While preliminary, our findings highlight potential targets and risk factors that could be used to distinguish youth at risk for BD from youth at risk for MDD, and also possibly improve approaches to prevention and treatment selection for youth at familial risk for mood disorders.

Keywords: Pediatric Bipolar Disorder, Thalamus, Reward Processing

Disclosure: National Institutes of Health, Brain and Behavior Research Foundation, Johnson and Johnson, Stanford University Maternal Child Health Research Institute, Stanford University Department of Psychiatry: Grant (Self)

Sunovion, Skyland Trail: Advisory Board (Self)

X, moonshot factory, Alphabet, Inc., Limbix: Consultant (Self)

American Psychiatric Association, Thrive Global: Royalties (Self)

P394. Structural MRI-Based Brain Age as a Reliable Biomarker of Response to Antidepressant Treatment: Findings From the EMBARC Study

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Background: Recent reports suggest that adults with major depressive disorder (MDD) have higher neuroimaging-predicted brain age as compared to their chronological age, i.e., have accelerated brain aging. Furthermore, accelerated brain aging in adults with MDD has been linked to greater impulsivity and increased depression severity. While promising, it remains unclear whether neuroimaging-predicted brain age can be a useful biomarker in prognosticating clinical outcomes. Therefore, to establish brain age as a clinically useful biomarker we first checked its reliability using structural brain scans that were acquired approximately one week apart, and then evaluated changes in depression severity based on pre-treatment brain age using data from the Establishing Moderators and Biosignatures of Antidepressant Response in Clinical Care (EMBARC) study.

Methods: Brain age was estimated using T1-weighted structural MRI scans and a previously-published Gaussian Processes Regression model (https://doi.org/10.5281/zenodo.3476365) at baseline ($n = 290$) and week-1 ($n = 260$) of EMBARC study. Participation in the EMBARC study was restricted to adults with MDD who were (1) in a currently depressive episode of at least moderate severity, (2) unmedicated and (3) willing to be randomized to either sertraline or placebo. Overall depression severity was measured with the 17-item Hamilton Depression Rating Scale (HAMD-17) at baseline, weeks 1, 2, 3, 4, 6, and 8. Δ brain age was computed as follows: (neuroimaging-predicted brain age) minus (chronological age). Comparison of brain age values at baseline and week-1 of EMBARC were conducted by using correlation analyses, Bland Altman analyses, and paired t-tests. Separate repeated measures mixed model analyses for sertraline and placebo were used to evaluate with changes in depression severity (dependent variable) differed on the basis of pre-treatment Δ brain age after controlling for age, race, ethnicity, sex and site.

Results: Mean [standard deviation (SD)] chronological age of EMBARC study participants at baseline was 37.1 (13.3) years. Mean (SD) neuroimaging-predicted brain ages at baseline ($n = 290$) and week-1 ($n = 260$) of EMBARC were 40.6 (13.1) and 41.0 (13.2) years, respectively. Brain age values at baseline and week-1 were highly correlated (Pearson's $r = 0.98$, $n = 260$, $p < 0.0001$). Furthermore, there was no significant difference in brain age at baseline and week-1 (mean difference = 0.23 years, 95% Confidence Interval: -0.09 years, 0.55 years).

On average, individuals with MDD had higher neuroimaging-predicted brain age than chronological age at the baseline visit of EMBARC [mean (SD) Δ brain age = 3.1 (6.1) years]. Δ brain age and overall depression severity were not associated at baseline (Pearson's $r = -0.06$, $p = 0.29$). However, Δ brain age at baseline significantly predicted changes in depression severity with sertraline (Δ brain age-by-week interaction: $F = 2.54$, $df = 6.695$, $p = 0.019$) but not with placebo ($p = 0.64$) after controlling for age, gender, race, ethnicity, and site. Among EMBARC participants treated with sertraline, higher Δ brain age was associated with smaller reductions in depression severity from baseline-to-week-8.

Conclusions: In this large sample of adults with MDD, we found that brain age values based on structural MRI scans 1-week apart were highly correlated and reliable. Additionally, accelerated brain aging (i.e., higher Δ brain age) was associated with poorer outcomes with sertraline. No such association between accelerated brain aging and treatment response was seen among participants who were treated with placebo.

Keywords: Biomarker, Brain age, Antidepressant Treatment

Disclosure: Alto Neuroscience, Axsome, Sage, GH Research Limited, GreenLight VitalSign6 Inc.: Advisory Board (Self)

Janssen, Otsuka, Acadia Pharmaceuticals, Inc., Alkermes Inc., Merck Sharp and Dohme Corp., Mind Medicine (MindMed) Inc., Neurocrine Biosciences Inc., Orexo US Inc., Signant Health, Titan Pharmaceuticals, Inc.: Consultant (Self)

Oxford University Press: Royalties (Self)

P395. The Role of Mitochondrial DNA in Mediating Chronic Stress-Induced Inflammation and Behavior

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Background: A better understanding of how stressful experiences accelerate behavioral abnormalities can help to improve the treatment options. Chronic stress can also provoke elevated inflammation and exaggerated inflammatory responses in both humans and animal models. Although much of the prior studies have focused on the innate immune system, the mechanisms that link adaptive immunity to chronic stress-induced behavioral abnormalities are not well understood. Mitochondria, the powerhouse of cell, become damaged and dysfunctional following chronic stress conditions, raising the question of whether inflammation associated with chronic stress-related neuropsychiatric conditions is due to mitochondria-induced inflammation. In the present study, we investigated the role of mitochondrial DNA (mtDNA) from CD4 + T cells in mediating chronic stress-induced behavioral changes.

Methods: Adult male ICR, mitochondrial antiviral signaling protein (MAVS) knock out (KO), and Toll-like receptor 9 (TLR9) KO mice were used. The chronic restraint stress (RS) model was used to examine the effects of chronic stress on neurobehavior. Social behavior was tested in a three-chamber test. mRNA levels were measured by quantitative RT-PCR. Neutrophil Extracellular Traps (NETs) formation was measured by ELISA. The data were analyzed by two-way ANOVA.

Results: Antibody-mediated depletion of CD4 + T cells (but not CD8 + T cells), as well as treatment with Rapamycin (a known stimulator of mitophagy), significantly attenuated ($p < 0.05$) RS-induced increases in cell-free (cf)-mtDNA, NETs formation, and social behavior deficits in mice. RS induced a decrease in the expression of NOD-like receptor X1 (NLRX1, a mitochondrial protein known to promotes autophagy, but attenuates IFN-I

production via promoting the degradation of MAVS) in CD4 + T cells. Further, activation of NLRX1 signaling using MAVS KO mice attenuated RS-induced increases in serum cf-mtDNA, NETs, and behavior deficits. In addition, Inhibition of TLR9 attenuated mtDNA-induced increases in NETs and behavior deficits.

Conclusions: Our findings show an important role of mtDNA in mediating chronic stress-induced inflammation and behavior in mice.

Keywords: mtDNA, Chronic Stress, Inflammation, Social Behavior, Mitophagy

Disclosure: Nothing to disclose.

P396. Alternative Splicing Mechanisms and Chronic Stress

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Background: Chronic stress is a significant risk factor for several psychiatric and neuropsychiatric disorders, including major depressive disorder, schizophrenia, and post-stress traumatic disorder. Preclinical and clinical studies substantiate the effect of stress on neurotransmission and synaptic plasticity in brain regions related with behavioral responses, leading to long-term neuronal dysregulation, and changed emotional and cognitive behaviors. More than 70% of human genes undergo alternative splicing, and about a third of diseases are the result of errors in pre-mRNA splicing. Splicing mechanisms are especially vital in the central nervous system (CNS), as vast numbers of genes that are critical for neuronal differentiation, function, and survival are known to undergo alternative splicing. This study will investigate the novel functional role of an essential splicing factor, CWC22 (spliceosome-associated protein CWC22), in alternative splicing of genesthat may contribute to neuronal dysfunction in chronic stress. CWC22 was initially shown to be a marker of antidepressant treatment response in Mexican-Americans (Wong ML et al. J Affect Disord. 2021;279:491-500), and it was subsequently identified as a male-associated suicidality risk locus in a multivariate gene-by-environment genome-wide interaction study (GEWIS) of suicidality in 123,633 individuals (Wendt FR et al. Neurobiol Stress. 2021 Feb 18;14:100309).

Methods: Male C57BL/6 mice were submitted to chronic restraint stress (CRS); control mice were non-stressed. Behavior despair and anxiety were assessed using behavioral tests. Plasma was collected to measure corticosterone levels. CWC22 levels in brain tissues were determined by Western blots, immunohistochemistry, and RT-PCR. CWC22 knockdown in primary hippocampal neurons were generated using shRNA and control lentiviruses. Day-in-vitro 14 neurons were recorded using Whole-cell patch clamp. All behavioral scoring and data analyses was conducted with the experimenter blinded to the group. Experiments involving cells were repeated at least 3 times with independent batches of cells. Viral vectors were generated by us and sequence-verified. Student's t-test was used to compare 2 groups and Mann Whitney test was used for non-normal data. α was set at 0.05; a p value of < 0.05 was considered significant as mean differences have a 95% probability of not being due to chance. Viral vector efficiency and sequence was verified before use.

Results: CRS animals displayed decreased time spent in the center of the open field ($P < 0.001$),

decreased sucrose preference ($P < 0.001$), increased immobility time in the forced swim test ($P < 0.001$) and corticosterone levels ($P < 0.05$) in comparison to non-stressed mice ($n = 12$-14). CWC22 is highly expressed in CNS tissues. Hippocampal CWC22 protein levels were reduced in CRS animals in comparison to non-stressed controls ($P < 0.05$; $n = 8$). CWC22 knockdown neurons had significantly reduced mean mEPSC (miniature excitatory post-synaptic current) frequency and amplitude ($P < 0.01$, $n = 20$). Both SYN1 (synapsin 1)-positive puncta and PDS95 (postsynaptic density protein 95)-positive puncta were increased in CWC22 knockdown neurons; $P < 0.001$ ($n = 4$-15 neurites/condition). The number of dendritic spines labeled with GFP was increased by CWC22 knockdown ($P < 0.01$), and further analysis of spine morphology revealed that mushroom spines' (considered to be 'memory' spines), and thin spines (called 'learning' spines) density, were increased (both at $P < 0.001$; $n = 4$-15 neurites/condition). Gria1 Pre-mRNA splicing was reduced in CWC22 depleted hippocampal neurons ($P < 0.001$). New and unpublished data include: 1) CWC22 expression in CNS tissues; 2) change in hippocampal CWC22 protein levels in CRS animals; 3) All molecular biology and in vitro data; 4) Viral vector constructs.

Conclusions: Splicing events have been reported for many genes in the central nervous system (CNS), and pre-mRNA splicing is a major neuronal mechanism for adaptation to stress. Elucidating the specific roles of spliceosomal components in the CNS may clarify how dysfunction of pre-mRNA splicing affects its functional integrity and provide novel insights into the etiology of stress-related neuropsychiatric disorders. Our studies indicate that CWC22 dysregulation in chronic stress can lead to hippocampal neuronal dysfunction and in vitro results also support the role of CWC22 in synapse formation and dendritic spine morphology. Taken together, our data suggest that CWC22 is a negative regulator of synaptic plasticity.

Keywords: Splice Variants, Stress, Synspsis, Hippocampus, Major Depression

Disclosure: National Institutes of Health, CART Foundation: Grant (Self & Spouse)

Springer Nature: Honoraria (Self)

eLife Sciences Publications: Honoraria (Spouse)

P397. Functional Network Connectome Abnormalities Associated With Familial Risk for Developing Bipolar I Disorder in Youth With Attention-Deficit/Hyperactivity Disorder

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Background: Comorbid attention-deficit/hyperactivity disorder (ADHD) is highly prevalent in youth with bipolar I disorder (BD-I), and ADHD symptoms commonly precede the initial onset of mania and reduce the age at onset of mood symptoms. Having a first-degree relative with BD-I substantially increases the risk of developing BD-I, and there is a high prevalence of ADHD in youth with a first-degree relative with BD-I. Although youth with a first-degree relative with BD-I and ADHD symptoms are at elevated risk for developing BD-I, associated central pathophysiological mechanisms are poorly understood. The initial onset of BD-I frequently occurs during adolescence, a developmental period associated with robust maturational changes in prefrontal cortex structural and functional connectivity. Graph theory analysis is increasingly being used to characterize topological properties of brain network organization and connectivity. In this cross-sectional resting-state MRI study, we characterized the topology of the functional connectome in ADHD youth with (high-risk) and without (mid-risk) a first-degree relative with BD-I and a healthy comparison group (low-risk). Exploratory analyses evaluated associations between functional topological metrics and clinical measures of ADHD and mood symptom severity.

Methods: Resting-state MRI scans were acquired from $n = 37$ adolescents with ADHD and at least one biological parent or sibling with BD-I (high-risk), $n = 45$ ADHD adolescents with no first- or second-degree relative with a mood or psychotic disorder (mid-risk), and $n = 32$ healthy adolescents with no personal or family history of a DSM-5 Axis I psychiatric disorder (HC, low-risk). All ADHD patients met DSM-5 criteria for ADHD (all types), had no exposure to psychostimulants for at least 3 months prior to scanning, and had no comorbid mood, anxiety, conduct, eating or psychotic disorders. Whole-brain functional connectivity network was constructed for each participant. Global- and nodal-level network topological properties were calculated based on individual functional networks using graph theoretical analysis. Global topological metrics included global efficiency (Eglob), local efficiency (Eloc), clustering coefficient (Cp), characteristic path length (Lp), normalized clustering coefficient (γ), normalized characteristic path length (λ) and small-worldness scalar (σ). The nodal topological metrics reflecting centrality included degree, betweenness, and efficiency. The comparison of network topological metrics across groups and post-hoc analyses were conducted using the non-parametric permutation analysis (10000 permutations) with a one-way ANOVA model. Bonferroni correction was applied for subsequent post-hoc pairwise comparisons. Correlation analyses evaluated associations between significantly different functional topological metrics and clinical measures of mood and ADHD symptom severity, and FDR correction was applied to control for false positives.

Results: A total of $n = 114$ psychostimulant-free adolescents were included in the functional connectome analysis, and there were no group differences in age, sex, or BMI. Significant group differences were observed for the global topological metrics Eglob ($p = 0.026$) and Lp ($p = 0.028$). Eglob was significantly decreased ($p = 0.005$) and Lp ($p = 0.003$) increased in mid-risk ADHD adolescents compared to low-risk HC subjects. Brain regions with significant group differences in at least two of three nodal topological metrics included the left dorsolateral superior frontal gyrus (SFG), right middle orbitofrontal cortex (OFC), right hippocampus, left amygdala, left inferior parietal lobule (IPL) and right middle temporal pole (MTG). Compared with low-risk HC subjects, high-risk ADHD adolescents exhibited abnormal nodal topological centralities in the left dorsolateral SFG, right hippocampus and right MTG, whereas mid-risk ADHD adolescents exhibited abnormalities in the right middle OFC, right hippocampus, and left amygdala. Significant differences between high-risk and mid-risk ADHD groups were found in the left IPL. Among all ADHD subjects, the degree ($r = -0.28$, $p = 0.002$) and efficiency ($r = -0.27$, $p = 0.003$) of the right hippocampus, and the degree ($r = -0.27$, $p = 0.003$) and efficiency ($r = -0.27$, $p = 0.003$) of the right middle OFC, were inversely correlated with ADHD-RS total score. Hyperactive/impulsive scores were inversely correlated with global efficiency ($r = -0.25$, $p = 0.007$) and right hippocampus degree ($r = -0.29$, $p = 0.002$), and positively correlated with characteristic path length ($r = 0.26$, $p = 0.005$) and betweenness ($r = 0.29$, $p = 0.001$) of left dorsolateral SFG. The degree ($r = 0.27$, $p = 0.004$) and betweenness ($r = 0.25$, $p = 0.007$) of the left dorsolateral SFG was positively correlated with YMRS total scores.

Conclusions: This cross-sectional analysis found that youth at mid- and high-risk for developing BD-I exhibit patterns of functional network abnormalities compared with low-risk youth. Common abnormal regional centralities were observed in the right hippocampus for all ADHD adolescents regardless of risk for developing BD-I, whereas high-risk subjects exhibited unique abnormalities in topological metrics in the inferior parietal gyrus. These findings suggest that familial risk for BD-I in conjunction with ADHD is associated with a pattern of regional functional network topological abnormalities that may represent a unique prodromal phenotype.

Keywords: Bipolar Disorder, Familial Risk, ADHD

Disclosure: Nothing to disclose.

P398. Brain Network Structural Connectome Abnormalities in Adolescents at Varying Risk for Bipolar I Disorder: A Cross-Sectional MRI Study

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Background: Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder that significantly increases risk for developing other psychiatric disorders including bipolar I disorder (BD-I). Comorbid ADHD is highly prevalent in youth with BD-I, and ADHD symptoms commonly precede the initial onset of mania and reduce the age at onset of mood symptoms. Having a first-degree relative with BD-I also significantly increases the risk for developing BD-I, and there is high prevalence of ADHD in youth with a first-degree relative with BD-I. Furthermore, the initial onset of mania, and by definition BD-I, frequently occurs during adolescence, a developmental period associated with robust maturational changes in prefrontal cortex structural and functional connectivity. Previous structural MRI studies have found that youth with BD-I or ADHD exhibit morphological abnormalities in prefrontal brain regions compared with healthy typically developing youth. However, the majority of prior studies did not control for familial risk of BD-I, ADHD comorbidity, and/or psychostimulant exposure, and there have been no studies that have directly compared psychostimulant-free ADHD youth with and without a first-degree relative with BD-I. In this cross-sectional study, we used graph-based network analysis based on structural MRI data to interrogate topological properties of brain networks in ADHD adolescents with and without a first-degree relative with BD-I and a healthy control group. We additionally evaluated relationships between topological metrics and mood and ADHD symptom ratings.

Methods: Three groups of psychostimulant-free adolescents (10-18 years) were recruited: adolescents with ADHD and at least one biological parent or sibling with BD-I (high-risk), adolescents with ADHD and no first- or second-degree relative with a mood or psychotic disorder (mid-risk), and healthy adolescents with no personal or family history of psychiatric illness (HC, low-risk). High-resolution 3D T1-weighted images were collected using a Philips 3.0 T MR scanner. Brain networks were constructed based on the similarity of morphological features across regions and analyzed using a graph theory approach. Network metrics, which include seven global-level graph metrics (characteristic path length, clustering coefficient, normalized clustering coefficient, normalized characteristic path length, small-worldness, global efficiency, and local efficiency) and three nodal-level properties (degree, efficiency and betweenness) were calculated. We used ANOVA models to compare the structural connectome characteristics across groups, and post hoc pairwise permutation tests were conducted for measures that differed between groups. Significant topological metrics with $p < 0.05$ were reported. Partial correlation analysis was used to assess the relationships between each topological metric and clinical measures of mood and ADHD symptom severity variables. The potential confounding effects of age and sex were controlled for in the models, and the threshold for results were set to an expected FDR of 5% to correct for multiple comparisons.

Results: A total of $n = 149$ adolescents (mean age: 14.1 ± 2.5 years, 36% female) were included in the analysis (low-risk, $n = 49$; mid-risk, $n = 50$; high-risk, $n = 50$). No significant group differences were observed for age, sex, or BMI. The mid-risk and high-risk ADHD groups exhibited similar differences when compared with low-risk HC subjects, mainly in the default-mode network

(DMN) and central executive network (CEN), including bilateral gyrus rectus, right superior parietal gyrus, and right triangular inferior frontal gyrus. Topological alterations in the salience network (SN), including right opercular inferior frontal gyrus and right pallidum, were found between the high-risk group and both mid- and low-risk groups. Significant abnormalities in global network properties were found only in the high-risk group when compared with low-risk HC group, which included reduced characteristic path length ($p = 0.008$), increased global efficiency ($p = 0.008$), and increased local efficiency ($p = 0.041$). ADHD-RS hyperactivity/impulsivity subscale scores were positively correlated with the degree ($r = 0.34$, $p = 0.001$) and nodal efficiency ($r = 0.27$, $p = 0.008$) of right triangular inferior frontal gyrus. There were no significant correlations between topological metrics and mood symptom ratings after correcting for multiple comparisons.

Conclusions: Both high- and mid-risk ADHD adolescents exhibit topological alterations in three major intrinsic connectivity networks (i.e., CEN, DMN and SN) compared to low-risk HC subjects. However, topological alterations in the SN were only observed in the high-risk group. These findings suggest that familial risk for BD-I in conjunction with ADHD is associated with different regional structural network abnormalities compared with ADHD alone and may represent a unique prodromal phenotype relevant to the risk for developing BD-I.

Keywords: Bipolar Disorder, Familial Risk, ADHD

Disclosure: Nothing to disclose.

P399. Cross-Sectional Evaluation of Prefrontal Neurochemistry and Prodromal Mood Symptoms in Youth at Varying Risk for Bipolar I Disorder

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Background: The initial onset of bipolar I disorder (BD-I) frequently occurs during adolescence, and having a first-degree relative with BD-I substantially increases the risk of developing BD-I. Additionally, attention deficit/hyperactivity disorder (ADHD) is a risk factor for psychopathology including BD-I, and there is a high prevalence of ADHD in youth with BD-I or a first-degree relative with BD-I. Furthermore, ADHD symptoms commonly precede the initial onset of BD-I and are associated with an earlier onset of mood symptoms. Although adolescents with ADHD and a first-degree relative with BD-I are at elevated risk for developing mood disorders, associated prodromal clinical and neurophysiological features remain poorly understood. Proton magnetic resonance spectroscopy (1H MRS) studies have previously found that youth with BD-I or ADHD exhibit abnormalities in prefrontal neurochemistry compared with healthy typically developing youth. However, the majority of prior studies did not control for familial risk of BD-I, ADHD comorbidity, and/or psychostimulant exposure, and there have been no studies that have directly compared psychostimulant-free ADHD youth with and without a first-degree relative with BD-I. In this cross-sectional study, we characterized mood and cognitive symptoms and used 1H MRS to investigate bilateral ventrolateral prefrontal cortex (VLPFC) neurochemistry in ADHD youth with (high-risk) and without (mid-risk) a first-degree relative with BD-I and a healthy comparison group (low-risk). Exploratory analyses evaluated associations between VLPFC neurochemistry and mood and ADHD symptom severity.

Methods: A total of $n = 150$ male and female (65% male) psychostimulant-free adolescents (mean age: 14.1 ± 2.5 years) were enrolled into three groups: adolescents with ADHD and at least one biological parent or sibling with BD-I (high-risk, $n = 50$), adolescents with ADHD and no first- or second-degree relative with a mood or psychotic disorder (mid-risk, $n = 51$), and healthy adolescents with no personal or family history of a DSM-5 Axis I psychiatric disorder (low-risk, $n = 49$). All ADHD patients met DSM-5 criteria for ADHD (all types), had no exposure to psychostimulants for at least 3 months prior to scanning, and had no comorbid mood, anxiety, conduct, eating or psychotic disorders. ADHD (ADHD-RS), mania (YMRS), depression (CDRS-R), and global functioning (CGAS) ratings were determined, and 1H MRS scans performed using a Philips 3.0 T MR scanner. MRS voxels ($20 \times 20 \times 20$ mm) were positioned in the right and left VLPFC, and glutamate (Glu), glutamate+glutamine (Glx), myo-inositol (mI), choline (Cho), N-acetyl aspartate (NAA), and phosphocreatine plus creatine (PCr + Cr) concentrations determined.

Results: There were no significant group differences in age ($p = 0.25$), sex ($p = 0.83$), race ($p = 0.28$), or BMI ($p = 0.40$). Significant group differences were observed for ADHD-RS total score ($p < 0.0001$), as well as inattentive ($p < 0.0001$) and hyperactivity/impulsivity ($p < 0.0001$) subscores, YMRS total score ($p < 0.0001$), CDRS-R total score ($p < 0.0001$), and CGAS total score ($p < 0.0001$). Pairwise comparisons found that both high-risk and mid-risk groups differed significantly from low-risk on all rating measures, and high-risk subjects had significantly higher hyperactivity/impulsivity subscores ($p = 0.004$), YMRS total scores ($p = 0.0006$), and CDRS-R total scores ($p = 0.006$) compared with low-risk subjects. For the 1H MRS analysis, a total of $n = 143$ subjects were included: high-risk, $n = 47$; mid-risk, $n = 48$; low-risk, $n = 48$. For the right VLPFC, no significant group differences were observed for Glu ($p = 0.27$), Glx ($p = 0.24$), mI ($p = 0.19$), Cho ($p = 0.84$), NAA ($p = 0.63$), or PCr + Cr ($p = 0.55$). For the left VLPFC, no significant group differences were observed for Glu ($p = 0.84$), Glx ($p = 0.82$), mI ($p = 0.15$), Cho ($p = 0.53$), NAA ($p = 0.53$), and PCr + Cr ($p = 0.69$). Among all subjects ($n = 143$), CDRS-R ($r = 0.18$, $p = 0.03$) and YMRS ($r = 0.19$, $p = 0.03$) total scores were positively correlated with right VLPFC NAA. CDRS-R scores were inversely correlated with left VLPFC Cho ($r = -0.18$, $p = 0.03$) and Glx ($r = -0.18$, $p = 0.03$), and YMRS scores were inversely correlated with left VLPFC Glx ($r = -0.21$, $p = 0.04$).

Conclusions: This cross-sectional analysis found that youth at high-risk for developing BD-I exhibit greater depression and manic symptom severity, as well as greater hyperactivity/impulsivity symptoms, compared with mid- and low-risk youth. These prodromal symptoms may represent early indices of progressive mood symptom dysregulation that could facilitate risk assessment and management. Although neither high-risk nor mid-risk youth exhibited significant abnormalities in prefrontal neurochemistry, associations between mood symptoms and prefrontal NAA, Cho, and Glx warrant additional investigation.

Keywords: Bipolar Disorder, Familial Risk, ADHD

Disclosure: Alkermes, Allergan, Sunovion: Consultant (Self)
Janssen, Johnson and Johnson, Lundbeck, Sage, Myriad: Advisory Board (Self)
Sunovion: Honoraria (Self),

P400. Ketamine Decreases Neuronally Released Glutamate via Retrograde Stimulation of Presynaptic Adenosine A1 Receptors

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Background: Ketamine produces a rapid antidepressant response in patients with major depressive disorder (MDD), but the underlying mechanisms appear multifaceted. One hypothesis, proposes that by antagonizing NMDA receptors on GABAergic interneurons, ketamine disinhibits afferens to glutamatergic

principal neurons and increases extracellular glutamate levels. However, ketamine seems also to reduce rapid glutamate release at some synapses. Therefore, clinical studies in MDD patients have stressed the need to identify mechanisms whereby ketamine decreases presynaptic activity and glutamate release.

Methods: The effect of ketamine and its antidepressant metabolite, (2R,6R)-HNK, on neuronally derived glutamate release was examined in rodents. We used FAST methodology to measure depolarization-evoked extracellular glutamate levels in vivo in freely moving or anesthetized animals, synaptosomes to detect synaptic recycling ex vivo and primary cortical neurons to perform functional imaging and to examine intracellular signaling in vitro.

Results: In all used approaches, ketamine and (2R,6R)-HNK reduced glutamate release in a manner which could be blocked by AMPA receptor antagonism. Antagonism of adenosine A1 receptors, which are almost exclusively expressed at nerve terminals, also counteracted ketamine's effect on glutamate release and presynaptic activity. Signal transduction studies in primary neuronal cultures demonstrated that ketamine reduced P-T286-CamKII and P-S9-Synapsin, which correlated with decreased synaptic vesicle recycling. Moreover, systemic administration of A1R antagonist counteracted the antidepressant-like actions of ketamine and (2R,6R)- HNK in the forced swim test.

Conclusions: By studying neuronally released glutamate, we identified a novel retrograde adenosinergic feedback mechanism that mediate inhibitory actions of ketamine on glutamate release that may contribute to its rapid antidepressant action.

Keywords: Ketamine, Glutamate, A1 adenosine Receptor, Antidepressant

Disclosure: Nothing to disclose.

P401. Neuronal Models of Circadian Rhythms in Major Depressive Disorder

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Background: Major depressive disorder (MDD) is a common and debilitating mental health condition that responds inconsistently to pharmacotherapy. Circadian rhythm abnormalities have been observed in patients with MDD including sleep and appetite disturbances and diurnal variation in mood. However, until recently, methods to study rhythms in live neurons from human subjects were not available and the mechanism underlying circadian rhythm disruption remains unknown.

Methods: We derived stem-cell lines from controls ($N = 3$) or MDD patients ($N = 6$) previously characterized for clinical response to escitalopram in a prospective clinical trial, choosing the best/worst responders to treatment out of a large cohort. Controls and patients were matched for demographic features. Using the embryoid body method, we derived TUJ1 + glutamatergic neurons from each donor. Circadian rhythms were examined in triplicate live cell cultures using a luminometer to measure rhythms over 7 days using the Per2-luc bioluminescent reporter. Rhythm parameters were calculated for each cell line (period, amplitude, phase). Analyses of variance (one-way ANOVA for case-control analyses or two-way ANOVA with repeated measures for time course studies) were used to determine statistical significance with $p < 0.05$.

Results: Compared to control neurons, Per2-luc rhythms in MDD neurons had a significantly shorter period under entrained conditions. Anti-depressant response correlated with lower amplitude rhythm in neurons from MDD patients whose symptoms did not remit on escitalopram. Compared to controls, MDD neurons had lower overall gene expression levels of CRY1 and PER2. Immunostaining revealed increased levels of BMAL1 in MDD neurons.

Conclusions: We conclude that neurons from MDD patients display abnormal period and dysregulated elements of the molecular clock. Overexpression of BMAL1 protein may lead to excess negative feedback of the molecular clock. Some of these differences may underlie differential outcomes in antidepressant treatment.

Keywords: Major Depression Disorder, Circadian Rhythm, Induced Pluripotent Stem Cells (iPSCs), Neuronal Stem Cells (NSCs)

Disclosure: Nothing to disclose.

P402. Sleep, Ketamine and Hyperarousal: Potential Influences on Suicidal Thoughts

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Background: Ketamine, a glutamatergic modulator, is associated with rapid-reductions in both depressive symptoms and suicidal thoughts. A growing research literature has suggested that sleep difficulties represent a critical suicide risk factor. Previous work by our group has demonstrated that nocturnal wakefulness is associated with next-day suicidal thoughts and this relationship is normalized by ketamine administration; such that suicide ideation (SI) responders to ketamine demonstrate decreased nocturnal wakefulness the night after ketamine administration. More recently, our group demonstrated that oscillations in overnight beta power, as evaluated using a functional data analytic approach, are associated with next-day SI. This relationship between oscillations in beta power and SI may be indicative of a sleep-related hyperarousal process associated with suicide risk. However, the influence of ketamine on sleep-related hyperarousal more generally, and beta fluctuations more specifically, is undefined. The aim of this analysis was to evaluate the relationship of ketamine on sleep hyperarousal in a sample of unmedicated patients with treatment resistant depression (TRD). We then evaluated whether changes in these arousal-related processes mediate the SI response to ketamine.

Methods: 35 medication-free participants with TRD who participated in a double-blind placebo-controlled crossover trial of ketamine and completed overnight polysomnography (PSG) the nights before and after ketamine and placebo infusions were included in this analysis. For each participant, we calculated traditional PSG macroarchitecture metrics, including wake after sleep onset (WASO), total sleep time (TST), sleep efficiency (SE) and REM latency. Additionally, we estimated power spectral density (PSD) and averaged beta frequencies (18- 25 hz) in each 30-second epoch over 5 hours from sleep onset. We then used a functional data analytic approach to identify the three eigenfunctions or principal components (PCs) that represent the three primary orthogonal axes or latent patterns of variation evident in oscillatory patterns in beta power over time. To test the effect of drug (ketamine v placebo) on arousal measures, we used the macroarchitecture and PC scores as outcome measures in separate mixed effect models. To test our mediation hypothesis, we regressed SI on each drug*arousal measure interaction effect in separate models. All models controlled for period-specific baseline, age, infusion, and sex.

Results: In preliminary analyses, ketamine was associated with greater reductions in WASO relative to placebo (beta = -0.35, $p =$

0.03), but no drug effects were detected for TST, SE or REM latency. When PSG macroarchitecture metrics were limited to the 5 hours after sleep onset, the effect became a trend ($p = .08$) in the expected direction. None of the macroarchitecture arousal measures mediated ketamine's effect on SI. After the removal of an outlier, ketamine was associated with lower scores on the second PC score, associated with oscillations of beta (beta = -0.37, $p = 0.03$). This sleep after ketamine administration was characterized by lower arousal shortly after sleep onset. Ketamine did not appear to be related to the first and third PCs which respectively reflected overall average levels of beta and declining beta over the course of the night. We did not find evidence to support temporal patterns of beta oscillations as mediators of ketamine's therapeutic effect on SI.

Conclusions: Preliminary results indicate that ketamine may reduce arousal during sleep as measured by both WASO and temporal patterns of beta oscillations. Contrary to expectations, ketamine's effect on SI was not mediated by arousal-related sleep metrics. We are pursing ongoing analysis of other frequency bands, including alpha and delta, to obtain a fuller picture of ketamine's effects on sleep-related arousal.

Keywords: Suicide, Ketamine, Sleep, Polysomnography

Disclosure: Nothing to disclose.

P403. Reinforcement Learning and Prediction Error in Pediatric Irritability

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Background: Irritability is a common, chronic, and impairing clinical phenotype in youth (Leibenluft, 2011; Stringaris et al., 2018). Alterations in reward processing, including frustrative nonreward (i.e., omission of expected reward), are a proposed mechanism of pediatric irritability (Brotman et al., 2017; Leibenluft, 2017). While a handful of studies have examined reward processing in irritability (e.g., Adleman et al., 2011; Deveney, 2019; Dougherty et al., 2018), no studies have focused on reward prediction error (RPE) during reinforcement learning. RPE operationalizes the difference between an expected outcome and a received outcome; negative RPE (i.e., an outcome that is worse than expected) is posited to have particular relevance to frustrative nonreward and irritability (Kircanski et al., 2019). In the current study, we used a novel, child-friendly fMRI paradigm to assess reinforcement learning before and after a frustration induction in the scanner. We used computational methods to derive estimates of RPE during reinforcement learning and map them onto brain activation on a trial-by-trial basis, testing preliminary associations with irritability.

Methods: Data collection is ongoing; to date, the transdiagnostic sample includes 34 children and adolescents varying in level of irritability (M age = 13.9 years, SD = 2.4; 29% female; 14 with symptoms of disruptive mood dysregulation disorder [DMDD], 13 with attention deficit hyperactivity disorder [ADHD], 7 with no psychiatric disorder). The "Carnival" fMRI paradigm simulates games at a virtual carnival and comprises a series of probabilistic reinforcement learning tasks administered before and after a frustration block with rigged feedback. In each task block, participants learn to select between pairs of stimuli (2-armed bandit tasks; 80:20 reward ratio) to maximize monetary winnings, delivered via trial-by-trial feedback. Subjects use handheld dynamometers to make selections, measuring motor force and arousal as clinically relevant to irritability. Halfway through the paradigm, unbeknownst to participants, a separate, rigged game occurs to induce frustration. Participants are debriefed after the task. Irritability is assessed using the parent-reported Affective Reactivity Index (ARI; Stringaris et al., 2012). For data analysis, a Rescorla-Wagner reinforcement learning model with two parameters (learning rate and inverse temperature) was fit to participants' choice data. Subject-specific, trial-by-trial RPE estimates were extracted and used in whole-brain and ROI-based amplitude modulation analyses (AFNI) to examine modulation of brain activity by RPE and associations of this modulation with irritability (voxel-wise threshold $p < .005$, clusters>27 voxels [422 mm3]).

Results: The in-scanner frustration induction was successful; frustration ratings were higher during the rigged game than during both the pre- and post-frustration games (Fquadratic[1,33] =14.77, $p = .001$), with a large effect size (partial η2 = .31). With respect to task effects, as expected, a general linear test indicated significant modulation of regional activity by RPE, including large clusters of positive RPE encoding in the striatum and OFC, and of negative RPE encoding in the bilateral insula and dorsal anterior cingulate cortex (all ps < .005, whole-brain corrected). In the whole-brain analysis, there were no significant associations between RPE modulation and irritability. However, for a priori anatomical ROIs in the bilateral insula and striatum, activity as modulated by RPE was correlated with irritability. Consistent small-to-medium effect sizes were shown for correlations between parent-reported ARI and activity in the insula and striatum post-frustration (r range = -.25 to -.17). The insula associations were in the hypothesized direction, in which higher irritability was associated with enhanced negative RPE encoding. There were no directional predictions for the striatum, but preliminary associations suggested that higher irritability is associated with diminished positive RPE encoding.

Conclusions: These preliminary findings support the feasibility and utility of computational methods for examining RPE during reinforcement learning as a function of irritability and frustration, bridging the reward processing and FNR literatures (Yu et al., 2014). Whole-brain analyses supported robust task effects for RPE modulation in neural circuitry known to be engaged in reinforcement learning. Further, ROI analyses suggested small-to-medium effect sizes for hypothesized associations between irritability and enhanced negative RPE encoding in the insula. Data collection is ongoing; subsequent analyses will use the established pipeline in a sample optimized for statistical power (projected $N = 120$).

Keywords: Functional MRI (fMRI), Pediatric Irritability, Frustrative Non-Reward, Reinforcement Learning, Reward Prediction Error

Disclosure: Nothing to disclose.

P404. Lack of Association Between Pretreatment Glutamate/GABA and Major Depressive Disorder Treatment Response

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Background: Studies have shown gamma-amino-butyric acid (GABA) and Glx (a combination of glutamate and glutamine) to be altered in major depressive disorder (MDD). Using proton Magnetic Resonance Spectroscopy (1H-MRS), this study aimed to determine whether lower pretreatment GABA and Glx levels in the anterior cingulate cortex (ACC), a region implicated in MDD pathophysiology, are associated with better antidepressant treatment response.

Methods: Participants with MDD ($N = 73$) were antidepressant naïve or medication-free for at least three weeks before imaging. Two MEGA-PRESS 1H-MRS acquisitions were collected in the ACC, interleaved with a water unsuppressed reference scan. GABA and

Glx concentrations were quantified from an average difference spectrum. Following imaging, participants were randomized to escitalopram or placebo for 8 weeks in a double-blind design. Multivariable logistic regression models were applied with treatment type and age as covariates. Bayes Factor hypothesis testing was used to interpret the strength of the finding.

Results: There was no significant association between pretreatment Glx, GABA, or Glx/GABA and remitter status or the continuous outcome, percent change in depression severity. In an exploratory analysis, no significant correlation was found between pretreatment Glx, GABA or Glx/GABA and days to response. Bayes factor analysis showed strong evidence towards the null hypotheses in all cases.

Conclusions: To date, there are no replicated biomarkers in psychiatry. To address this, well-powered, placebo-controlled trials need to be undertaken and reported. The present analysis

suggests GABA, Glx, or their ratio cannot predict antidepressant treatment response.

Keywords: Glutamate GABA, Depression, Magnetic Resonance Spectroscopy

Disclosure: Nothing to disclose.

P405. Prophylactic Effects of (R)-Ketamine on Schizophrenia-Relevant Phenotypes in Adult Offspring After Maternal Immune Activation: A Role of TRKB Signaling

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Background: A number of studies demonstrated that maternal immune activation (MIA) might play a role in the development of neuropsychiatric disorders such as schizophrenia and autism spectrum disorder in offspring. It is demonstrated that pregnant mice exposed to polyinosinic:polycytidylic acid [poly(I:C)] resulted in schizophrenia-like behavioral and neurochemical abnormalities in their offspring.

We demonstrated that (R)-ketamine, (R)-enantiomer of the N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine, would be a novel rapid-acting and sustained antidepressant without side effects of ketamine (reviews by Hashimoto K. Biochem. Pharmacol. 2020; Wei Y, et al. Mol. Psychiatry 2021). Recently, we reported that phencyclidine (PCP)-induced cognitive deficits in mice could be ameliorated after subsequent repeated intermittent administration of (R)-ketamine, but not (S)-ketamine, and that brain-derived neurotrophic factor (BDNF)-TrkB signaling plays a role in the beneficial effects of (R)-ketamine in PCP model of schizophrenia (Tan Y, et al. Pharmacol. Biochem. Behav. 2020). Clinical study of (R)-ketamine in depressed patients is underway.

In this study, we examined whether repeated intermittent administration of (R)-ketamine during juvenile and adolescent stage ameliorates abnormal behavior, parvalbumin (PV)-immunoreactivity and dendritic spine density in the adult offspring after MIA.

Methods: Pregnant female mice ddY (5 days) were purchased from SLC Company Japan (Hamamatsu, Shizuoka Prefecture, Japan). From E12 to E17, every 6 consecutive days, the pregnant mice were intraperitoneally injected with Poly(I:C) (5.0 mg/kg/day), dissolved in 0.2 ml physiological saline, per 20 g body weight or an equivalent volume of physiological saline (Han M, et al. Sci. Rep. 2016; Fujita Y, et al. Sci. Rep. 2016; Matsuura A, et al, Sci. Rep. 2018). After 3 weeks, the offspring were separated from their mother mice, and male mice were kept in cages in groups of three to five. Saline (10 ml/kg/d, twice a week, for 4 weeks) or (R)-ketamine (10 mg/kg as hydrochloride salt, twice a week, for 4 weeks) was intermittent intraperitoneal injection (i.p.) into the mice from 4-weeks old to 8-weeks old. In the experiment using TrkB inhibitor, the vehicle or ANA-12 (0.5 mg/kg; TrkB inhibitor) was injected 30 minutes before saline (10 ml/kg) or (R)-ketamine (10 mg/kg). Novel object recognition test (NORT), PV-immunohistochemistry, and Golgi staining were performed as previously reported (Han M, et al. Sci. Rep. 2016; Matsuura A, et al, Sci. Rep. 2017; Zhang J, et al. Int. J. Neuropsychopharmacol. 2019). The data shown are the mean ± standard error of the mean (S.E.M.). Data were analyzed using one-way analysis of variance (ANOVA), followed post-hoc Tukey test.

Results: In the NORT, administration of poly(I:C) caused cognitive deficits in adult offspring. In the training session, there was no difference among the three groups. In the retention test, the repeated intermittent administration of (R)-ketamine significantly ameliorated the decreased exploratory preference in adult offspring after MIA. PV-immunohistochemistry showed that PV-immunoreactivity in the PrL of medial prefrontal cortex (mPFC) of poly(I:C) + vehicle group was significantly lower than that of vehicle + vehicle group or poly(I:C) + (R)-ketamine group. Furthermore, Golgi staining showed that dendritic spine density in the PrL of mPFC from poly(I:C) + vehicle group was significantly lower than that of vehicle + vehicle group or poly(I:C) + (R)-ketamine group.

Pretreatment with ANA-12 significantly antagonized the prophylactic effects of (R)-ketamine in the adult offspring of prenatal mice exposed to poly(I:C). In contrast, ANA-12 alone did not improve MIA-induced cognitive deficits.

Conclusions: The current data suggest that repeated intermittent administration of (R)-ketamine during juvenile and adolescent stages could prevent the onset of behavioral abnormalities, the reduction of PV-immunoreactivity and dendritic spine density in the mPFC of adult offspring after MIA. Therefore, treatment with (R)-ketamine in young subjects at high risk for psychosis may prevent conversion to psychosis.

Keywords: R-(-)-Ketamine, Ketamine, Maternal Immune Activation, Child Offspring, Schizophrenia-Like Behavior

Disclosure: Taisho, Dainippon Sumitomo, Otsuka,: Contracted Research (Self)

Perception Neuroscience: Grant (Self)

Patents: Patent (Self)

P406. Baseline Memory Function Affects Memory Outcome in Older Adults With Major Depressive Disorder Treated With Acute Course Electroconvulsive Therapy and Venlafaxine

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Background: Major depressive disorder (MDD) is a significant public health challenge worldwide. Major depression in the older adult populations worsens overall health, results in functional impairment, and leads to increased morbidity, and mortality. Moreover, depression in older adults tends to be treatment-resistant. For such treatment-resistant depression, and in acute crises cases such as the presence of suicidality and psychotic features, electroconvulsive therapy (ECT) is often prescribed. While ECT is highly beneficial in older adults with depression, it can also produce adverse cognitive effects. We previously assessed broad neurocognitive and specific verbal learning and memory component effects of a combined regimen of ECT and venlafaxine in

older adults with depression during Phase 1 of the Prolonging Remission in Depressed Elderly (PRIDE) study. Prior research and our findings demonstrated that ECT adversely impacts multiple neurocognitive functions including verbal learning and memory. However, there is limited information regarding how baseline memory function relates to memory outcomes following treatment with ECT. The purpose of this analysis was to examine how baseline cognitive function, specifically verbal memory, affected ECT-associated memory outcome. We explored whether, following acute treatment with a regimen of ECT and venlafaxine, older adults with relatively intact baseline memory function would retain stable memory performance better than those with impaired baseline memory.

Methods: The PRIDE study was a NIMH funded, multicenter, randomized study of an individualized continuation ECT schedule combined with pharmacotherapy to enhance long-term outcomes in older adults with MDD. In Phase 1 of the study, patients received acute ECT thrice weekly combined with venlafaxine. ECT parameters were standardized as: right unilateral (RUL) electrode placement using a Somatics Thymatron System IV with an ultrabrief pulse width of 0.25ms and current of 0.9Amps or a MECTA SPECTRUM device with an ultrabrief pulse width of 0.3ms and current of 0.8Amps. Older adults (age > 60) with MDD were included. All participants provided written informed consent for this IRB approved investigation before completing study procedures. For the purposes of this analysis, the neurocognitive outcome data were collected with the 2nd edition of the California Verbal Learning Test (CVLT-II). The CVLT-II is a psychometrically sound measure of verbal learning and memory. The CVLT-II Standard and Alternate forms were used in order to minimize practice effects, with form order counterbalanced across study sites, subjects, and the two study time points (baseline, end) by a computerized algorithm. The CVLT-II was administered at Phase 1 baseline and within 72 hours following the last ECT session. CVLT-II raw scores were converted into demographic-adjusted scores. Based on baseline performance using the demographic-adjusted scores, the study cohort was characterized as having strong (z-score > 0.5), average (z-score > -0.5 and < 0.5), or impaired (z-score < -1) memory performance. Descriptive statistics were used to characterize the demographic, memory function, and clinical features of the sample, as well as the impact on memory performance following treatment. Percentages may not add up to 100% due to missing data.

Results: The study sample ($n = 203$) had a mean age of 69.9 (SD = 7.6), 14.5 (SD = 3.3) years of education, 57.5% were female and 95% were Caucasian. Verbal memory scores at baseline revealed that 32.1% showed strong performance, 35.4% showed average performance, and 32.5% showed impaired performance. Following acute treatment with RUL ultrabrief pulse ECT and venlafaxine, the three memory function groups showed differential outcomes. For those with strong baseline verbal memory function, 56.9% remained strong, 23.1% had average performance, and 9.2% displayed impaired performance. For those with average baseline verbal memory function, 31.9% remained average, 36.1% showed strong performance, and 15.3% had impaired performance. For those with impaired baseline verbal memory function, 36.4% remained impaired, 43.9% showed intact performance, and 6.1% had strong performance.

Conclusions: To our knowledge, this is one of the first studies to examine how baseline verbal memory function relates to performance following treatment with ECT and venlafaxine in older adults with MDD. Those with average and strong baseline performance showed relative stability and limited impairment after treatment. While some older adults with impaired baseline performance showed intact or strong performance after treatment, approximately 33% remained impaired. As such, average and strong baseline memory function may be a protective factor and impaired baseline memory function may be a risk factor for ECT-associated effects on memory performance. These findings are consistent with other treatments with known adverse cognitive effects where baseline cognitive function has been found to moderate treatment-associated cognitive outcomes. These findings highlight the importance of collecting neurocognitive information in older adults with depression who receive ECT. As this study only focused on verbal memory function, future research is warranted to examine other aspects of cognitive function such as attention, executive function, and working memory to examine how such baseline function may impact outcomes after treatment.

Keywords: Electroconvulsive Therapy, Depression, Memory, Cognition, Older Adults

Disclosure: Pearson Assessment: Consultant (Self)

P407. Orally Administered Choline Analog 2-(4-((1-phenyl-1H-pyrazol-4-yl)methyl)piperazin-1-yl)ethan-1-ol Attenuates Pentylenetetrazol-Induced Seizure-Like Effect in Mice

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Background: A transient and recurrent seizure often leads to a long-lasting behavioral change and low quality of life in epileptic patients. The roles of cholinergic and GABAergic neuronal projections in epileptic seizures inspire the synthesis of choline analog 2-(4-((1-phenyl-1H-pyrazol-4-yl)methyl)piperazin-1-yl)ethan-1-ol (LQFM032) through molecular hybridization of 4-(1-Phenyl-1h-pyrazol-4-ylmethyl)-piperazine-1-carboxylic acid ethyl ester. With the cholinergic and significant cytoprotective effects of LQFM032 in the previous studies, we hereby conduct a preliminary evaluation of its anti-seizure-like effect.

Methods: Acute oral administration of LQFM032 (5, 15 or 45 mg/kg), diazepam (DZP 5 mg/kg) or vehicle 10 ml/kg was carried out before the exposure of 4-week-old male and female Swiss mice [Weight = 28 ± 3 g; $n = 6$ (3 mice per sex) randomly distributed into five groups] to the sodium pentobarbital (50 mg/kg i.p)-induced barbiturate sleep, open field and pentylenetetrazol (PTZ 60 mg/kg i.p.) - induced convulsion. In a separate experimental session, mice were pretreated intraperitoneally with flumazenil 2 mg/kg (FLU; competitive antagonist of benzodiazepine site of the GABA-A receptor), atropine 10 mg/kg (ATR; nonselective muscarinic receptor antagonist), or vehicle before the oral administration of LQFM032, DZP or vehicle. The NIH Guidelines for the Care and Use of Laboratory Animals as approved by the Ethics Committee of the Federal University of Goiás (protocol number 104/08) were adhered to in all experimental procedures. Data were subjected to ANOVA followed by Dunnett's s or Bonferroni's post hoc tests and expressed as mean ± SEM ($p < 0.05$ was considered statistically significant).

Results: The oral administration of LQFM032 (15 and 45 mg/kg) and DZP 5 mg/kg potentiated barbiturate sleep [Sleep latency with F (4, 25) = 21.4, and sleep duration with F (4, 25) = 19.8, $p < 0.05$], thereby showing CNS depressant property without alterations in locomotor activities in the open field arena [Total crossing with F (4, 25) = = 5.9, and freezing time with F (4, 25) = 12.1, $p > 0.05$]. Like DZP 5 mg/kg, an increase in the time to the first PTZ-induced myoclonic jerk [Latency with F (4, 25) = 17.7, $p < 0.05$] and a reduction in the duration of seizure [F (4, 25) = 10.5, $p < 0.05$] suggest the attenuation of seizure-like behavior by LQFM032. The involvement of the benzodiazepine

site and muscarinic receptor in the activities of LQFM032 was shown by the blockade of its antiseizure-like property following FLU and ATR pretreatments.

Conclusions: Together, LQFM032 reduced seizure-like behavioral manifestations that involve cholinergic and GABAergic systems. Further evaluation of the specific modulator, receptor, or circuit underlying this anti-seizure-like mechanism remains pertinent. Keywords

Keywords: Choline Analog, Convulsion-Like Model, Cholinergic System, Gabaergic System, LQFM032

Disclosure: Nothing to disclose.

P408. Opposing Roles of Mu Opioid Receptors in Dopamine D1 and D2 Expressing Neurons in Opioid Mediated Analgesia

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Background: There is extensive interaction between systems involved in pain processing and motivation systems, where the aberrant functioning of salience circuits likely contributes to chronic pain as well as increased susceptibility for opioid misuse and opioid use disorder. The mesolimbic circuitry also contributes to opioid analgesia where an injection of intra-striatal morphine alleviated nociceptive behaviors associated with the chemical irritant formalin. In this study we asked to what extent mu opioid receptors (MOR) in dopamine D1 and D2 receptor expressing neurons contribute to opioid analgesia and negative reinforcement.

Methods: We ablated MOR in dopamine receptor expressing neurons by breeding D1-cre, D2-cre and A2A-cre with MORloxP mice. Mice were subjected to chronic pain followed by conditioned place preference to oxycodone or were tested in an inflammatory pain model induced by formalin.

Results: Ablating MORs from D1 receptor expressing neurons significantly reduced oxycodone (3 mg/kg, i.p.) mediated analgesia in the formalin test, whereas deleting MOR from D2 or A2A receptor expressing neurons enhanced oxycodone mediated analgesia in this test. In contrast, ablation of MOR from any of these neurons had no effect on formalin-induced pain, but only modified the opioid-mediated analgesia. Separate cohorts of mice underwent conditioned place preference to oxycodone following either sham or induction of neuropathic pain via sciatic nerve constriction. Conditioning was conducted over 6 days counterbalancing for day and chamber. Oxycodone produced a greater preference score in pain animals than sham control groups. Deleting MORs from D1 receptor expressing neurons prevented place preference in both sham and neuropathic pain groups. Whereas deleting MOR from D2 and A2A receptor expressing neurons enhanced oxycodone induced place preference in pain animals.

Conclusions: These data demonstrate that MORs have opposing roles in dopamine receptor expressing neurons (likely striatal) on opioid mediated analgesia and negative reinforcement.

Keywords: Pain, Ventral Striatum, Prescription Opioids, Negative Reinforcement, Analgesia

Disclosure: Nothing to disclose.

P409. Association Between Lower Body Temperature and Increased Tau Pathology in Cognitively Normal Older Adults

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Background: Rodent model and in vitro studies suggest brain temperature has the potential to bidirectionally interact with tau pathology in Alzheimer's Disease (AD): tau phosphorylation is robustly increased by small (<1°C) reductions in temperature within the human physiological range, and lower brain thermoregulatory areas may be among those first affected by AD pathology. Here, we evaluated the cross-sectional association between body temperature (Tb), as a proxy for brain temperature, and clinically accessible markers of tau pathology in cognitively normal older adults.

Methods: Tb was measured continuously over 48 hours with ingestible telemetry combined with a novel pre-processing algorithm. This period included 2 nights of nocturnal polysomnography to facilitate delineation of Tb–tau pathology relationships according to waking vs sleeping time intervals. Tau pathology was assessed with both soluble markers including plasma P-tau (P-tau 181) and cerebrospinal fluid (CSF) P-tau, both sampled the following day, and aggregated tau, namely neurofibrillary tangle (NFT) burden in early (I-III) Braak stage areas imaged with MR-PET using the [18F]MK-6240 radio tracer on average ~ one month later

Results: Plasma and CSF P-tau levels were highly correlated with one another and with tau tangle radio tracer uptake (NFT burden), $p < 0.05$ for all comparisons. Lower Tb (quantified by lower mean Tb and a greater proportion of time Tb was under 37.0°C) was associated with increased NFT burden and increased plasma and CSF P-tau levels, $p < 0.05$ all comparisons. For aggregated tau, lower Tb – tau pathology associations were seen during for Tb recorded during waking, but not during sleeping intervals.

Conclusions: Preliminary results suggest that lower body temperature in older adults may be associated with increased aggregated and soluble tau pathology.

Keywords: Temperature, Tau, Alzheimer's, Circadian, Sleep

Disclosure: Nothing to disclose.

P410. Effects of Reproductive Experience on Cost-Benefit Decision Making in Females

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Background: After decades of neglect, a growing body of preclinical behavioral neuroscience research is including female subjects, with significant findings that underscore the importance of this inclusion. These efforts have largely neglected the variable of reproductive experience, however, in that almost all studies employ reproductively naïve animals. Notably, the majority of women undergo reproductive/maternal experience at some point in their lives, and pregnancy and childbirth are associated with alterations in risks of several psychiatric disorders. Research in rodents shows that maternal experience affects spatial learning and other aspects of hippocampal function. In contrast, there has been little investigation of how reproductive experience affects cost-benefit decision making, despite the relevance of this aspect of cognition for psychiatric disorders.

Methods: To begin to address the aforementioned issues, female Long-Evans rats (nulliparous, $n = 8$; parous, $n = 8$) were evaluated in tests of impulsive and risky decision making in standard operant chambers. Rats in the parous group were mated, gave birth, and nursed for 21 days until pup weaning, whereas rats in the nulliparous group were unmated. One week after pups were weaned (or at the equivalent time point in nulliparous rats), rats

began behavioral testing. Rats were initially tested in a delay discounting (impulsive choice) task, in which they made discrete trial choices between a small immediate food reward and a large food reward delivered after a variable delay period ranging from 0 to 60 s. Next, in a risky decision-making task, rats made discrete trial choices between a small, "safe" food reward and a large food reward accompanied by variable probabilities of mild footshock punishment. To investigate effects of reproductive experience on food motivation, rats were tested on a progressive ratio schedule of reinforcement. Data from the decision-making tasks were analyzed via two-factor, repeated measures ANOVA (group x delay or probability of shock), whereas data from the progressive ratio task were analyzed via Welch's t-test.

Results: On the delay discounting task, there was an interaction between group and delay duration ($F(4,56) = 3.57$, $p < .05$). Specifically, parous rats chose the large reward less frequently than nulliparous in the first block of trials when delays were absent, suggestive of a deficit in discrimination between the large and small rewards. On the risky decision-making task, there was an interaction between group and shock probability ($F(4,56) = 2.73$, $p < .05$); on this task, however, parous rats chose the large reward more frequently than nulliparous, suggestive of greater risk taking in this group. Data from the progressive ratio task revealed no differences between groups ($p = 0.51$), indicating that nulliparous and parous rats were comparably motivated to obtain the food reward.

Conclusions: Together, these results show distinct effects of reproductive experience on different forms of cost-benefit decision making in females and highlight reproductive status as a variable that may influence aspects of cognition relevant for psychiatric disorders. Future work will determine the contributions of different components of reproductive experience to these effects, and whether reproductive experience influences cost-benefit decision making in males as well.

Keywords: Reproduction, Decision Making, Rodent Models

Disclosure: Nothing to disclose.

P411. Intrathecal Inflammatory Responses in the Absence of SARS-CoV-2 Nucleic Acid in the CSF of COVID-19 Hospitalized Patients

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Background: Little is known about cerebrospinal fluid (CSF) profiles in patients with acute COVID-19 infection and neurological symptoms. Here, CSF was tested for SARS-CoV-2 RNA and inflammatory cytokines and chemokines and compared to controls and patients with known neurotropic pathogens.

Methods: CSF from twenty-seven consecutive patients with COVID-19 and neurological symptoms was assayed for SARS-CoV-2 RNA using quantitative reverse transcription PCR (RT-qPCR) and unbiased metagenomic sequencing. Assays for blood-brain barrier (BBB) breakdown (CSF: serum albumin ratio (Q-Alb)), and proinflammatory cytokines and chemokines (IL-6, IL-8, IL-15, IL-16, monocyte chemoattractant protein -1 (MCP-1) and monocyte inhibitory protein – 1β (MIP-1β)) were performed in 23 patients and compared to CSF from patients with HIV-1 (16 virally suppressed, 5 unsuppressed), West Nile virus (WNV) ($n = 4$) and 16 healthy controls (HC).

Results: The median CSF cell count for COVID-19 patients was 1 white blood cell/μL (range 0-7250); two patients were infected with a second pathogen (Neisseria, Cryptococcus neoformans). No CSF samples had detectable SARS-CoV-2 RNA by either detection method. In patients with COVID-19 only, CSF IL-6, IL-8, IL-15, and MIP-1β levels were higher than HC and suppressed HIV (corrected-$p < 0.05$). MCP-1 and MIP-1β levels were higher, while IL-6, IL-8, IL-15 were similar in COVID-19 compared to WNV patients. Q-Alb correlated with all proinflammatory markers, with IL-6, IL-8, and MIP-1β ($r \geq 0.6$, $p < 0.01$) demonstrating the strongest associations.

Conclusions: The lack of SARS-CoV-2 RNA in CSF despite neurological symptoms is consistent with current literature on hospitalized COVID-19. Intrathecal proinflammatory cytokines and chemokines correlate with markers of BBB breakdown in a subset of patients despite minimal CSF pleocytosis and may explain some of the neurological sequelae in COVID-19.

Keywords: Neuroinfection, Pro-Inflammatory Cytokines, Novel Coronavirus (SARS-CoV-2), CSF Biomarkers, Next Generation Sequencing

Disclosure: Nothing to disclose.

P412. Identification of Plasma Biomarkers Involved in Regulation of Inflammatory Pain

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Background: Physicians are challenged in treating pain patients due to the lack of quantifiable, objective methods of measuring pain in the clinic; pain sensation is multifaceted and subjective to each individual. There is a critical need for point-of-care quantification of accessible biomarkers to provide objective analyses beyond the subjective pain scales currently employed in clinical care settings. In the present experiment, we employed an animal model to test the hypothesis that blood-born regulators of the inflammatory response directly associate with an objective behavioral response to inflammatory pain. Upon induction of localized paw inflammation, we measured various cytokines and matrix metalloproteinases (MMPs) that are known to participate in the inflammatory response and investigated their relationship to the behavioral response across a 24-hour period.

Methods: Male Sprague-Dawley rats ($n = 6$/group) outfitted with indwelling jugular catheters were injected with 0.1 ml of saline or 0.1 ml of the pro-inflammatory compound λ-carrageenan (1%) into the left hindpaw. Quantification of carrageenan-evoked paw inflammation (paw thickness) and locomotor activity, as well as collection of blood (500 μl) for later biochemical analyses occurred 24 hrs prior to intraplantar injection (baseline) and at four timepoints post-injection (1-24 hrs). Plasma was aliquoted from each sample for detection of cytokines (BioPlex Rat Cytokine Panel) and MMPs.

Results: Intraplantar injection with 1% λ-carrageenan induced a significant increase in paw thickness across 24 hrs, with peak inflammation observed at 8 hrs ($p < 0.05$ vs saline). Pearson's correlation analyses revealed that locomotor activity counts negatively correlated with paw inflammation at 8 hrs ($r2 = 0.5645$, $p < 0.05$), demonstrating impaired locomotion at the peak of paw inflammation. Expression of the chemokines C-X-C motif chemokine ligand 1 (Gro KC) ($r2 = 0.7061$, $p < 0.05$) and monocyte chemoattractant protein-1 (MCP-1) ($r2 = 0.5050$, $p < 0.05$) positively correlated with paw inflammation and

negatively correlated with locomotor activity at 8 hrs (Gro KC, r2 = 0.6386, $p < 0.05$; MCP-1, r2 = 0.4150, $p < 0.05$). The ratio of MMP9 to MMP2 activity negatively correlated with paw inflammation (r2 = 0.4759, $p < 0.05$) at 8 hrs. No relationship between MMP9/MMP2 activity and locomotor activity was observed this timepoint. Interestingly, a mixed model ANOVA of the time course of MMP9/MMP2 activity revealed a significant increase at 1 hr ($p < 0.05$ vs saline).

Conclusions: We postulate that the chemokines Gro KC and MCP-1 as well as the ratio of MMP9 to MMP2 activity may serve as predictive biomarkers for the timecourse of inflammation-associated behavioral deficits. These data define opportunities for the future development of a point-of-care device to objectively quantify biomarkers for inflammatory pain states.

Keywords: Inflammation, Pain Sensitivity, Immune Biomarkers

Disclosure: Nothing to disclose.

P413. Deep Brain Optical Imaging of Subcellular cAMP Signaling Dynamics During Striatum-Dependent Learned Behaviors

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Background: Striatal neuromodulators have critical roles in motor control, decision-making, and substance use disorders. These neuromodulators exert their functions through G-protein-coupled receptors (GPCRs) on striatal neurons that activate second messenger intracellular cascades to affect neuronal signaling. Understanding how neuromodulation involving intracellular signaling molecules can shape synaptic efficacy and circuit functions involved in decision making and behavior is critical to advancing our knowledge of basal ganglia function. The interaction of dopamine and the cAMP-PKA signaling pathway is important in the modulation of synaptic plasticity. The spatial and temporal changes of these striatal intracellular molecules during the induction of synaptic plasticity and behavior are unclear.

Methods: To study the subcellular cAMP dynamics in rodent brain slices and in vivo during behavior, we expressed adeno-associated virus expressing a genetically encoded fluorescent cAMP biosensor, cAMP Difference Detector in situ (cADDis) in a Cre recombinase dependent manner in male and female mice expressing Cre in D1 dopamine receptor-expressing (D1rda) and D2 receptor expressing (A2a) medium spiny neurons (MSNs). Fast-scan cyclic voltammetry and cADDis were used to simultaneously measure dopamine release and subsequent downstream GPCR-mediated cAMP-PKA signaling in striatal brain slices. To examine the relationship between postsynaptic cAMP-PKA activity and motor actions, we measured cAMP-PKA dynamics using fiber photometry during skill and procedural learning.

Results: MSN-cAMP transients can be altered by changes in stimulation duration and are blocked by tetrodotoxin, indicating that they are driven by neuronal activation. During afferent stimulation, MSN-cAMP signaling is primarily influenced by dopamine transmission and seems to be directly modulated by dopamine receptor activation measured with simultaneous recordings of dopamine and cAMP-PKA signaling. Specifically, single pulse electrical stimulation generates increased cAMP in D1 MSNs and decreased cAMP in D2 MSNs. Using fiber photometry and cADDis, we observed real-time movement- and trial-related changes in MSN-subcellular cAMP signaling during motor-skill learning on the accelerating rotarod. Similar changes in cAMP-PKA dynamics have been implicated in complex procedural learning, thus we are beginning to examine subcellular changes during goal-directed behaviors.

Conclusions: Our findings indicate the feasibility of using cADDis combined with traditional methods to investigate the precise temporal changes in striatal dopamine-cAMP signaling in real-time. My work aims to link changes in subcellular GPCR function to events relevant for behavior in vivo; thus, providing a causal link between neuronal signaling and behavior.

Keywords: Basal Ganglia, cAMP Signaling, Dopamine

Disclosure: Nothing to disclose.

P415. LYT-300: An Orally Bioavailable Prodrug of Allopregnanolone With Anticonvulsant Activity

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Background: Neuroactive steroids, which function as allosteric modulators of GABAA receptors, are in clinical development for post-partum depression, intractable epilepsy, tremor, status epilepticus, and some genetic epilepsy disorders. (3α,5α)-3-Hydroxy-pregnan-20-one (allopregnanolone or ALLO) is an endogenous neuroactive steroid which is a positive allosteric modulator of GABAA receptors. An intravenous formulation of allopregnanolone (brexanolone) was recently approved for treatment of severe postpartum depression (marketed as Zulresso®) where it is given in a hospital setting over a 60-hr infusion period. The duration of antidepressant effect has been reported out to 30 days.

The low oral bioavailability of allopregnanolone was the rationale underlying the development of an intravenous dosing formulation. The ability to provide ALLO at therapeutic drug levels by the oral route could have significant advantages and expand its applicability beyond postpartum depression (PPD).

Methods: A novel prodrug strategy was used to facilitate sufficient exposure of ALLO by leveraging lymphatic absorption of dietary lipids. PureTech Health's Glyph technology reversibly connects an active drug to a dietary fat molecule, which shifts the route of intestinal absorption from the liver to the gut-draining lymphatics. Typically, molecules are absorbed into intestinal epithelial cells, passed to the liver and then into the systemic blood circulation. However, lipids and other highly lipid-soluble molecules are transported via the lymphatic route via a specific set of fat-processing pathways, bypassing the liver. The prodrug technology mimics this system by the reversible attachment of the drug to a triglyceride. This system uses proprietary chemistry to form the reversible attachment to the parent compound (ALLO). A self-immolative portion controls systemic release of the parent compound once absorbed, a linker tunes recognition for triglyceride resynthesis to maximize lymphatic transport levels, and a terminal glyceride integrates prodrug into chylomicrons for lymphatic transport. Using this approach, a series of prodrugs were synthesized and characterized in pharmacokinetic studies by oral dosing in various species. Male cynomolgus monkeys ($n = 6$) or beagle dogs ($n = 4$) were fed a standardized diet prior to drug administration. Compounds were formulated in gelatin capsules using a long-chain lipid-based self-emulsifying drug delivery system. Levels of free ALLO in plasma were determined by LC-MS/MS.

Behavioral observations (by blinded observers) of anticonvulsant activity were assessed to determine if LYT-300 could recapitulate the effects of ALLO. Experimental work using animals was under the approval and oversight of an institutional committee and veterinary staff. Male, albino CF-1 mice were used to study of the effects of orally-administered LYT-300 on anticonvulsant activity at multiple

time points after LYT-300 dosing ($n = 4$ for each time point). The 6 Hz seizure model was used to detect compounds with novel mechanisms of action and potential activity against pharmacoresistant seizures and detects effects of ALLO.

Results: A series of lipid prodrugs of ALLO were synthesized and evaluated for their ability to provide systemic exposure in several species after oral administration. Plasma exposure of free allopregnanolone was observed after oral administration of lymphatic targeting prodrugs in both dogs and non-human primates. Apparent bioavailability of free allopregnanolone in the plasma from oral administration of prodrug versus IV administration of free ALLO was calculated to be over 30 percent in both species. Further studies in anesthetized rats supported the lymphatic mechanism of uptake. In these studies ($n = 3$), ALLO prodrug was found to be present in the mesenteric lymph following intraduodenal infusion of prodrug.

In the 6Hz seizure test, orally dosed LYT-300 was confirmed to have anti-convulsant activity. Pharmacokinetic analysis of ALLO levels in the mice in the 6Hz assay showed significant, dose-dependent exposure.

Conclusions: LYT-300, when given orally to a range of species, provides significant release of parent compound, allopregnanolone (ALLO). In mice, oral administration of LYT-300 produced dose-dependent increases in plasma exposure of ALLO and evidence of anticonvulsant efficacy. These data provide proof of concept that LYT-300 (p.o.) can deliver the pharmacologically active compound ALLO at levels that produce ALLO-related pharmacological effects. An orally-bioavailable ALLO is likely to be a major advance in therapeutics for wide-ranging neuropsychiatric disorders. A Phase 1 clinical trial in healthy volunteers is expected to begin in 2021 to characterize the safety, tolerability, and pharmacokinetics of orally administered LYT-300. This study may include exploratory endpoints such as beta wave power electroencephalography, a marker of GABAA receptor target engagement.

Keywords: GABA-A, Positive Allosteric Modulator, MDD, GABA-A, Positive Allosteric Modulators, GABAA Receptor Positive Allosteric Modulator, Epilepsy, Mood

Disclosure: PureTech Health: Employee (Self)

P416. The Role of Male-Specific Perinatal Sex Hormones in the Development of Sex-Biased Social Behavioral Dysregulation

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Background: Immune alterations such as aberrant activation of microglia, the innate immune cells of the brain, are described in patients with neurodevelopmental disorders (NDDs). Our lab has demonstrated that microglia from male rodents play a critical role in the development of neural circuits underlying social behavior through selective phagocytosis of synapses in male but not female rodents. Microglial homeostatic and inflammatory processes are intimately linked to mitochondrial function, and mitochondrial abnormalities have also been reported in early-onset NDDs, suggesting that microglial mitochondria play a role in brain vulnerabilities underlying NDD risk. The incidence of early-onset neurodevelopmental disorders such as Autism Spectrum Disorder is strongly male biased. One well-characterized sex-specific developmental program that may underlie this male-biased incidence is a surge of gonadal hormones that occurs only in males during a perinatal critical period of brain development. This surge of gonadal hormones masculinizes the male body and brain. Importantly, we can divert the female brain towards a male-like state by injecting female mouse pups during this perinatal critical period with estradiol, the aromatized form of testosterone that has been shown to masculinize the male brain. This allows us to determine whether gonadal sex hormones impart male-typical vulnerability independent of the chromosomal milieu. Here, we hypothesized that gonadal sex hormones during a perinatal critical period of brain development induce vulnerability to immune alterations in male microglia that lead to impaired brain development and aberrant social behavior.

Methods: Perinatal immune challenge of male, female, and masculinized female mice was performed at postnatal day (PN) 9. Mice were subcutaneously injected with 10 mg/kg lipopolysaccharide (LPS) or vehicle saline. Wild type mice or mice lacking toll like receptor 4 (TLR4), a receptor recognizing LPS, specifically in microglia (Cx3cr1-CreBT:TLR4flox/flox (TLR4 cKO)) were used to study effects of perinatal immune challenge on social behavior, microglial function, and mitochondrial function. For masculinization studies, a subset of female mice were masculinized by subcutaneous injection on postnatal days (PN) 0 and PN1 with 100 ug estradiol benzoate dissolved in sterile sesame seed oil. Mice underwent 3-chambered sociability and social novelty preference testing starting at PN30. A cohort of mice were sacrificed on PN30 for molecular and immunohistochemical assays. Microglia were isolated from prefrontal cortex using positive bead selection. Mitochondrial gene expression was assessed by PCR array. Microglial cellular oxygen consumption was determined using MitoXpress Oxygen Consumption Assay. Immunostaining for mitochondria within microglia and astrocytes was performed, and cellular mitochondrial morphologies (mitochondrial length, volume, and network connectivity) were assessed using Fiji and Imaris volumetric reconstructions. $N = 6$-12 per group for social behavior experiments, 3-4 per group for PCR array experiments, and 3-8 per group for imaging experiments. Two-way ANOVAs (sex x treatment) followed by Bonferroni's post-hoc analyses were performed to determine significance.

Results: PN9 LPS challenge induced male-specific deficits in sociability and social novelty preference ($p < 0.0001$) that were prevented by microglia-specific ablation of TLR4 signaling (TLR4 cKO mice do not show sociability deficits in response to LPS; $p > 0.99$). Masculinizing female pups at birth induced male-typical vulnerability to immune challenge-induced social deficits ($p < 0.0001$). Microglia isolated from the prefrontal cortex of PN30 male and masculinized female mice injected at PN9 with LPS demonstrated significantly downregulated expression of 54% of mitochondrial electron transport chain (mtETC) genes, whereas this same challenge did not alter female mtETC gene expression. PN9 LPS challenge also induced significant decreases in microglial mitochondrial length, volume, and network connectivity measures in male and masculinized female, but not in female, mice ($p < 0.05$).

Conclusions: These findings suggest that there are sex differences in social behavior and microglial mitochondria function that are dependent upon both microglial inflammatory signaling as well as the organizational perinatal sex hormone surge. We are currently exploring the mechanisms through which perinatal sex hormones induce this sex-specific vulnerability to perinatal immune challenge.

Keywords: Microglia, Mitochondria, Sex Hormones, Brain Development, Neuroimmunology

Disclosure: Nothing to disclose.

P417. A Female-Specific Role of RGS20 in Transcriptional, Epigenomic and Behavioral Responses to Chronic Pain

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Background: The signal transduction protein named Regulator of G-protein Signaling-20 (RGS20) modulates functional responses of several GPCRs in the brain, by acting as a GTPase activator or as an effector antagonist for G□ subunits. It is found highly expressed in the periaqueductal gray (PAG), a key component of the descending pain pathway. Using genetic mouse models, genomic and proteomic approaches we are studying the female-specific role of RGS20 in sensory hypersensitivity associated with chronic pain states.

Methods: Constitutive knockout of RGS20 (RGS20KO), as well as conditional knockdown of RGS20 in the mouse PAG were used along with RGS20WT animals.

Peripheral inflammation was induced by intraplantar injection of Complete Freud's adjuvant (CFA)) and chronic pain by spared nerve ligation (SNI). Mechanical allodynia and thermal hyperalgesia were measured using Von Frey and Hargreaves respectively.

Following 3 or 10 days after CFA injection in the hind paw, PAGs were collected. RGS20 expression was verified by qPCR and western blot. To better understand the underlying sex specific mechanisms we utilized ChIP-qPCR on female PAG with the estrogen receptors alpha (Esr1) and beta (Esr2), and we measured Esr1 and Esr2 protein levels by western blot. We also performed RNA-sequencing on male and female PAG and identified candidate genes using IPA (QIAGEN) and validated them by qPCR. Fast-Scan Cyclic Voltammetry was used to measure changes of serotonin PAG projections following CFA injection and neuronal activity was monitored by whole-cell patch clamp. Finally, bottom-up mass-spectometry was used to study histone mark changes across all conditions and the mark of interest was chosen was chosen to pursue with ChIP-sequencing.

Results: Both RGS20KO and conditional knockdown of RGS20 in the PAG, exacerbate mechanical allodynia and thermal hyperalgesia in models of peripheral inflammation and nerve injury in female but not male mice. Furthermore, we show that prevention of RGS20 action delays recovery from thermal hypersensitivity in female mice after CFA treatment. In PAG tissue, biochemical studies revealed a sex-specific upregulation of RGS20 levels three days after the induction of peripheral inflammation. Chromatin Immunoprecipitation (ChIP)-qPCR at this time point reveals there is no change in ER elements binding to the RGS20 promoter. RNA Sequencing analysis of PAG samples from RGS20WT and RGS20KO mice under naïve states or 10 days after CFA, uncovered that exacerbated sensory hypersensitivity upon RGS20 knockdown is associated with changes in the expression of genes and pathways associated with central sensitization and pain maintenance, including molecules involved in serotonin synthesis and release. This was confirmed by voltammetry where stimulated release and readily releasable pool were increased in the rostral ventromedial medulla of RGS20WT following CFA injection, but this effect was significantly diminished in RGS20KO. Whole cell patch clamp recordings from rostral ventromedial medulla neurons also reveal decreased activity in CFA RGS20KO groups compared to RGS20WT mice. Proteomic analysis of epigenetic marks in the female PAG, identified that following CFA treatment, H3K14ac was increased in RGS20WT and decreased in RGS20KO animals. On the contrary, H3K9me2/H3K14ac expression decreased following CFA treatment in RGS20WT animals, and decreased in naïve RGS20KO animals vs RGS20WT. Ongoing ChIP-seq analysis of the selected histone marks is expected to provide details on key genes affected by dysregulation of RGS20.

Conclusions: Our studies highlight a novel female-specific intracellular pathway in the PAG which controls the severity of sensory hypersensitivity and recovery from chronic pain states.

Keywords: Chronic Pain, GPCR, Sex-Specific

Disclosure: Nothing to disclose.

P418. Resting-State Individual Variability in Youth and Young Adults With Autism Spectrum Disorder: Insights From a Transcranial Magnetic Stimulation Trial

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Background: Individuals with autism spectrum disorder (ASD) exhibit greater variability of resting-state functional connectivity than typically developing controls (TDC). Recent work also suggests that fronto-parietal network engagement during a spatial working memory (SWM) task is more variable in ASD vs. TDC. Repetitive Transcranial Magnetic Stimulation (rTMS) is a neuromodulatory intervention, wherein brain variability among targeted networks may influence optimal network engagement. We used two approaches to characterize individual variability of resting-state connectivity in youth and young adults with ASD, and examined the effect of a 4-week (20-session) clinical trial of active vs. sham rTMS to the dorsolateral prefrontal cortex (DLPFC). Our primary objective was to evaluate if variability metrics change following rTMS intervention. We also explored whether change in variability from pre-to-post rTMS correlates with improved SWM task performance.

Methods: Resting state fMRI was acquired in 37 ASD participants [22.8 (4.55) years; 12-female] pre and post-rTMS, and in 20 TDC participants [23.9 (4.34) years; 6-female] at a single time-point (baseline). Mean correlational distance, a similarity/difference metric that captures the pattern of functional connectivity between the rTMS stimulation site (8mm region of interest, L/R-DLPFC) and the remainder of the brain, was calculated between each pair of participants from the active and sham groups separately (so as to assess change in variability among those exposed to the same treatment). Distance metrics derived from Personalized Intrinsic Network Topography (PINT) were used to measure variability in spatial locations of fronto-parietal resting-state network nodes. SWM was assessed with the Cambridge Neuropsychological Test Automated Battery (CANTAB). All variability metrics were compared between ASD and TDC groups at baseline using ANCOVAs. PINT-derived distances were compared from pre-to-post rTMS between the active vs. sham ASD groups, whereas mean correlational distance and SWM task performance were examined from pre-to-post rTMS in the active and sham group separately, using linear mixed models. Age and framewise displacement (for variability analyses) were included as covariates.

Results: ASD and TDC groups did not differ in either variability metric at baseline. PINT distances did not differ from pre-to-post rTMS in either treatment group, though frontal nodes were more variable than parietal or temporal network nodes, across all participants and treatment groups (all $p < 0.05$). Mean correlational distance of R-DLPFC functional connectivity changed from pre-to-post rTMS in both the active ($F(1,15) = 5.04$, $p = 0.04$), and sham ($F(1,18) = 28.54$, $p < 0.001$) ASD groups; participants in the active group became less variable, and those in the sham group became more variable (this pattern of change was consistent for L-DLPFC, despite no significant change from pre-to-post rTMS in either group). Although improved SWM performance from pre-to-post rTMS did not reach statistical significance in the active group ($F(1.16) = 3.6$, $p = 0.07$), change in mean correlational distance (L-DLPFC) from pre-to-post rTMS was positively correlated with change in SWM performance ($r = 0.53$, $p = 0.03$); participants in the active group who became less variable post-rTMS exhibited the greatest improvements in SWM performance.

Conclusions: Our results suggest that active rTMS may reduce/limit variability of functional connectivity, with the DLPFC, in ASD. Moreover, it is promising that we found a relationship between variability of L-DLPFC functional connectivity and SWM performance in participants treated with active rTMS. Given that rTMS was delivered to the DLPFC, and that we found frontal network ROIs to be most variable, it may be critical to account for variability, whether pre-emptively prior to rTMS delivery, or when relating measures of brain activation to behavioral and clinical outcomes following rTMS.

Keywords: Autism Spectrum Disorder, Repetitive Transcranial Magnetic Stimulation (rTMS), Clinical Trial, Functional MRI (fMRI), Individual Variability

Disclosure: Nothing to disclose.

P419. SARS-CoV-2 Acutely Infects Sensory Nervous System Tissue and Triggers an Injury Response Underlying Hypersensitivity in a Hamster Respiratory Infection Model

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Background: COVID-19 results in variable sensory symptoms in a notable percentage of the patient population, including headache, myalgias, and nerve pain. Understanding the mechanisms underlying these symptoms in sensory structures such as dorsal root ganglia (DRGs) and spinal cord (SC) can help identify treatments for acute and prolonged somatosensory COVID-19 symptoms and potentially highlight novel pain pathways.

Methods: Male golden hamsters received 1000 plaque-forming units of intranasal SARS-CoV-2 or saline. Cervical and thoracic dorsal root ganglia (DRGs) and spinal cord (SC) were harvested at 1-, 4-, 7-, and 14-days post-infection (dpi). Real-time PCR and RNAscope were used to validate DRG and SC infection and measure SARS-CoV-2 co-receptor expression. Bulk RNA sequencing was used to identify infection-related maladaptive pathways. Existing DRG RNA sequencing data from spared nerve injury (SNI) and complete Freund's adjuvant (CFA) models was meta-analyzed against our SARS-CoV-2 DRG sequencing data. The Von Frey assay was used to assess mechanical hypersensitivity. $N = 4$-8 hamsters were used per experiment in this study. T-tests, one-way and two-way ANOVAs were used for molecular and behavioral analyses. DESeq2 was used for differential gene expression list generation for bulk RNA sequencing experiments.

Results: SARS-CoV-2 spike and nucleocapsid RNA was detected in cervical and thoracic DRGs and SC at 1dpi, but subsided in almost all samples by 4dpi except for thoracic SC (RM two-way ANOVA, $p < 0.05$). Interestingly, Tmprss2 co-receptor expression was upregulated in mock thoracic versus cervical SC (t-test, $p = 0.017$), suggesting vulnerability to prolonged infection at this SC level. 894 upregulated and 136 downregulated genes were identified in 3dpi combined cervical/thoracic SARS-CoV-2 DRGs compared to saline ($\log2(FC) > |0.5|$, p-adj<0.05). Top canonical pathways associated with these changes included: Opioid Signaling Pathway ($-\log(p) = 6.362$), Synaptogenesis Signaling Pathway ($-\log(p) = 5.259$), Axonal Guidance Signaling ($-\log(p) = 5.053$), and GABA Receptor Signaling ($-\log(p) = 4.863$) (Qiagen IPA). Meta-analysis between SARS-CoV-2 and CFA revealed 68 commonly upregulated genes associated with interferon response and extracellular matrix reorganization. Similar pathways were commonly upregulated between SARS-CoV-2 and SNI (79 genes), although 161 genes were contra-regulated. The opposing gene set suggests upregulated sodium channel activity and synaptic plasticity pathways in DRGs from SARS-CoV-2-infected hamsters that are downregulated in SNI. Von Frey revealed mechanical hypersensitivity in SARS-CoV-2 hamsters at 4dpi (one-way ANOVA, $p = 0.013$; Tukey's m.c., $p = 0.012$).

Conclusions: SARS-CoV-2 actively infects DRGs and the SC in the golden hamster model of COVID-19. Infection-related transcriptomic maladaptations reflect molecular signatures seen in peripheral neuropathy models, which is demonstrated by mechanical hypersensitivity in hamsters. However, unique transcriptional changes induced by SARS-CoV-2 infection of the DRG could point to novel pain pathways. Future work will focus on SARS-CoV-2-induced, sustained peripheral neuropathy signatures and the contribution of prolonged sensory perturbations to long-term affective components of COVID-19, a phenomenon commonly experienced by chronic pain patients.

Keywords: Novel Coronavirus (SARS-CoV-2), Sensory Processing, Dorsal Root Ganglia, Spinal Cord, Tissue Transcriptomics

Disclosure: Nothing to disclose.

P420. Efforts to Accelerate Development of Non-Opioid, Non-Addictive Pain Therapeutics Within the NIH Heal Initiative Preclinical Screening Platform for Pain: Validating Two Models of Chemotherapy-Induced Painful Neuropathy in the Rat

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Background: With the goal of accelerating the discovery and development of new non-opioid, non-addictive pain therapeutics, the National Institutes of Health Helping to End Addiction Long-term Initiative, or NIH HEAL Initiative, Preclinical Screening Platform for Pain (PSPP) program PSPP is collaborating with PsychoGenics, Inc. to validate preclinical models and endpoints to enable screening and profiling of assets. Here, we describe the validation and optimization of the paclitaxel and oxaliplatin models of chemotherapy-induced peripheral neuropathy in the rat. Repeated dosing of the chemotherapeutic agents paclitaxel or oxaliplatin in rats has been shown to produce hind paw hypersensitivity to tactile and cold stimuli, and these effects are believed to resemble sensory neuropathy observed in the clinic.

Methods: Adult male and female Sprague Dawley rats ($N = 10$, each sex) were used in these studies. For the paclitaxel model studies, paclitaxel was injected at several doses (2 mg/kg, i.p.; 4 mg/kg, i.p.; 2 mg/kg, i.v.) on alternate days (Day 0, 2, 4, 6) to determine the optimal dose and route of administration. For the oxaliplatin model studies, oxaliplatin (3 mg/kg, i.v.) was injected 2 days per week for a period of 4 weeks. Hind paw tactile sensitivity was determined with von Frey filaments using the up-down testing method as described by Chaplan et al., 1994, and hind paw cold sensitivity was determined using the acetone test. Effects of chemotherapeutic agents on hind paw sensitivity were evaluated for a maximum period of 8 weeks, and effects of mechanical and cold priming of the hind paws on the development of hypersensitivity were also examined.

Statistical analysis, effect size, and power analysis: Data were analyzed using two-way repeated measures ANOVA with Bonferroni's or Dunnett's post hoc test when appropriate. Effects of $p < 0.05$ were considered to be statistically significant. Power analysis and effect size were determined using SAS/STAT, and appropriate sample size was based on a power value of 0.8 to ensure adequate power for F-tests for two-way interactions.

Results: In the paclitaxel model studies, 4 injections of paclitaxel on alternate days (Day 0, 2, 4, 6) produced bilateral hind paw tactile and cold hypersensitivity which was maximal by Week 5 and persisted through Week 6. The paclitaxel dose of 4 mg/kg, i.p. was found to be optimal for this model, and results from rat

pharmacokinetic studies demonstrated that Cmax and AUC values associated with this dose were similar to the values associated with efficacy in the clinic. Interestingly, hind paw priming with acetone during Week 2 enhanced acetone cold hypersensitivity in this model during Weeks 3-6, while mechanical priming with von Frey filament stimulation did not affect the development of tactile hypersensitivity. In the oxaliplatin model studies, oxaliplatin injection (3 mg/kg, i.v.) 2 days per week for a period of 4 weeks produced bilateral hind paw tactile and cold hypersensitivity which was maximal by Week 6 and persisted through Week 8. The magnitude of tactile and cold hypersensitivity was similar in the paclitaxel and oxaliplatin models.

Initial pharmacological characterization was performed in these models by examining the effects of morphine sulfate (0.3-3 mg/kg, s.c.) on bilateral hind paw hypersensitivity. Morphine sulfate (3 mg/kg, s.c.) was found to significantly inhibit bilateral tactile or cold hypersensitivity in the paclitaxel and oxaliplatin models at Week 6 and Week 7, respectively. Thus, these studies demonstrate that optimizing the dose of paclitaxel (4 mg/kg, i.p.) to be consistent with plasma exposure at clinically effective doses, enabled optimization of parameters in the model.

Importantly, both the paclitaxel and the oxaliplatin models showed (1) reproducible bilateral hind paw tactile and cold hypersensitivity in male and female rats in the paclitaxel and oxaliplatin models of chemotherapy-induced peripheral neuropathy, (2) bilateral hind paw hypersensitivity was enhanced through the use of hind paw priming procedures, and (3) hypersensitivity to both cold and tactile stimuli was significantly inhibited by morphine sulfate, demonstrating the effectiveness of an opioid in these models.

Conclusions: The validation of the paclitaxel and oxaliplatin models of chemotherapy-induced peripheral neuropathy further highlights efforts within the NIH HEAL Initiative's PSPP program to validate endpoints and models to be incorporated into evaluating novel assets towards accelerating the development of novel non-opioid, non-addictive therapeutics.

Keywords: Pain Therapeutics, Preclinical Screening Platform for Pain, Chemotherapy-Induced Peripheral Neuropathy, Neuropathic Pain

Disclosure: PsychoGenics, Inc.: Employee, (Self)

P421. Automated Detection of Eye Tics Using a Computer Vision Approach

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Background: Tourette Syndrome (TS) a chronic, childhood-onset neurodevelopmental disorder that affects 1-3% of people. TS is characterized by tics: involuntary, repetitive movements and vocalizations. The most common and bothersome motor tics in treatment-seeking adults and children are visible movements of the face and upper body. Effective diagnosis and treatment depends upon accurate detection of tic presence and severity, yet there is a lack of objective, quantitative tools to measure tics. Video-based observational methods of measuring tics in research contexts have yielded critical insights into the symptomatology of TS. These methods produce accurate quantification of tics but are not readily scalable to the clinic due to reliance on trained human raters. Advances in computer vision and machine learning have enabled researchers to develop fully automated algorithms to detect clinically relevant abnormal movement in disorders such as autism and Parkinson's disease. The aim of the current study was to develop and preliminarily test a computer vision approach to automate detection of eye tics.

Methods: A dataset of $N = 39$ videos of individuals with eye tics (eye blinking, eye darting/rolling, and/or eyebrow movements) was acquired from public YouTube postings using search terms of "Tourette" and "tic." Videos were screened for relevance and quality during consensus team meetings and coded by human raters who met a training target of >80% reliability. A fine-grained tic coding scheme captured distinct aspects of observable tics (e.g., tic complexity, anatomical location). Codes were captured using DataVyu, a computer-assisted behavioral coding software program. A supervised machine learning approach was used to train a binary classifier to detect eye tics. Classifier development included four phases: data preprocessing, preparation of a convolutional neural network (CNN) based on DenseNet architecture, training the CNN to detect eye tics on a "training" set of coded videos, and applying the classifier to a "test" dataset (i.e., videos never before seen by the computer).

Results: The coded dataset included 198 images extracted from the $N = 39$ videos. The classifier demonstrated accuracy (agreement with human coder) of 84% with a Sensitivity of 75% and Specificity of 88%.

Conclusions: The binary classifier showed accuracy well within the accepted threshold of 80% for a human rater to be considered reliable. This preliminary study suggests that computer vision methods hold promise for automating detection of motor tics, an approach that could eventually improve screening, diagnosis, and treatment monitoring. Limitations are the focus only on eye tics and absence of clinical information for individuals in the videos. Use of single images as input data instead of multiple images from the video stream limited our ability to capture temporal information of the motor event. Future research using larger datasets that include videos of tic expression alongside clinical phenotyping and novel computer vision analytic techniques will further our ability to create scalable, quantitative clinical tools.

Keywords: Tourette Syndrome, Tic Disorders, Machine Learning Classification, Computer Vision

Disclosure: Posit Science: Grant (Self)

P422. Neuroimaging Probe Development for Receptor-Interacting Serine/Threonine-Protein Kinase 1

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Background: Necroptosis is a complex and regulated caspase-independent cell death mechanism mediated by various protein members, e.g. Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) involving inflammation. Notably, RIPK1 is dysregulated by microglial cells in human brains and mediates a disease-associated microglial response (DAM), which is related to an inflammatory response. Interestingly, down-regulation of RIPK1, by both pharmacological and genetic means, reduced disease pathology, corrected altered inflammatory changes in transgenic mouse models and in cell models. Thus, RIPK1 is an important therapeutic target for both understanding and treatment of human diseases. Here, our overarching goal is to develop the first positron emission tomography (PET) radiotracer of RIPK1/necroptosis. PET has provided a useful and indispensable way to both assist the patient selection for trials in medicine and improve pharmacological performance in drug development. However, there are no PET tracers that can image RIPK1 and necroptosis in the human brain and the mechanisms for in vivo selectivity, distribution and involvement of necroptosis in the neuropathophysiology remain elusive.

Despite the preclinical success and early clinical trial of RIPK1 inhibitors, a large knowledge gap exists between animal models and human diseases. In our opinion, this knowledge gap can in large part

be filled by translational neuroimaging with the availability of a RIPK1 imaging biomarker. In order to fully support the therapeutic development for neurological disease, characterization of RIPK1 density and its dysregulation in the living human brain is required. In addition, understanding the relationship between RIP1 drug engagement in the brain and inhibitor dosing is essential to advancing the RIPK1 therapeutic discovery and dose selection process.

Methods: For PET imaging study, mice (both male/female) are utilized, anesthetized with inhalational isoflurane (Forane) at 3% in a carrier of 1.5-2 L/min medical oxygen and maintained with isoflurane for the duration of the scan. The mice are arranged in a Triumph Trimodality PET/CT/SPECT scanner and injected with radiolabeled compounds with/without pre-treatment of unlabeled RIPK1 inhibitors via a lateral tail vein catheterization at the start of PET acquisition. Dynamic PET acquisition is last for 60 min and followed by CT for anatomic co-registration. PET data are reconstructed using a 3D-MLEM method. Reconstructed images are exported from the scanner in DICOM format along with an anatomic CT for rodent studies. These files are imported to PMOD 4.01 (PMOD Technologies, Ltd.) and manually co-registered using six degrees of freedom. Volumes of interest (VOIs) are drawn manually as spheres in brain regions guided by high resolution CT structural images and summed PET data, with a radius no less than 1mm to minimize partial volume effects. Time-activity curves (TACs) are exported in terms of decay corrected activity per unit volume at specified time points with gradually increasing intervals.

Results: In past years, we have designed, radiosynthesized and evaluated several RIPK1 inhibitors as potential PET imaging probes, and some candidates displayed good brain uptake (%ID/cc > 1 for mice brain imaging), strong RIPK1 binding affinity (KD < 10 nM), good specificity and selectivity (no significant binding towards over 400 kinases), and appropriate kinetics and distribution profiles, strongly supporting the application as PET tracers in humans. We also further characterized the age and gender effects of RIPK1/ necroptosis in mice to support potential human imaging.

Conclusions: In summary, we fully expect that our RIPK1 probes will be useful for human neuroimaging study and are also able to serve as a putative biomarker that can significantly improve understanding of the pathogenesis of human diseases and rapidly enhance the drug development. The further characterization of these probes is undergoing and the best candidate will be moved forward for human imaging study.

Keywords: In Vivo Imaging, PET Probe, Neuroinflammation

Disclosure: Nothing to disclose.

P423. Characterizing Disease-Associated Missense Mutations in the GABA Transporter, GAT1

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Background: Dysregulation of neural circuit excitatory/inhibitory balance may underlie multiple neuropsychiatric disorders. Genes involved in GABAergic neurotransmission, the main regulator of inhibition in the central nervous system, have been increasingly implicated in disease. SLC6A1 encodes the GABA transporter GAT1, one of two GABA transporters expressed in the brain. GAT1 primarily localizes to presynaptic terminals of GABAergic neurons, where it regulates GABAergic signaling through the re-uptake of GABA from the synaptic cleft. Knockout mouse studies have demonstrated impairment of GAT1 leads to abnormal GABA receptor signaling, alterations to hippocampal oscillations, abnormal social behavior, impairments in learning and memory, as well as deficits in prepulse inhibition. To date, over 85 unique mutations in the coding sequence of GAT1 have been identified in patients and associated with a wide variety of disorders including epilepsy, neurodevelopmental delay (NDD), autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), schizophrenia (SCZ), and Tourette Syndrome (TS). Previous studies have investigated a select cohort of variants primarily associated with epilepsy, ASD, and/or NDD and found them to all be loss-of-functions mutations. Here, we set out to characterize an extensive list of missense mutations across the complete spectrum of disorders associated with GAT1.

Methods: We introduced 42 missense mutations associated with a variety of disorders into the wild-type human GAT1 coding sequence and transiently overexpressed these variants in HEK293T cells. After 48 hours, cells were fixed, stained, and imaged on an Opera Phenix High-Content Screening System to quantify total GAT1 intensity and transfection efficiency. In parallel, transfected HEK293T cells were exposed to radiolabeled 3H-GABA to functionally measure GABA uptake. Radiouptake measurements were then normalized by transfection efficiency for each variant ($n = 9$, 3 independent transfections, 3 replicates per transfection). Data were analyzed using a one-way ANOVA with a Dunnett correction for multiple comparisons and graphed as the mean +/- SEM. The significance level was set at $p < 0.05$.

Results: Mean GAT1 intensity was decreased from wild-type only for the single mutation associated with TS, T46M (69.3 +/- 3.9%). No other variants showed a significant difference in GAT1 intensity. Radiouptake analysis demonstrated that 41/42 (97.6%) mutations significantly impacted GABA uptake. Most variants (39/42, 92.9%) were characterized as loss-of-function, with radiouptake scores ranging from 65.9% to 2.2% of wild-type. In addition, two mutations (4.8%) were gain-of-function with scores of 125.8% and 293.7% of wild-type.

To gain insight on how GAT1 function correlates with clinical phenotypes, we compared the level of GABA uptake for all mutations associated with a variety of disorders. Mutations associated with epilepsy, NDD, ASD, and ADHD showed on average severe loss-of-function (13.1%, 14.8%, 21.7%, and 15.1% respectively), whereas those associated with SCZ were only mildly impacted (71.5%). The unique mutation associated with TS showed a robust increase in function (293.7%).

Conclusions: Using a radiouptake assay, we functionally assessed 42 missense mutations in the GABA transporter, GAT1. Consistent with previous studies, most of the mutations were loss-of-function, yet two variants demonstrated enhanced GABA uptake. To our knowledge, this is the first observation of GAT1 gain-of-function mutations indicating bi-directional modulation of GABA uptake is relevant for neuropsychiatric disease. Furthermore, by correlating the uptake scores with clinical phenotypes, we were able to stratify disorders based on functional severity. Future experiments will investigate mechanisms underlying the changes to GAT1 function, including membrane localization and endoplasmic reticulum retention, and assess these mutants in GABAergic neurons derived from human induced pluripotent stem cells.

Keywords: GABA, SLC6A1/GAT1, Epilepsy, Schizophrenia (SCZ), Tourette Syndrome

Disclosure: Janssen Research and Development LLC. Employee (Self)

P424. Neuroprotective Effects of Cannabidiol in Human Neural Cell Lines Infected With SARS-CoV-2

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Background: COVID-19 has shown to be more than respiratory disease. Several good studies have demonstrated that the SARS-CoV-2 can cause long-term neurological symptoms such as headache, dizziness, stroke, cognitive and neuropsychiatric symptoms. Cannabidiol is the main non-psychotomimetic compound of Cannabis sp., with putative neuroprotective properties in different contexts of neurological disorders associated with infectious diseases (severe malaria, encephalomyelitis, meningitis). In rodent models, CBD prevented the long-term neurological and psychiatric effects of severe cerebral malaria. In the present study, we evaluate if Cannabidiol can prevent the deleterious effects of SARS-CoV-2 neuroinfection in an in vitro model.

Methods: In a first experiment, we evaluated in postmortem brain samples of COVID-19 patients (obtained by a minimally invasive technique) the presence and pattern of neuroinfection produced by SARS-CoV-2 (neurons and glial cells) and the presence of inflammatory markers (cytokines and chemokines). In a second experiment, the human glioblastoma cell line SH-SY5Y was incubated for 2h with SARS-CoV-2. After this period, cells were incubated with different concentrations of Cannabidiol, at concentrations of 100, 300, 1000, 3000, and 10000 nM at different time points (0h, 2h, or 4h). After the end of the experiments, we performed: a) MTT viability assay, b) BrdU proliferation assay, and qPCR for the detection/determination of SARS-CoV2 replication and cytokine production (cells and supernatant). In a final experiment, we conducted an in silico molecular docking to test the putative interaction of CBD with proteins and the primary cells targets of SARS-CoV-2.

Results:. The analysis of brain samples demonstrated that SARS-CoV-2 infects neurons and astrocytes, increasing the production of cytokines and chemokines in the tissue. In the in vitro experiments, CBD (at the doses of 1000nM and 3000nM) increased the viability and proliferation rate of SH-SY5Y infected with SARS-CoV-2. CBD (at the concentration of 1000 and 3000nM) increased the neuronal-like dendritic morphology of SH-SY5Y. In the qPCR assay, CBD increased the production of IL-10 mRNA. CBD did not decrease SARS-CoV-2 infection at any tested concentration. This latter effect was confirmed by the molecular docking results showing that CBD does not interact with the Spike and the *N* proteins of the virus or with the type 2 angiotensin-converting enzyme and the serine protease TMPRSS2.

Conclusions: Our results suggest a possible neuroprotective effect of Cannabidiol in the context of the neurological disorder burn associated with COVID-19. Our results are still preliminary, and more experiments are needed to confirm and better elucidate the mechanism behind CBD's neuroprotective effects in models of SARS-CoV-2 neuroinfection.

Keywords: Cannabidiol, Novel Coronavirus (SARS-CoV-2), Neuroprotection

Disclosure: Nothing to disclose.

P425. Greater Monoamine Oxidase B Distribution Volume in the Cortex in Traumatic Brain Injury With Persistent Symptoms: An [11C]SL25.1188 Positron Emission Tomography Study

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Background: Traumatic brain injury (TBI) has a lifetime prevalence of more than 10%, and a third of cases have long term symptoms, making TBI the most important cause of disability from neurological disease. There are no established pharmacological treatments for long term symptoms and a key barrier is a lack of targetable processes in community-based samples of human TBI, which are of mild to moderate severity representing about 90% of TBI cases.

Gliosis (proliferation and activation of glial cells) occurs in chronic traumatic encephalopathy, but it is unknown whether gliosis occurs in the more common TBI of mild to moderate severity. Astroglial activation (activation and proliferation of astrocytes) is correlated with monoamine oxidase B (MAO-B) level in neuropsychiatric disease and may be applied as a marker of gliosis. [11C]SL25.1188 positron emission tomography (PET) may be applied to measure MAO-B total distribution volume (MAO-B VT), an index of MAO-B density.

It is hypothesized that MAO-B VT is elevated in the prefrontal cortex as well as in other cortex, regions. Cortex regions are close to the skull and receive high levels of deformation stress in TBI models of direct and contra-coup injury, and the prefrontal cortex additionally plays an important role in cognitive functions often persistently affected by TBI.

Methods: Seventeen healthy and 23 TBI cases with persistent symptoms were recruited from the community in Toronto, Canada. [11C]SL25.1188 PET was applied to measure MAO-B VT, prioritizing the prefrontal cortex then other cortical regions, although a number of cortical and subcortical grey matter regions were also assessed. TBI cases had persistent symptoms since their last injury and no history of cigarette smoking, substance abuse, or psychotic illness.

Results: Patients with TBI had significantly greater MAO-B VT in the PFC (19%, $F1,38 = 10.76$, $P = 0.002$) and throughout the cortical regions (15%, $F1,38 = 9.31$; $P = 0.004$). The effect sizes (Cohen's d) were robust in the cortical regions, ranging from 0.97 to 1.24.

Conclusions: MAO-B VT was considerably elevated in the TBI group with an effect size of ~1.1 across cortical regions. This is strongly supportive of astrogliosis in TBI with persistent symptoms from community-based samples. The effect size is particularly notable, being the largest among brain markers comparing mild to moderate severity TBI to controls. [11C]SL25.1188 PET shows intriguing promise for stratifying TBI cases with gliosis and monitoring treatments of TBI to reduce gliosis.

Keywords: Astrogliosis, Positron Emission Tomography (PET), Traumatic Brain Injury, Monoamine Oxidase B, Gliosis

Disclosure: Exceltis: Other Financial Or Material Support (Self)
Sanofi: Grant (Self),
CAMH: Patent (Self)

P426. The Effect of 5-HT1A Agonists on Psychiatric Symptoms in a Mouse Model of Parkinson's Disease by Suppressing Activity of the Median Raphe Nucleus

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Background: Parkinson's disease (PD) is a neurodegenerative disorder in which the degeneration of dopaminergic nerves in the substantia nigra is central to the pathogenesis of the disease. Psychosis in PD (PPD) such as hallucinations and delusions occurs in the advanced stages. The psychosis is now considered to be related to internal factors such as disease progression, external factors such as medications, and facilitating factors such as environmental changes and stress. Treatment is usually started with environmental adjustments and reduction of PD medications. Some of the drugs that have been reported

to be effective for PD psychosis include clozapine, quetiapine, cholinesterase inhibitors and pimavanserin.

Dopamine-deficient (DD) mice are tyrosine hydroxylase knockout and do not produce dopamine, so they are maintained on L-dopa. DD mice move well after L-dopa administration, but show bradykinesia when dopamine in the brain is decreased. Surprisingly, they become hyperactive in a novel environment when DA level in the brain is below its detection. The hyperactivity of DD mice was suppressed by clozapine and donepezil.

In general, hyperactivity in mice is said to reflect psychiatric symptoms. We hypothesized that hyperlocomotion in DD mice would be a model mouse for PPD, since abnormal behavior (psychiatric symptoms) occurs under dopamine deficiency and is ameliorated by the common drugs clozapine and donepezil.

To investigate the relationship between serotonin and psychosis with PD, which remain largely unexplored, serotonin-based drugs were administered to DD mice exhibiting abnormal hyperlocomotion. For the effective drugs, immunohistochemical staining of brain samples using c-Fos was performed to investigate the cause.

Methods: DD mice are maintained with administration of L-dopa. Seventy-two hours after interruption of L-dopa administration, when DA level in the brain was below its detection, open field test (OFT) was performed; the mice were placed in a novel environment (35 × 40 × 25 cm) and locomotor activity was counted. Saline, quetiapine (multi-acting receptor-targeted antipsychotics; 20 mg/kg), pimavanserin (5-HT2A/2C inverse agonist; 5 mg/kg), tandspirone (5-HT1A agonist; 3 mg/kg) and paroxetine (selective serotonin reuptake inhibitor; 8 mg/kg) were injected into DD mice ($n = 29$, 20, 11, 10, 10, respectively) and wild type mice ($n = 16$, 21, 11, 12, 10, respectively) three hours after exposure to the novel environment. For each mouse, locomotion was monitored for additional three hours.

The drug was administered three hours after the start of OFT, and brain samples were collected from DD and wild-type mice one hour after that. We also collected brain samples from mice not exposed to the novel environment and brain samples taken four hours after the start of OFT without drug administration. c-Fos immunohistochemistry was performed to observe neuronal activity in the raphe nuclei. In the median raphe nucleus, c-Fos positive cells were counted in an area of 400 μm long x 100 μm wide, and in the rostral linear nucleus, cFos-positive cells were counted in an area of 200 μm long x 200 μm wide.

Results: DD mice gradually showed hyperactivity that could be regarded as a psychiatric symptom. Among the drugs administered, only quetiapine suppressed hyperactivity in DD mice. Administration of WAY100635 (5-HT receptor antagonist) 30 minutes before quetiapine partially cancelled the effect, suggesting quetiapine function may be mediated by serotonin receptors. To find out which subtype of receptor is involved, 8-OH-DPAT (5-HT1A agonist) and EMD281014 (5-HT2A antagonist) were administered, and 8-OH-DPAT was more effective.

Immunohistochemical staining was performed under quetiapine or 8-OH-DPAT treatment. During hyperactivity in DD mice, there was an increase in c-Fos-positive cells in the median raphe nucleus, which was reduced by quetiapine and more reduced by 8-OH-DPAT. In contrast, few c-Fos-positive cells were found in the rostral linear nucleus.

Conclusions: These results suggest that 5-HT1A receptors may be involved in PPD, and that drugs targeting 5-HT1A receptors may be useful in treating psychiatric symptoms. It is also possible that the median raphe nucleus, but not the rostral liner nucleus, is involved in the suppression effect of the drugs on hyperactivity.

Keywords: Parkinson's Disease, Parkinson's Disease and Dementia Psychosis, Mouse Models, Raphe Nucleus

Disclosure: Nothing to disclose.

P427. Vesicular Glutamate Transporter 2 Expression Mediates Sex Differences in Dopamine Neuron Resilience by Modifying Mitochondrial Oxidative Stress

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Background: Neurodegenerative illnesses including Parkinson's disease (PD) is characterized by dopamine (DA) neuron loss, but some DA neurons are relatively protected compared to others. Clues for better understanding mechanisms of DA neuron resilience come from a distinct subpopulation of midbrain DA neurons that co-transmit glutamate. Multiple observations have shown: 1) midbrain DA neurons in the medial VTA are spared in brains of PD patients and in both primate and rodent models of PD; and 2) these resilient DA neurons express the vesicular glutamate transporter 2 (VGLUT2). Indeed, we and colleagues discovered that VGLUT2-expressing DA neurons are more likely to survive neurotoxic insults compared to DA neurons that do not express dVGLUT2. We previously demonstrated that DA neuron VGLUT2 enables DAergic synaptic vesicles to dynamically tune their vesicle content and enhance vesicular DA loading and release (Aguilar et al., 2017). These data therefore suggest that DA neuron VGLUT2 may be employed by surviving neurons to maintain adequate synaptic DA neurotransmission to compensate for ongoing DA neurodegeneration. However, to date, the mechanisms behind this protection conferred by VGLUT2 is still unknown. Considering the important role of mitochondrial dysfunction in DA neuron loss and PD etiology, we hypothesized that VGLUT2 expression may improve mitochondrial resilience to oxidative stress observed in DA neurons in animal models of PD and human PD. To test this hypothesis, we employed the genetically tractable Drosophila melanogaster model which expresses a single ortholog of VGLUT, Drosophila VGLUT (dVGLUT).

Methods: Drosophila strains: All Drosophila strains were maintained at 24C, ~50% humidity under a 12:12 hour light/dark cycle in humidity- and temperature-controlled incubators. We used the TH-GAL4 strain (gift of Dr. S. Birman, Université Aix-Marseille II-III, Marseille, France) (Friggi-Grelin et al., 2003) to drive expression in DA neurons via the GAL4/UAS binary expression system.

Brain imaging: To measure reactive oxygen species (ROS) in DA neurons, we imaged whole intact adult central brains expressing the MitoTimer ROS biosensor (Laker et al., 2014) via multiphoton microscopy.

dVGLUT knockdown: To ascertain effects of dVGLUT RNA interference (RNAi) KD on ROS in DA neurons, we used TH-GAL4-driven UAS-VGLUT-RNAi (UAS-Vglut-RNAi) co-expressed with MitoTimer. All fly strains were outcrossed for 10 generations into the w1118 wild-type genetic background. For all experiments utilizing drug treatments, flies were randomly assigned to vehicle or drug treatment groups.

RNAscope: Conduced in adult fly central brain as described earlier (Buck et al., 2021).

Results: We first validated DA neuron-specific RNAi KD of dVGLUT which caused a 36.1%-51.4% decrease in average number of dVGLUT mRNA grains in TH + neurons compared to control genotypes. Importantly, DA neuron knockdown of dVGLUT resulted in a 44.0%-63.6% decrease in TH + /dVGLUT+ neuron density, and a 51.3%-69.0% decrease in percent of TH + neurons that are dVGLUT + , reinforcing dVGLUT's role as a neuroprotective factor in DA neurons. To test if dVGLUT's neuroprotective properties are linked to its ability to lower neurotoxic ROS in DA neurons, we analyzed the impact of DA neuron dVGLUT

knockdown on mitochondrial oxidative stress using flies that express MitoTimer, a mitochondria-targeted ROS biosensor in DA neurons. Notably, we discovered sex differences in mitochondrial ROS levels in vivo in DA neurons, finding that males possess ~40% more mitochondrial ROS compared to females ($n = 5$-8/group, $p < 0.05$). This suggests females' lower ROS levels may contribute to their greater DA neuron resilience. We then tested our hypothesis that DA neuron dVGLUT lowers mitochondrial ROS to potentially contribute to these sex differences by knocking down dVGLUT in DA neurons via RNAi. dVGLUT KD indeed raised mitochondrial ROS. This was significantly pronounced in females compared to males ($p < 0.01$). Last, we examined effects of DA neuron dVGLUT KD in response to paraquat, a neurotoxicant that raises DA neuron ROS. dVGLUT RNAi KD exacerbated mitochondrial ROS in DA neurons in a region-specific manner ($p < 0.05$) with the greatest increase observed in the male dVGLUT RNAi group ($P < 0.05$).

Conclusions: Our findings uncover a novel mechanism of dVGLUT-mediated DA neuroprotection, highlighting VGLUT's role in mediating sex differences in mitochondrial oxidative stress in DA neurons. These data suggest that an important source of increased DA neuron resilience in females is the capacity of these cells to diminish levels of toxic mitochondrial ROS via heightened dVGLUT expression versus males. This sex difference is relevant since PD in women is less prevalent and has a later age of symptomatic onset. Ultimately, determining the mechanisms of increased DA neuron resilience in females can be transferred to males to boost DA neuron survival in neurodegenerative diseases including PD. This may lead to new, effective treatments to either slow or stop DA neurodegeneration in both men and women.

Keywords: Dopamine, Glutamate, VGluT2 Neurons, Neurodegeneration

Disclosure: Nothing to disclose.

P428. Distinct Neuropsychiatric Features Define Three Subgroups of Female FMR1 Premutation Carriers

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Background: Cytosine-guanine-guanine (CGG) trinucleotide repeats in the 5′ untranslated region of Fragile X mental retardation 1 (FMR1) gene over 200 results in a "full mutation" of Fragile X Syndrome (FXS), whereas 55-200 repeats results in a "premutation". FMR1 premutation carriers (PMC) are characterized by a range of psychiatric, neurocognitive, and physical conditions, though different than those features associated with full mutation FXS. For example, previous studies have documented both intact and impaired executive function, social processing, and psychiatric features in female PMCs relative to age- and sex-matched typically-developing controls (TDC). CGG repeat length, Fragile X mental retardation protein (FMRP) levels, and degree of toxicity resulting from CGG repeat containing FMR1 mRNA, and environmental factors are thought to play a role in the variable neuropsychiatric feature presentation of PMC. Studies have reported both linear and non-linear relationships with CGG repeat length and neuropsychiatric symptoms. A bottom-up, data-driven approach to identify naturally occurring clusters of neuropsychiatric features in female PMCs may be an important step in parsing this heterogeneity, ultimately offering better insight into understanding underlying pathology and developing better screening, diagnostic, and treatment planning for this unique population.

Methods: Forty-one females (17-78 years) with 50-200 CGG repeats, or premutation carriers (PMC), and fifteen age- and-sex matched controls completed the study. PMC status (50-200 CGG repeats) was confirmed via genetic analysis or medical record review. All participants completed: 1) neurocognitive testing via the Test of Attentional Performance for Children (KiTAP), 2) self-report measures including Beck Depression Inventory, Second Edition (BDI-II), Anxiety Sensitivity Index (ASI), and Adult Sensory Profile (SP); 3) eye tracking using Tobii T120 of Farzan faces and social preference scenes; and 4) five-minute resting state EEG using EGI NetAmp400. First, we examined the feasibility to use cluster analysis to identify potential subgroups of female PMCs using K-means cluster analysis, in order to classify non-overlapping clusters with the lowest within-cluster variance and the highest between-cluster variance. Second, we examined inter-correlations between neuropsychiatric features and CGG repeat count within each cluster membership. Last, we conducted separate univariate ANOVAs for each variable of interest for each task with the between subjects' factor group (PMC vs TDC).

Results: Female PMC and TDC did not differ on IQ, depression or anxiety symptoms, or social or sensory processing. Across frequency ranges, groups also did not differ on relative power. Only subtle impairments in specific executive function domains, including processing speed and distractibility, were observed for female PMCs. We found a three cluster solution using k-means clustering. Cluster 1 represented a psychiatric feature group (27% of our sample); Cluster 2 represented a group with executive dysfunction and elevated high frequency neural oscillatory activity (32%); and Cluster 3 represented a relatively unaffected group (41%). In addition, female PMCs within Cluster 1 demonstrated significant relationship between psychiatric symptoms and higher CGG repeat count. Similarly, female PMCs in Cluster 2 with more aberrant theta and gamma power had higher CGG repeat counts, although this subgroup had the lowest percentage of females in the high CGG repeat category. Although Cluster 3 demonstrated increased theta power and reduced alpha power compared to TDC, this subgroup was observed to have relatively spared neuropsychiatric functions.

Conclusions: Consistent with prior reports, our overall sample of female PMCs has relatively preserved psychiatric, cognitive, social, and electrophysiological functioning. However, by using a bottom-up approach to subgroup clustering using a wide array of quantitative assessments, we were able to identify three distinct clinically-meaningful clusters with good clinical face-validity. We also showed that CGG repeat count and its association with neuropsychiatric features differed across clusters. Thus, our findings indicate the feasibility of using a data-driven approach to identify naturally occurring clusters in female PMCs using a multi-method assessment battery. Ultimately, our findings provide important insight into potential diverging pathophysiological mechanisms and risk factors for each female PMC cluster, which may help provide new insights into pathology associated with FMR1 gene and potential treatment options.

Keywords: FMR1 Premutation, Cluster Analysis, Neuropsychiatric Disorders, Psychiatric Genetics

Disclosure: Nothing to disclose.

P429. NIH HEAL Initiative: National Institute of Neurological Disorders and Stroke's Early Phase Pain Investigation Clinical Network (EPPIC-Net)

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Background: The NIH Helping to End Addiction Long-term SM (HEAL) Initiative focuses on efforts to advance scientific solutions to eradicate the opioid crisis by improving prevention and treatment of opioid misuse/addiction and enhancing pain

management by accelerating early-phase clinical trials of non-addictive pain therapeutics ("assets").

Methods: Within the HEAL Initiative, NINDS has been tasked with identifying, developing, and testing non-addictive pharmacologic and non-pharmacologic therapeutics ("assets") targeted to pain conditions of high unmet need. NINDS established the Early Phase Pain Investigation Clinical Network (EPPIC-Net) to accelerate and enhance pain therapeutic development by carrying-out phase 2 clinical trials of non-addictive pain therapies. EPPIC-Net evaluates new, as well as repurposed, small molecules, biologics, drugs, natural products, and devices submitted by industry, academic, or other partners for studies across the age and pain condition spectrum. The ideal asset has adequate data to support a phase 2 clinical trial and has an existing IND/IDE or is IND/IDE-ready. EPPIC-Net infrastructure includes a Clinical Coordinating Center, a Data Coordinating Center, and 12 Specialized Clinical Sites with access to broad, inclusive patient populations to provide phase 2 clinical trials, incorporating proof-of-concept testing, biomarkers validation, novel study design, and protocol development and implementation.

Results: EPPIC-Net aims to provide a robust and readily accessible infrastructure with a network comprised of pain experts who will design, conduct, and provide data analysis for phase 2 trials built around submitted potential pain therapeutic assets at no cost to the asset provider. The applicant is expected to provide the asset and matched placebo for the study. The asset owner retains intellectual property rights to their asset. It is anticipated that successful phase 2 trials will enable the applicant to continue to phase III studies and further development of the asset outside of EPPIC-Net. Ultimately, EPPIC-Net will reduce reliance on opioids by accelerating development of non-addictive pain therapeutics.

Conclusions: This abstract describes the NIH HEAL Initiative EPPIC-Net program, charged with evaluating pain therapeutics in phase 2 clinical development. EPPIC-Net accepts applications on a rolling basis; EPPIC-Net seeks innovative non-addictive treatments for any pain condition that lacks adequate treatment, spanning the age spectrum from children to old age.

Keywords: Opioid Addiction, Phase II Clinical Trial, Non-Opioid, Non-Addictive Therapeutics

Disclosure: Nothing to disclose.

P430. Aberrant Anterior Cingulate Cortex Activity in HIV + Adults With High Early-Life Stress Exposure: Relation to Cognitive Efficiency

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Background: High early-life stress (ELS) exposure is associated with greater cognitive impairments in adults living with HIV, yet the neural correlates underlying these effects remain poorly understood. The anterior cingulate cortex (ACC) is one of few brain regions in which abnormalities in neural structure and function are observed in both HIV + adults and adults with high ELS. Although aberrant activity within the subgenual ACC (sgACC) is known to contribute to cognitive dysfunction, the combined effects of HIV and high ELS exposure on sgACC activity have received little investigation. Here, we examined the independent and combined effects of HIV and high ELS exposure on sgACC function and its relation to cognitive efficiency.

Methods: We included 49 HIV + adults (26 with high ELS) and 45 demographically-matched healthy control adults (24 with high ELS). fMRI was used to assess sgACC function during a verbal working memory task, the N-back. Our task included several N-back conditions (e.g., 1back, 2back, 3back) where larger Ns provide increased cognitive load. To capture neural response associated with increased cognitive load, we examined sgACC function during the 3back relative to the 1back. Participants with low N-back accuracy (A' < .70), low N-back response rate (<80%), excessive movement during the N-back (>2mm), or positive hepatitis C status were excluded. We utilized a behavioral index of cognitive efficiency previously shown to be sensitive to cognitive impairment in HIV + samples, reaction time intra-individual variability (RT-IIV), to measure cognitive efficiency during the 3back, where higher RT-IIV scores indicate poorer efficiency. Using a region of interest (ROI) approach, we calculated the mean BOLD response within a 5-mm radius sphere centered on coordinates for the sgACC. The independent and combined effects of HIV and high ELS status on sgACC function were examined using ANCOVA, controlling for variables on which the groups differed significantly (e.g., current stress). Associations between sgACC and cognitive efficiency (RT-IIV) were examined using linear regression.

Results: Across the entire group, the 3back elicited expected patterns of verbal working memory activation (e.g., middle frontal gyrus) and deactivation (e.g., ACC, fully encompassing our sgACC ROI) ($p < .05$, FDR corrected) consistent with active suppression of the default mode network (DMN). Analyses of sgACC function revealed a significant interaction effect between HIV and ELS status ($F = 5.67$, $p = .020$, partial eta-squared = .09), as well as a significant main effect of ELS status ($F = 7.57$, $p = .008$, partial eta-squared = .11). Follow-up analyses indicated that these effects were driven by greater 3back-induced sgACC deactivation in the HIV + high ELS group relative to all other groups (p's < .032). In the HIV + high ELS group, sgACC function correlated significantly with RT-IIV (beta = .510, $p = .037$), such that participants who exhibited greater 3back-induced deactivation also exhibited greater cognitive efficiency. This association was maintained (beta = .496, $p = .046$) even when controlling for 3back performance (accuracy).

Conclusions: These findings demonstrate for the first time ELS-related functional abnormality in the sgACC in HIV + adults. Further, our data align with studies linking cognitive difficulty to a failure to suppress DMN functions. Greater sgACC deactivation in HIV + high ELS adults may reflect an adaptive dynamic down-regulation of DMN-related processes (e.g., internal state monitoring) during high cognitive-load tasks. The observed association between sgACC deactivation and cognitive efficiency thus implicates ELS-related DMN abnormalities in cognitive difficulties experienced by HIV + high ELS adults.

Keywords: HIV, Adverse Childhood Experiences (ACE), Early-Life Stress, Cognitive Symptoms, Subgenual Anterior Cingulate

Disclosure: Nothing to disclose.

P431. Aberrant Cortical Neurophysiology in a Patient-Derived NMDA Receptor GRIN1 Y647S +/- Mutant Mouse: Characterization and Pharmacological Interventions

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Background: NMDA receptors (NMDARs) are essential for brain function and enable supralinear integration of glutamatergic inputs to generate dendritic plateau potentials. GRIN1 neurodevelopmental disorder is a rare condition caused by de novo mutations in the gene encoding the obligate GluN1 NMDAR subunit. This genetic disorder is characterized by profound neurological deficits and seizures. However, little is known about the neurophysiological deficits as the consequences of various human GRIN1 mutations on NMDAR function and dendritic integration are unknown. Therefore, we characterized the neurophysiological consequences of a patient-derived GRIN1 mutation (Y647S), using a heterozygous transgenic mouse model.

These experimental results give new insight into dynamic interactions between NMDARs and proximal ion channels and identify a new research direction for GRIN disorder treatment.

Methods: Transgenic mutant mice (Grin1 Y647S +/-) and wildtype littermate mice of both sexes were used. Patch clamp electrophysiology of layer 5 pyramidal neurons in the prefrontal cortex examined three different aspects of NMDA receptor functionality: (1) whole-cell currents evoked by bath application of NMDA as a broad measure of NMDAR levels and activity; (2) electrically-evoked excitatory postsynaptic currents (EPSCs) for functional assessment of cortical synapses; and (3) dendritic plateau potentials for characterization of NMDAR mediated dendritic integration.

Results: We found unexpected complexities in the prefrontal cortical neurophysiology in mice heterozygous for the patient-variant Grin1 Y647S +/- mutation, with paradoxical gain- and loss-of-function of different aspects of cortical NMDAR signaling. Whole-cell currents evoked by bath applied NMDA were dramatically enhanced in the Grin1 Y647S +/- mice ($t(17) = 3.88$, $p = 0.0012$). By contrast, electrically-evoked pharmacologically-isolated NMDAR EPSCs show significant reduction in the Grin1 Y647S +/- mice (Interaction between stimulus intensity and genotype: $F(7,112) = 3.809$, $p = 0.001$). Ongoing examination of NMDAR-mediated dendritic plateau potentials shows reduced burst firing, but greatly prolonged depolarization in Grin1 Y647S +/- mice. The combined data suggests deficient NMDAR together with a dysregulation in their typical recruitment of calcium-activated potassium channels. Drugs that potentiate calcium-activated potassium channels, such as NS309, show promise in restoring appropriate timing of NMDAR-dependent integration.

Conclusions: We have characterized the consequences of the patient-derived Grin1 Y647S +/- mutation for multiple aspects of prefrontal neurophysiology. It results in a multi-faceted dysregulation arising via reduction of NMDARs themselves but also their recruitment of tightly linked ion channels. Ongoing work is refining a pharmacological strategy to restore appropriate NMDAR signaling in these mice

Keywords: GRIN-Related Disorders, NMDA Receptor, GRIN1 Mutation, Cortical Excitability, Ion Channels

Disclosure: Nothing to disclose.

P432. The NIH HEAL Initiative Preclinical Screening Platform for Pain (PSPP) Efforts to Accelerate Development of Non-Opioid, Non-Addictive Pain Therapeutics: Validation of the Monoiodoacetate Model of Osteoarthritis Pain in the Rat

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Background: The National Institutes of Health Helping to End Addiction Long-termSM Initiative, or NIH HEAL InitiativeSM, Preclinical Screening Platform for Pain (PSPP) program aims to accelerate the discovery and development of new non-opioid, non-addictive pain therapeutics. Towards this goal, PSPP is collaborating with PsychoGenics, Inc. to validate preclinical models and endpoints to enable screening and profiling of assets, including small molecules, biologics, natural products, and devices. Here, we describe the validation of one such effort to optimize the monoiodoacetate (MIA) model of osteoarthritis pain in the rat. Intraarticular injection of MIA into the rat hindlimb knee joint results in cartilage degeneration, localized inflammation, and pain behaviors that are thought to resemble those seen in human osteoarthritis (OA).

Methods: Adult male and female Sprague Dawley rats ($N = 10$, each sex) were used in these studies. MIA (0.3 – 3 mg) was injected intraarticularly into the left hindlimb knee joint. Tactile sensitivity (von Frey testing using the up down method as described by Chaplan et al., 1994), weight bearing, changes in gait, and paw pressure were systematically evaluated in both sexes for 4-6 weeks. Following this systematic phenotyping, pharmacological validation of the model was established using morphine (3 mg/kg), duloxetine (60 mg/kg), and ketoprofen (6 mg/kg) after acute and repeated dosing.

Statistical analysis, effect size, and power analysis: Data were analyzed using two-way repeated measures ANOVA with Bonferroni's or Dunnett's post hoc test when appropriate. Effects at $p < 0.05$ were considered to be statistically significant. Power analysis and effect size were determined using SAS/STAT, and appropriate sample size was based on a power value of 0.8 to ensure adequate power for *F*-tests for two-way interactions.

Results: Intraarticular injection of MIA (1 and 3 mg) into the hindlimb knee joint produced unilateral hind paw tactile hypersensitivity which was maximal at week 2 post MIA injection in both male and female rats. Changes in gait were also observed in male and female rats following MIA injection at weeks 1 and 2 post MIA injection. Both static and dynamic weight bearing were evaluated in both sexes. Deficits were modest when measuring static weight bearing, but more pronounced when measuring dynamic weight bearing in both male and female rats at 1 week post MIA injection. While female rats showed hypersensitivity to pressure and pinch stimuli at week 2 post MIA injection, this effect was not observed in male rats.

We then evaluated the efficacy of several analgesics on dynamic weight bearing in rats injected with MIA (1 mg). Acute subcutaneous injection of morphine (3 mg/kg) reduced hind paw tactile hypersensitivity and weight bearing deficits in male and female rats, whereas acute oral administration of ketoprofen (6 mg/kg) and duloxetine (60 mg/kg) were less effective. In contrast, repeated treatment with ketoprofen or duloxetine (4 days, b.i.d.) significantly reduced tactile hypersensitivity and weight bearing deficits.

These studies further helped establish inclusion and exclusion criteria for using various endpoints in this model and will be described in detail in this presentation.

Conclusions: The results from these validation studies using evoked and non-evoked endpoints in the rat MIA model suggest that both tactile sensitivity and dynamic weight bearing in the acute and repeated treatment paradigms of the MIA model may be used to identify and differentiate novel therapeutics for treatment of osteoarthritis. This example highlights efforts within the NIH HEAL Initiative's PSPP program to validate endpoints and models to be incorporated into evaluating novel assets towards accelerating the development of novel non-opioid, non-addictive therapeutics.

Keywords: NIH HEAL Initiative, Preclinical Screening Platform for Pain, Non-Opioid, Non-Addictive Therapeutics, Monoiodoacetate Model of Osteoarthritis

Disclosure: National Institutes of Health: Employee (Self)

Retiree, Eli Lilly and Company: Other Financial or Material Support (Self)

P433. Transdiagnostic Anxiety-Related Increases in Information Sampling Associated With Altered Valuation

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Background: The ability to gather an appropriate amount of information about one's environment and use this information

effectively is critical for adaptive decision-making. In certain psychiatric disorders characterized by high trait anxiety, excessive information seeking can drive maladaptive responses to the environment (e.g., compulsive checking in obsessive-compulsive disorder [OCD], eliciting reassurance in generalized anxiety disorder).

Increased information seeking in psychiatric patients has been linked with alterations in inferential and valuation processes, although findings have been inconsistent. In part this is attributable to the use of information sampling tasks that lack appropriate controls for decision-making processes or task design elements that could influence task performance (e.g., time-pressure to make a decision, lack of motivation). To more precisely clarify the mechanisms associated with increased information seeking in individuals with high trait anxiety, computational models of inference and valuation were applied to data collected from a transdiagnostic sample of adults using a version of the beads task that improved on previous designs by accounting for potential confounds.

Methods: Participants (55% female, 45% male; M(SD) age = 27.6(7.3)) included 18 individuals diagnosed with OCD, 17 individuals with an anxiety disorder, and 23 healthy controls. No participants were taking psychotropic medications for at least 4-8 weeks prior to study enrollment.

Participants were told that at the start of each trial, either a jar with mostly blue beads or mostly green beads was selected at random and concealed from them, and their goal was to correctly guess the identity of the jar. Each jar had one of four majority-to-minority ratios of green or blue beads ("bead-ratio" conditions: 60:40, 75:25, 90:10, or 100:0 [control condition]) that was told to the participant at the start of the trial. Participants were able to draw up to a maximum of eight beads from the hidden jar. Within each trial, participants provided an estimate of the hidden jar's identity prior to drawing any beads (i.e., a baseline measure of "prior belief"), then given the choice of drawing a bead or guessing the jar's identity. Probability estimates were provided again after the bead was displayed. Participants were incentivized to earn up to $30 cash, with a $0.30 penalty for requesting an additional bead and a $15 penalty for incorrectly guessing the identity of the hidden jar.

First, a model of belief updating was fit to reported draw-by-draw probability estimates. Informed by Bayes' theorem, this model quantified the iterative process by which new information (i.e., bead color) and prior beliefs were integrated to influence current beliefs (i.e., jar identity). This model consisted of four key parameters, including ω1 (the prior weight), reflecting the degree to which evidence presented earlier in the trial contributed to the posterior belief, and three ω2 parameters, one for each bead-ratio condition (60:40, 75:25, 90:10), representing the contribution of new information on posterior beliefs.

Next, a parameterized partially-observed Markov decision process ideal-observer model was used to understand the contribution of value-based decision making to sampling behavior (i.e., draws-to-decision; DTD). This model was comprised of three subjective cost parameters, Csub, one for each bead-ratio condition, and γ, the inverse-temperature parameter reflecting choice stochasticity.

Results: A repeated-measures ANOVA revealed a difference in DTD across bead-ratio conditions by trait anxiety, $F(2, 114) = 3.17$, $p = 0.04$. Post-hoc analyses of this significant interaction showed that trait anxiety was correlated with mean DTD only in the 90:10 bead-ratio condition, $r = 0.29$, $p = 0.03$.

Of the key parameters from potential explanatory models (ω1, ω2(0.9), Csub(0.9), γ), the only parameter that satisfactorily accounted for the association of trait anxiety and DTD behavior in the 90:10 bead-ratio condition was Csub(0.9). Specifically, lower Csub(0.9) was associated with more trait anxiety, $r = -0.51$, $p < 0.0001$, and more DTD in the 90:10 bead-ratio condition, $r = -0.76$, $p < 0.0001$, suggesting that individuals who perceived the cost of drawing a bead to be smaller (or the cost of an incorrect guess to be larger) were higher in trait anxiety and engaged in greater information seeking.

Of note, lower ω1 was associated with higher trait anxiety, $r = -0.28$, $p = 0.03$, suggesting that individuals with greater trait anxiety were more likely to demonstrate a recency bias, but ω1 was not associated with DTD in the 90:10 condition. Although ω1 and Csub(0.9) shared variance, $r = 0.29$, $p = 0.03$, in a linear regression model predicting trait anxiety from ω1 and Csub(0.9), only Csub(0.9) retained significance, $p = 0.005$.

Conclusions: Dimensional trait anxiety correlated with increased information sampling (more DTD) in a condition with high objective evidence strength (90:10 bead-ratio condition). This was not explained by differences in weighting of new or old information, but rather by a different cost-benefit analysis suggesting that individuals with greater trait anxiety placed a higher subjective cost to incorrect guesses in relatively unambiguous contexts. Findings align with prior research that has identified alterations in valuation processes among patients with OCD and anxiety disorders and provide insight into potential mechanisms of maladaptive clinical characteristics of these disorders.

Keywords: Computational Modeling, Transdiagnostic, Anxiety, Obsessive Compulsive Disorder

Disclosure: Nothing to disclose.

P434. How Do Obsessive-Compulsive Symptoms and Traits Impair Adjustment to the Easing of COVID-19 Restrictions?

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Background: Adjusting to a changing lifestyle as COVID-19 restrictions ease will be important for public mental health, psychosocial wellbeing and economic recovery. Our previous study surveyed adult members of the public ($n = 438$) during a temporary release of lockdown (July to November 2020) and showed that those with a history of mental disorder and obsessive-compulsive (OC) personality traits and symptoms, alongside those showing cognitive rigidity on an objective task, found adjustment problematic (Fineberg et al., 2021; Journal of Psychiatric Research). In this secondary analysis of the original study data, we aim to clarify the specific OC traits and symptoms impacting most on three key COVID-related behaviors: problematic adjustment, avoidance and disinfecting, as a further step toward identifying those most at risk and how they are affected.

Methods: A series of correlational analyses, followed by multiple regression analyses, was performed to determine the associations between individual OC personality traits and symptoms, measured on the self-rated Compulsive Personality Assessment Scale (CPAS) and Obsessive-Compulsive Inventory Revised (OCI-R), respectively, and self-rated problematic adjustment, avoidance and disinfecting behaviors. Statistical analyses were conducted using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, N.Y., USA). Bonferroni correction for multiple comparisons was applied.

Results: Difficulties adjusting in general correlated with perfectionism, preoccupation with details and rules, over-conscientiousness, need for control, contamination obsessions and compulsions, checking compulsions, difficulties controlling one's thoughts and counting compulsions; social avoidance correlated with perfectionism, preoccupation with details and rules, contamination obsessions and compulsions, checking compulsions and difficulties controlling one's thoughts; while disinfecting

behaviors correlated with preoccupation with details and rules, miserliness, contamination obsessions and compulsions, checking compulsions and difficulties controlling one's thoughts (Pearson's r - all $p < .001$). Regression analysis of the scores on these specific items explained a greater amount of the variance than did analysis of the total CPAS or OCI-R. Intriguingly, self-rated rigidity did not relate to any of these problematic behaviors.

Conclusions: Several OC personality traits and specific symptoms from two of the four phenotypic OCD dimensions (contamination and/or cleaning, obsessions and/or checking) (Mataix-Cols et al., 2005; American Journal of Psychiatry) predict problems adjusting. This finding may be used by policy makers to identify those members of the public potentially most at risk as lockdown restrictions ease. The failure to detect an association between adjustment difficulties and a self-rated measure of cognitive rigidity (CPAS item 8) may reflect the impairment in metacognition and insight previously recognized as being integral to this personality trait (Oltmanns et al., 2005; Consciousness and Cognition), and suggests that rigidity may be better evaluated using an objective test. General adjustment difficulties were accompanied by problematic social avoidance and disinfecting behaviors, which could represent targets for public-health intervention.

Keywords: OCD Phenotypes, Obsessive-Compulsive Personality Traits, COVID-19, Prevention, Adjustment

Disclosure: Nothing to disclose.

P436. Abnormal Midfrontal Theta-Band Activity During Response Monitoring in Children With Obsessive-Compulsive Disorder

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Background: The error-related negativity (ERN), one of the most investigated event-related potentials (ERPs), is the negative deflection in midfrontal electroencephalogram (EEG) recording after a commission of an error. Abnormal ERN activity occurs in various psychiatric conditions, most consistently observed as abnormally enhanced in individuals with obsessive-compulsive disorder (OCD), presumably linked to underlying neurophysiological mechanisms. ERPs reflect the summation of neural oscillatory activities that underlie brain region communications and could arise from increase in total power and/or increase in intertrial phase coherence (ITPC) of neural oscillatory activity. The ERN, in particular, is thought to reflect increased total power and partially by increased ITPC in theta-band (4-8 Hz) activity. Therefore, this study sought to investigate the theta-band power and ITPC abnormalities in OCD patients during response monitoring to enable a richer understanding into the underlying neurophysiology of this condition.

Methods: EEG was recorded from 99 pediatric patients with OCD and 99 healthy control (HC) participants while they completed the arrow flanker task. Participants were selected from a larger data, matching for sex (OCD 61% female; HC 55% female) and age (OCD $M = 13.75$; HC $M = 13.68$) across groups. Time-frequency analysis was conducted to extract theta-band power and ITPC from a midfrontal channel (Cz). Two mixed ANCOVAs, including sex and accuracy as covariates, were conducted to investigate the effects of group (OCD, HC) and response type (error, correct) on post-response theta-band (1) power and (2) ITPC. We hypothesized that there would be main effects of Group and Response Type, as well as an interaction effect. Specifically, we hypothesized that OCD patients will have larger theta-band power and ITPC than HC participants, error trials will have larger theta-band power and ITPC than correct trials, and OCD patients will have a larger difference in theta-band power and ITPC between error and correct trials than HC participants.

Results: Theta-band power was larger on error trials than on correct trials ($F[1,198] = 416.659$, $p < .001$), and larger in OCD patients than in HC participants ($F[1, 198] = 4.720$, $p = .031$). There was no Group x Response Type interaction ($F[1,198] = 1.014$, $p = .315$) on power. Theta-band ITPC was also larger on error trials than correct trials ($F[1,198] = 17.832$, $p < .001$). However, there was no main effect of group ($F[1, 198] = 0.018$, $p = .894$) and there was no Group x Response Type interaction ($F[1,198] = 1.172$, $p = .280$) on ITPC

Conclusions: Increase in both theta-band power and ITPC seems to underlie the ERN. OCD patients, however, only have enhanced theta-band power, regardless of response type, and did not differ from HC participants in ITPC. These data suggest that pediatric patients with OCD have general difficulty in regulating response monitoring or general compensatory mechanism. At the same time, this difficulty seems to be specific to neural mechanisms that would underlie increase in power, but not an increase in ITPC. The findings are expected to help identify novel brain-based treatment targeting neural oscillatory activity, such as transcranial alternating current stimulation.

Keywords: Obsessive Compulsive Disorder, EEG Electrophysiology, Time-Frequency, Error Processing

Disclosure: Nothing to disclose.

P437. Distinct Roles for Norepinephrine and Dopamine Receptor Signaling in the Control of Stress-Induced Repetitive Behaviors

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Background: Tourette's syndrome (TS) is a childhood onset neurological disorder that affects 1% of the population and is highly co-morbid with obsessive-compulsive disorder (OCD). TS is characterized by uncontrollable, repetitive behavioral tics that are preceded by aversive premonitory sensations and exacerbated by stress. Antipsychotic drugs which block dopamine (DA) receptors are the first line of treatment for reducing motor and vocal tics in TS, but the effectiveness of these medications is limited by a wide range of adverse side effects. Because these drugs are poorly tolerated, antipsychotic therapy is often discontinued in pediatric TS patients, which comprise the majority of cases. Although not FDA-approved for the treatment of TS, clonidine is widely used off-label for this disorder because it reduces tic severity and has milder side effects than antipsychotic drugs, although the mechanism by which clonidine attenuates TS symptoms remains unknown. Clonidine belongs to a class of drugs that suppress norepinephrine (NE) transmission by activating inhibitory α2 autoreceptors on NE neurons. The locus coeruleus (LC) is the primary source of NE in the central nervous system, projecting extensively within forebrain circuits that govern attention, arousal, stress responses, and motivated behavior. We have previously shown that NE is required for repetitive behaviors induced by cage-change stress, such as nestlet shredding and marble burying, using NE-deficient dopamine β-hydroxylase knockout (Dbh-/-) mice and their NE-competent littermate controls. In this study, we used predator odor to induce repetitive behaviors, arousal, and exploratory behavior, and examined abnormalities in these innate stress responses and neuronal activity related to NE deficiency

and/or augmented DA release in Dbh-/- mice and their Dbh +/- littermate controls.

Methods: Dbh-/- and Dbh +/- control mice ($n = 30$ per genotype) of both sexes were exposed to a new clean cage with a nestlet treated with either bobcat urine or a novel neutral odorant (essential oil) for 90 min, and repetitive behaviors, arousal, exploratory behavior, and time spent in contact with the nestlet were measured. Immediately following the test, mice were euthanized, and brains were processed for c-fos immunohistochemistry in the LC and several target regions implicated in behavioral responses to innately stressful predator odorants.

Results: Dbh-/- mice showed high levels of grooming ($p < 0.001$) and low levels of digging ($p < 0.05$) relative to controls regardless of odorant. However, while control mice sustained high levels of arousal throughout the predator odor condition, Dbh-/- mice also fell asleep within 90 min ($p < 0.001$). The odor-induced high grooming/low digging phenotype of the Dbh-/- mice was recapitulated in controls pretreated with the DBH inhibitor nepicastat. Excessive grooming behavior in Dbh-/- mice elicited by novel odorants was blocked by systemic administration of the DA receptor antagonist flupenthixol. In control mice, systemic administration of a cocktail of NE receptor antagonists resulted in virtually complete suppression of all typical repetitive and exploratory behaviors, including digging, in response to novel odorants. Compared with controls exposed to predator odor, Dbh-/- mice demonstrated increased c-fos induction in the LC and the medial amygdala, but decreased c-fos in the anterior cingulate cortex, lateral septum, periaqueductal gray, and bed nucleus of the stria terminalis. Intriguingly, c-fos induction in the paraventricular nucleus of the hypothalamus was similarly high in mice of both genotypes.

Conclusions: These findings indicate that DA receptors control grooming, while NE receptors control exploratory behavior and digging elicited by odorant stress. Further, the results of our current study using odorant stress support our previous findings that anti-adrenergic drugs exhibit anti-compulsive effects in the marble burying and nestlet shredding models of TS/OCD. Because the neurobiological basis of TS remains poorly understood, there is a paucity of efficacious drugs for the treatment of this disorder. We propose that NE and DA may contribute separable roles in the expression of repetitive behaviors in TS and OCD, particularly following exposure to stress. Thus, anti-adrenergic drugs could be effective for the treatment of compulsive disorders.

Keywords: Tourette syndrome, OCD, Acute Stress, c-Fos, Norepinephrine

Disclosure: Nothing to disclose.

P438. Independent and Distinct Patterns of Abnormal Lateral Orbitofrontal Cortex Activity During Compulsive Grooming and Reversal Learning Normalize After Fluoxetine

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Background: Patients with obsessive-compulsive disorder (OCD) display disrupted performance and abnormal lateral orbitofrontal cortex (LOFC) activity during reversal learning tasks, yet it is unknown whether compulsions and reversal learning deficits share a common neural substrate. To answer this question, we measured neural activity with in vivo calcium imaging in LOFC during compulsive grooming and reversal learning before and after fluoxetine treatment.

Methods: Sapap3-knockout (KO) mice were used as a model for OCD-relevant behaviors. Sapap3-KOs and wildtype (WT) littermates (KO: $n = 8$; 5 female; WT: $n = 6$; 3 female) were injected with virus encoding GCaMP6f (AAV5-synapsin-GCaMP6f-WPRE-SV40, titer 1.82x10^12) and implanted with gradient-index lenses to visualize LOFC activity using miniature microscopes. Grooming, reversal learning, and neural activity were measured pre- and post-fluoxetine treatment (18mg/kg, 4 weeks).

Results: In KOs, baseline increases in the number of grooming bouts (WT = 42 +/- 5: KO = 159 +/- 26; $p < 0.003$) and impairments in reversal learning as measured by the number of correct lever presses (WT = 68 +/- 7; KO = 33 +/- 10; $p = 0.02$) improved after fluoxetine treatment (Grooming bouts: KOpre = 159 +/- 26; KOpost = 107 +/- 24; $p = 0.01$; Reversal learning – correct lever presses: KOpre = 33 +/- 10; KOpost = 59 +/- 8; $p = 0.04$). Additionally, KOs displayed distinct patterns of abnormal LOFC activity during grooming and reversal learning, both of which normalized after fluoxetine. During grooming, baseline increases in the percentage of KO LOFC neurons inhibited by grooming (WT = 22.1 +/- 3.6%; KO: 32.1 +/- 1.6%; $p = 0.02$) decreased after fluoxetine (KOpre = 32.1 +/- 1.6%; KOpost = 25.3 +/- 1.8%; $p = 0.001$). During reversal learning, baseline decreases in the strength by which KO LOFC neurons were modulated by the correct lever press (WT = 0.33 +/- 0.03; KO = 0.27 +/- 0.02; $p = 0.01$) improved after fluoxetine (KOpre = 0.27 +/- 0.02; KOpost = 0.30 +/- 0.01; $p = 0.01$). Finally, encoding of reversal learning and compulsive behavior are independent, as reversal learning-associated neurons are distributed randomly amongst grooming-associated neurons (i.e. overlap is what would be expected by chance).

Conclusions: In OCD, the LOFC is disrupted during both compulsive behaviors and reversal learning, yet whether these behaviors share common neural underpinnings is unknown. We find that the LOFC plays distinct and independent roles in compulsive grooming and impaired reversal learning and their improvement with fluoxetine in a mouse model. These findings suggest that LOFC plays separate roles in pathophysiology and treatment of different perseverative behaviors in OCD.

Keywords: Obsessive Compulsive Disorder, In Vivo Calcium Imaging, Reversal Learning

Disclosure: Nothing to disclose.

P439. Effects of Rapid Face Stimuli Presentation on Dorsal and Ventral Visual Stream Connectivity in Body Dysmorphic Disorder

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Background: Body dysmorphic disorder (BDD) is marked by preoccupation with misperceived appearance flaws, causing individuals to believe that they are ugly and disfigured. Consequences are often severe, with high suicide attempt and hospitalization rates. Disturbances of visual information processing in BDD may be core neurobiological contributors to perceptual appearance distortions. Previous neuroimaging studies found abnormally reduced dorsal visual stream (DVS) activity when viewing images filtered to convey only configural/holistic information. These have contributed to a model of imbalances in global vs. local visual processing in BDD. In general, there is evidence that magnocellular pathways in the DVS are tuned to rapid presentation of images, thereby facilitating global/holistic visual processing. Moreover, with higher stimulus frequency/shorter stimulus duration, ventral visual stream (VVS) regions seem to reduce activation magnitude. Accordingly, we conducted a pre-translational investigation to determine the effects of rapid face presentation on DVS and VVS systems, responsible for global and

local processing, respectively. Specifically, we investigated the effects on functional brain connectivity of viewing rapidly-presented, short-duration face stimuli in individuals with BDD and healthy controls, employing dynamic effective connectivity modeling.

Methods: Thirty-eight unmedicated adults with BDD and 29 healthy controls aged 18-40 participated. BDD participants met DSM-5 criteria for BDD, all with face concerns. Symptom severity was quantified with the Yale-Brown Obsessive-Compulsive Scale Modified for BDD (BDD-YBOCS), and the Brown Assessment of Beliefs Scale (BABS), assessing BDD symptoms and insight, respectively. Functional magnetic resonance imaging data were obtained while participants viewed photos of their own faces at different angles for short (125ms, 250ms, 500ms) and long (3000ms) durations.

Data preprocessing was done using fMRIPrep 1.4.0. Fourteen regions of interest (ROIs) in DVS and VVS were selected: 2 ROIs in primary visual cortex (V1) [bilateral calcarine], 6 ROIs in VVS [bilateral inferior occipital gyrus (IOG), fusiform gyrus (FG), and inferior temporal gyrus (ITG)], and 6 ROIs in DVS [bilateral superior occipital gyrus (SOG), inferior parietal gyrus (IPG), and superior parietal gyrus (SPG)]. Hemodynamic deconvolution was performed on the ROIs timeseries to minimize intra-subject hemodynamic response function variability, and to improve the estimation of effective connectivity. Dynamic effective connectivity, a dynamic measure of directional connectivity between pairs of ROIs, was computed at each time point using Kalman-filter based time-varying Granger causality. Twelve intra-hemispheric connections were chosen and divided into 4 categories: 1) VVS Lower (Calcarine to IOG), 2) VVS Higher (IOG to FG; IOG to ITG), 3) DVS Lower (Calcarine to SOG), and 4) DVS Higher (SOG to IPG; SOG to SPG). Linear mixed model was used to analyze the data (fixed factors: group [BDD or healthy controls], duration [125ms, 250ms, 500ms or 3000ms], category [VVS Lower, VVS Higher, DVS Lower or DVS Higher]; random factor: participant). For the follow-up pairwise comparisons, *p*-values were Bonferroni corrected. Spearman correlation was used to determine associations between dynamic effective connectivity and symptom severity measures of BDD-YBOCS and BABS in BDD participants.

Results: BDD individuals exhibited weaker dynamic effective connectivity than controls in DVS Higher during all stimuli presentation durations (125ms: $p = 0.085$; 250ms: $p = 0.004$; 500ms: $p = 0.003$; 3000ms: $p = 0.064$). There were no significant between-group differences for the other connectivity categories (i.e. DVS Lower, VVS Lower, and VVS Higher). Considering the within-group analyses, controls exhibited weaker dynamic effective connectivity for VVS Lower during short duration compared to during long duration (125ms < 3000ms, $p = 0.018$; 500ms < 3000ms, $p = 0.007$). Further, controls showed weaker dynamic effective connectivity for DVS Higher during the 125ms duration compared to during the 250ms duration (125ms < 250ms, $p = 0.018$). BDD showed weaker dynamic effective connectivity for DVS Lower during 125ms duration compared to during 500ms duration (125ms < 500ms, $p < 0.001$). Yet, there were no significant within-group differences for DVS between short and long durations. There was a significant negative correlation between BABS and dynamic effective connectivity during the long (3000ms) duration for DVSHigher (rho = -0.335, $p = 0.040$); those with weaker connectivity in the DVS Higher had poorer insight.

Conclusions: Those with BDD have aberrant, weaker connectivity in higher regions of the dorsal visual stream while viewing their faces. The clinical relevance of this pattern is underscored by the observation that those with poorer insight had weaker DVS connectivity during long duration viewing of their faces. There was no direct evidence that rapid face presentation using the parameters tested in this study enhances DVS connectivity, responsible for global/holistic visual processing. Nevertheless, the within-group results in healthy controls provide promise that rapid face presentation could potentially suppress VVS connectivity, responsible for local/detailed visual processing. For similar effects to occur in BDD, different parameters such as a higher number of stimuli may be necessary and will be explored in future studies.

Keywords: Body Dysmorphic Disorder, Visual Modulation, Dorsal Visual Stream, Ventral Visual Stream, fMRI Effective Connectivity

Disclosure: NOCD, LLC: Consultant (Self)

P440. Obsessive Compulsive Disorder During the COVID-19 Pandemic

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Background: The impacts of the COVID-19 pandemic have been legion in all corners of the globe. The impact on mental health has been well-documented and evidence continues to emerge. Obsessive-compulsive disorder (OCD) affects 1%–3% of the general population [1]. It is a chronic condition and is associated with significantly lower quality of life and major societal costs, [1]. OCD is characterized by unwanted and intrusive thoughts, images, or urges (obsessions) and repetitive behaviours (compulsions) [2]. A significant cluster of OCD symptoms includes fear of contamination, which often triggers ritualized hand-washing and other cleaning rituals in order to neutralize the thought [3]. The primary psychological treatment for OCD is Exposure and Response Prevention (ERP), which, in the case of contamination typically involves exposure to the thought of being contaminated and not engaging in the washing/cleaning rituals [3]. Recent reports have indicated that individuals with OCD may be particularly vulnerable to the mental health impact of the pandemic [4]. The widespread public health demands for increased and 'proper' hand-washing techniques, cleaning, and encouragement to use disinfectants as a result of the COVID-19 pandemic, may have been confusing for patients with OCD [5]. However, survey studies assessing the impact of the COVID-19 pandemic on OCD symptoms have yielded contrasting results, with some studies indicating no change and others indicating a worsening of OCD symptoms. The current cross-sectional study aims to comprehensively examine the psychological impact of the pandemic on individuals who suffered from OCD during the first phase of the COVID-19 pandemic across 3 continents: North America (NA), South America (SA) and Europe.

Methods: Adults aged 16 and over with a diagnosis of OCD, completed an online survey. Surveys were posted between May, 2020 and July, 2021 in English, Italian and Portuguese. After signing an electronic informed consent, completed a questionnaire battery, containing the Obsessive-Compulsive Inventory, Short Version (OCI-R) to assess OCD symptom severity, the Generalized Anxiety Disorder-7 (GAD-7) to assess anxiety severity, Patient Health Questionnaire (PHQ-9) to assess depression severity and the Sheehan Disability Scale to evaluate functional impairment. The impact of social supports and demographic variables on psychiatric symptoms and general behaviour of OCD individuals during the COVID-19 pandemic was also examined. Validated cut scores on the GAD-7, PHQ-9 and OCI-R were used to estimate rates of GAD, Major Depressive Disorder (MDD) and OCD. Contamination symptoms were evaluated using the Washing subscale of the OCI-R [6]; a score of ≥ 8 was considered high.

Statistical methods included descriptive statistics, ANCOVA and logistic regression analyses.

Results: The survey was completed by 417; 75% female, with a mean age of 31.4 ± 11.3. Most were from NA (56%), (SA: 22%; Europe: 22%). The mean scores on all symptom severity scales were above the clinical threshold, including the OCI-R: 28.9 ± 12.4, with no significant differences found between continents on ANCOVA. Most (76%) reported a worsening of their OCD symptoms during the first wave of the pandemic, with increases in both intrusive thoughts and rituals. Nearly 70% reported current OCD treatment (56% medication 43% psychotherapy) and 45% of this group reported increasing their treatment dose or frequency during the pandemic. Predictors of worsening OCD included being younger than 40 ($p < .01$), having comorbid GAD or MDD ($p < .001$) and having high contamination symptoms, ($p < .01$), while being from Europe was associated with decreased risk of worsening symptoms ($p < .05$). The symptom severity scale scores for individuals with high contamination were significantly higher even when controlling for demographic factors, including continental residence via ANCOVA. Those with high contamination also reported significantly less perception of emotional support, more anxiety about contracting and transmitting COVID-19 or a loved one contracting COVID-19.

Conclusions: In this international sample of OCD individuals, most perceived a worsening of their OCD symptoms during the pandemic, as evidenced by the 45% who increased their psychological or pharmacological treatment. Although no differences were found across continents in terms of OCD severity, having European residency was associated with a decreased risk of perceived worsening of symptoms. This may be attributed to the timing of survey, such that COVID-19 cases were decreasing in Europe, while North and South America were at the height of the first wave.

Keywords: OCD, COVID-19, Comorbidity

Disclosure: Biohaven: Contracted Research (Self)

P441. ACC-striatal Projections are Uniquely Positioned to Interact With an Unusually Functionally Diverse Set of Cortical Areas

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Background: Cortico-striatal projections play a central role in providing the basal ganglia with integrated information relevant to action selection and execution, making them a key candidate for the treatment of goal-directed and habitual disorders such as addiction and OCD. Probabilistic models have successfully summarized the spatial topography of this circuitry (Averbeck et al., 2014). For example, projections originating caudally from motor control areas terminate in the dorsolateral striatum, whereas rostral projections from ventromedial prefrontal cortex (PFC) terminate in ventromedial striatum. However, unlike the dorsolateral and ventromedial striatal sectors, the rostral and central striatum receives converging projections from multiple areas. This region is particularly complex and serves as an interface between functionally diverse cortical regions, the orbitofrontal cortex (OFC), ventrolateral PFC (vlPFC), and anterior cingulate cortex (ACC). Although there is an underlying topography to these connections, this alone may not be sufficient to understand the complexity of their interactions. The ACC is a particularly good example with its functional diversity and distributed striatal projections. The goal of this study was to develop a data-driven methodology that works in tandem with the more traditional topographic approach, to uncover additional key organizational principles of cortico-striatal circuitry. We focus specifically on the relationship between ACC-striatal projections to those originating in other areas of PFC.

Methods: We analyzed 30 anterograde injections sites evenly distributed throughout macaque PFC (Averbeck et al., 2014). For each injection, we tabulated the loci of its terminal projections according to a striatal grid composed of 600 μm isotropic voxels. We then computed the similarity of the projections among every pair of cortical injections using a metric that corrects for chance agreement (adjusted rand index), and clustered cortical regions using spectral methods on the resulting similarity matrix. This identified two dominant gradients of cortical organization. We then visually examined the pattern of projections of every cortical cluster. Finally, based on previous qualitative observations that PFC projections to the striatum are mirrored along a dorso-ventral axis in ACC (Tang et al., 2019), we asked whether PFC regions could be similarly organized based on their connectivity to this region. We replicated the clustering analysis using 10 cortico-cortical retrograde injections distributed along the ACC. For each injection site, we computed the proportion of cells that projected to it from each area in PFC, and clustered cortical areas by the similarity of their projection pattern across injection sites.

Results: The primary gradient organized cortical areas based on the location of their terminal projections along a ventromedial to dorsolateral axis in striatum. The secondary gradient captured the size of these projections. In line with classic topographic findings, the combined gradients positioned areas in ventromedial PFC as focally projecting to ventromedial striatum, whereas motor and dorsal PFC areas projected focally to dorsolateral striatum. In contrast, regions in ACC and portions of OFC had broad projections in central striatum, displaying partial overlap with the two more focal clusters. We found no significant relationship among injection volume, size of terminal projections, or the mean similarity of a given area (all pairwise correlations $p > 0.1$). Clustering performed on retrograde ACC injections resulted in a comparable primary gradient in PFC; motor and limbic areas primarily projected to dorsal and subgenual ACC, respectively, with remaining areas mostly projecting to rostral ACC.

Conclusions: In this project, we used a data-driven approach to further characterize the underlying organizational principles of cortico-striatal circuitry. We used this approach and identify that ACC stands out in its connectivity within this network. We show that projections to striatum are organized by the size of their terminal fields in addition to their rostro-caudal location. The combination of these axes of organization distinguished ACC as an area with particularly widespread connections to the striatum. These results were not explained by methodological issues, such as injection volume. Further, we note commonalities between the organization of connections between prefrontal areas and both the ACC and the striatum. Based on these findings, we conclude that the ACC integrates information from diverse functional areas and relays this information to other functional groups via its relatively large projections to striatum. The mirrored cortico-cortical organization observed here raises the possibility that an individual's topography of cortico-striatal projections, which can be difficult to precisely map using neuroimaging methods, could be approximated using cortico-cortical connectivity with ACC. This possibility could facilitate the identification of cortical targets for transcranial stimulation. Future work will test these findings using networks derived from diffusion and functional MRI in human and non-human primates.

Keywords: Neuroanatomy, Cortico-Striatal Connectivity, Anterior Cingulate Cortex (ACC), Connectivity Gradients

Disclosure: Nothing to disclose.

P442. Similar Valence Processing Alterations Associated With Compulsive Behavior in SAPAP3 Knockout Mice and Human OCD

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Background: Abnormalities in valence processing – the processing of aversive or appetitive stimuli – may be an underrecognized component of obsessive-compulsive disorder (OCD). Independent experimental paradigms have suggested disturbance of emotional valence systems in OCD, yet there is a dearth of studies assessing both negative and positive valence processing in clinical studies of OCD patients, either at baseline or in response to therapeutic interventions. Additionally, preclinical rodent models are critical for treatment discovery in OCD, yet investigations examining whether rodent models of compulsive behavior similarly show alterations in valence systems have been limited. In this study, we assessed valence processing in both human OCD patients and a preclinical rodent model of compulsive behavior, the SAPAP3 knockout (KO) mouse model.

Methods: In OCD patients ($N = 41$), we utilized a standardized, computer-based cognitive testing platform to examine both explicit (conscious) and implicit (nonconscious) processing of fear-related facial expressions (negative valence) and socially-rewarding happy expressions (positive valence). Subject-level performance was quantified with reference to age-, biological sex-, and years of education-matched norms derived from a previously acquired healthy norm cohort ($N = 1317$) and assessed in relation to clinical measures including the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) and Depression Anxiety Stress Scales (DASS). In the SAPAP3 KO mouse model of compulsive behavior, we used auditory fear conditioning and extinction in SAPAP3 KO mice ($N = 11$) and wild-type (WT) littermate controls ($N = 11$) to assess alterations in negative valence processing. We additionally used reward-based operant conditioning in SAPAP3 KO mice ($N = 8$) and WT littermate controls ($N = 11$) to assess alterations in positive valence processing. Both sexes were included in both the human and rodent studies.

Results: OCD patients were found to have speeded (Wilcoxon Rank Sum Test, $p = 0.025$) and more accurate (Wilcoxon Rank Sum Test, $p < 0.001$) identification of fearful compared to happy facial expressions on the explicit emotional processing task. On the implicit emotional processing task, OCD patients were significantly less accurate at identifying previously seen faces when implicitly influenced by fearful expressions compared to happy expressions (Wilcoxon Rank Sum Test, $p < 0.0001$). Speeded explicit responses to fearful expressions were correlated with greater subjective anxiety (Spearman's correlation, rho = 0.46, $p = 0.003$) while decreased facial recall accuracy under the implicit influence of fearful relative to happy expression correlated with OCD severity by Y-BOCS (Spearman's correlation coefficient, rho = -0.38, $p = 0.015$). SAPAP3 KO mice showed enhanced fear learning and impaired fear extinction as evidenced by increased freezing compared to WT mice during both fear conditioning (Two-way Repeated Measures ANOVA, Time x Genotype $p < 0.05$, Time $p < 0.0001$, Genotype $p = 0.0001$) and extinction (Two-way Repeated Measures ANOVA, Time x Genotype $p < 0.01$, Time $p < 0.0001$, Genotype $p < 0.0001$). In reward-based operant conditioning, SAPAP3 KO mice were significantly slower than wild-type mice at acquiring positive-reinforced nose-poke behavior (Two-way Repeated Measures ANOVA, Genotype x Training Day $p < 0.0001$, Training Day $p < 0.0001$, Genotype $p < 0.0001$) and were unable to maintain goal-directed responding for rewards (Two-way Repeated Measures ANOVA, Genotype x Training Day $p < 0.0001$, Training Day $p < 0.0001$, Genotype $p < 0.0001$).

Conclusions: We find that OCD patients and SAPAP3 KO mice both show evidence of enhanced negative valence processing and impaired positive valence processing. Our results reveal similar valence processing abnormalities in OCD patients and a preclinical rodent model of compulsive behavior, which suggest valence processing alterations as novel therapeutic targets across a translational research spectrum.

Keywords: Negative Valence, Positive Valence, OCD, SAPAP3 KO Mice

Disclosure: Nothing to disclose.

P443. Tolcapone in Obsessive Compulsive Disorder: A Randomized Double-Blind Placebo-Controlled Crossover Trial

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Background: Despite the availability of evidence-based treatments for obsessive-compulsive disorder (OCD), not all patients experience sufficient benefit or are able to tolerate them. Tolcapone is a catechol-o-methyl-transferase (COMT) enzyme inhibitor that augments cortical dopaminergic transmission. Conduct a proof of concept study to examine whether a COMT inhibitor would reduce OCD symptoms to a greater extent than placebo.

Methods: We conducted a randomized, placebo-controlled, double-blind cross-over trial in adults with OCD ($N = 20$). This was a fully counter-balanced, double-blind, placebo-controlled cross-over design in which participants received EITHER tolcapone 100mg twice daily for two weeks, followed by one-week washout, followed by placebo twice daily for two weeks; OR placebo twice daily for two-weeks, followed by one-week washout, followed by tolcapone 100mg twice daily for two weeks. The order of treatment was randomized. The washout period was intended to prevent possible carryover effects that might compromises the assessments and conclusions. The study had low risk of bias using the Cochrane criteria. Because tolcapone at these lower doses has few side effects, a cross-over design was considered as side effects should not jeopardize the blinding. The analysis followed Intent-To-Treat principles, with Last-Observation-Carried-Forward for dropouts and missing data. The analytic approach was determined prior to data unlock. Changes in YBOCS total scores for placebo treatment and tolcapone treatment were compared using paired sample t-tests, which constituted the primary analysis. This method was appropriate because the design was fully counter-balanced and randomized. Changes in total depression and anxiety symptom scores, and disability, were examined, again using paired t-tests, as secondary analyses. Statistical significance was defined as $p < 0.05$ two-tailed.

Results: When taking tolcapone, participants experienced a mean decrease in their total YBOCS scores of -4.24 (SD = 6.20), which differed significantly from the change observed under placebo treatment of -1.10 (SD = 4.71) ($t = 2.194$, df = 19, $p = 0.0409$). The mean percentage decreases in the total YBOCS scores for the entire sample over the corresponding two-week periods were 16.4% for tolcapone and 3.6% for placebo.

Conclusions: This randomized, placebo-controlled cross-over trial indicated that two-week treatment using tolcapone (100mg twice daily) was associated with significant symptomatic improvement in OCD as compared to two-week administration of placebo. The likely mechanism is that tolcapone enhanced cortical dopamine transmission and therefore top-down executive control over habitual patterns of behavior. The finding of significant

separation from placebo after two weeks may suggest that longer studies using tolcapone for OCD may be worthwhile to see if even greater benefit is possible. This small study was intended merely to see if tolcapone produced a signal and therefore would merit further study.

Keywords: COMT Inhibitor, Obsessive-Compulsive Disorder (OCD), Pharmacotherapy

Disclosure: Otsuka, Biohaven: Grant (Self)

P444. Different Brain Activation in Patients With OCD Compared to Healthy Participants During Conditioning: An fMRI Fear Extinction Paradigm

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Background: Patients with obsessive-compulsive disorder (OCD) have shown a fear processing pattern characterized by partial or total failure to recall extinction and hypoactivation of the ventromedial prefrontal cortex during early recall. Also, increased fear renewal was observed in a drug-free sample of OCD patients compared with their healthy counterparts. However, the brain activation underlying those processes is not well established, especially during the renewal phase, and studies investigating patterns of brain activation during fear conditioning/extinction tasks are warranted. Our main objective was to compare the behavior performance and brain activation during fear processing between adult, symptomatic and drug-free patients with OCD and healthy participants (HP) in a fear conditioning/extinction paradigm. We expected to replicate previous findings regarding worse extinction retention in a sample of drug-free OCD patients.

Methods: We measured skin-conductance (SCR) and blood oxygenation level-dependent response (BOLD) obtained in a 3T Philips Achieva scan in 37 drug-free adult OCD patients with current symptoms of moderate severity and 32 HP matched according to biological sex and age. Participants underwent a two-day, event-related, fear-conditioning fMRI paradigm comprising four different phases: conditioning and extinction (day 1), and recall and renewal (day 2). The tactile aversive (unconditioned) stimulus was an electric shock.

The paradigm was developed by Milad et al. (2013): there are two contexts (pictures of different rooms): one for conditioning and renewal, and another for extinction and recall. In these contexts, the visual cues (lights in one of the three basic colors) are presented. Cues are classified as conditioned to shock (CS+) or not conditioned to shock (CS-). There are two types of CS + , one of them is extinguished (CS + E) while the other is not (CS + U). For analytical purposes, the CS-s were divided into CS-E when paired with CS + E or CS-U when paired with CS + U, even though both CS-s correspond to the same stimulus.

The fear response (SCR) was measured through electrodermal activity (Biopac® Systems) and was determined as the difference between the maximum skin conductance level (SCL) reached during the 6s of CS presentation and the minimum skin conductance level during the 2s prior to context presentation.

Whole-brain BOLD contrasts included: the average maps for each group on each phase; a between-groups two-sample t-test (for each phase), and comparisons between the contexts in all four phases using two-way ANOVA, evaluating the effects of phase and its interaction with group comparing the phases two-by-two. Probability maps were generated using a stringent voxel threshold of $Z > 3.1$ and a (corrected) cluster $p < 0.05$.

Patients and HP were evaluated by experienced psychologists or psychiatrists that collected demographic information and applied the SCID-IV, supplemented with modules for tic and impulse control disorders. In addition, OCD patients were evaluated with the following clinical structured instruments: the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS), the Dimensional Y-BOCS, the Beck Depression and Anxiety Inventories.

Results: There were no significant group differences regarding SCR signal quality ($X2 = 1.45$, $p = 0.23$) or response congruence with stimuli ($X2 = 0.02$, $p = 0.88$). Both groups presented effective fear conditioning, and there were no between-group differences regarding the prevalence of CS learning ($X2 = 2.05$, $p = 0.15$). Across all phases, OCD patients presented higher SCR than HP for all types of stimuli.

Significant group differences were detected in fMRI analyses during conditioning, in the contrast between late conditioned stimuli (last four presentations of the CS + E) and unconditioned stimuli (CS-): a cluster encompassing inferior frontal, superior temporal, and pre and post-central gyri ($Zmax = 4.54$, $p < 0.0001$). There were also significant between-group differences in response to late CS + E (compared with baseline) during conditioning in regions of the right hemisphere, presented in three clusters, including the pre and post-central gyri and inferior frontal gyrus ($Zmax = 4.14$, $p = 0.001$), the insular cortex, and putamen ($Zmax = 4.17$, $p = 0.009$), and the lingual gyrus ($Zmax = 4$, $p = 0.016$). In addition, during late extinction, OCD patients presented higher activation of the left fusiform gyrus (CS + E late: $Zmax = 4.04$, $p = 0.014$). There were no significant differences between groups in early phases of conditioning or extinction, nor in early or late phases of recall or renewal.

Conclusions: Different patterns of brain activation during fear conditioning could explain differences in behavior between OCD patients and HP. Also, regions associated with OCD psychopathology and recognition of painful and visual emotional stimuli may participate in fear learning and extinction and may affect the expression of fear responses during recall and renewal. These findings underscore the relevance of the interaction between OCD psychopathology and fear processing. Future studies should investigate the relevance of contextual information in the processes of fear learning, extinction recall, and fear renewal in OCD patients.

Keywords: Obsessive-Compulsive Disorder (OCD), Functional Neuroimaging, Fear Physiology, Fear Conditioning and Extinction

Disclosure: Nothing to disclose.

P445. The Genetic Architecture of Obsessive-Compulsive Disorder: Alleles Across the Frequency Spectrum Contribute Liability to OCD

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Background: Obsessive-compulsive disorder (OCD) is known to be substantially heritable; however, the contribution of common genetic variation across the allele frequency spectrum to this heritability remains uncertain. We use two new, homogenous cohorts to estimate heritability of OCD from common genetic variation and contrast results with prior studies.

Methods: The sample consisted of 2090 Swedish-born individuals diagnosed with OCD and 4567 controls, all genotyped for

common genetic variants, specifically >400,000 single nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) ≥ 0.01. Using genotypes of these SNPs to estimate distant familial relationships among individuals, we estimated heritability of OCD, both overall and partitioned according to MAF bins.

Results: We estimated narrow-sense heritability of 29% (SE = 4%). The estimate was robust, varying only modestly under different models. Contrary to earlier reports, however, SNPs with MAF between 0.01 and 0.05 accounted for 10% of heritability and estimated heritability per bin roughly follows expectations based on a simple model for SNP-based heritability.

Conclusions: These results indicate that common inherited risk variation (MAF ≥ 0.01) accounts for most of the heritable variation in OCD. SNPs with low MAF contribute meaningfully to the heritability of OCD and the results are consistent with expectation under the "infinitesimal model," where risk is influenced by a large number of loci across the genome and across MAF bins.

Keywords: Genetic Architecture, SNP Variation, Rare Genetic Variation

Disclosure: Nothing to disclose.

P446. Developmental Impact of Glutamate Transporter Overexpression on Dopaminergic Neuron Activity and Stereotypic Behavior

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Background: Obsessive-compulsive disorder (OCD) is a disabling condition that often begins in childhood. Genetic studies in OCD have pointed to SLC1A1, which encodes the neuronal glutamate transporter EAAT3, with evidence suggesting that increased expression contributes to risk. In mice, midbrain Slc1a1 expression supports repetitive behavior in response to dopaminergic agonists, aligning with neuroimaging and pharmacologic challenge studies that have implicated the dopaminergic system in OCD. These findings suggest that Slc1a1 may contribute to compulsive behavior through altered dopaminergic transmission; however, this theory has not been mechanistically tested. To examine the developmental impact of Slc1a1 overexpression on compulsive-like behaviors, we therefore generated a novel mouse model to perform targeted, reversible overexpression of Slc1a1 in dopaminergic neurons.

Methods: Slc1a1-tetO mice were crossed to the Th-tTA line to obtain Slc1a1-Th-overexpressing (OE) and control mice. EAAT3 expression was regulated by doxycycline-supplemented chow (400mg/kg) and was quantified using real-time qRT-PCR and immunoblots. Mice were evaluated for baseline anxiety-like and repetitive behaviors (elevated plus maze, light-dark emergence, and marble-burying), AMPH-induced locomotion and stereotypy, and for D1-agonist (SKF-38393)-induced grooming behavior. Dopamine transmission was evaluated using single unit dopamine neuron recordings in anesthetized mice and via fiber photometry using the dLight1.1 dopamine sensor in behaving mice. Adult male and female mice and littermate controls were used for all experiments.

Results: Mice with life-long overexpression of Slc1a1 showed a significant increase in amphetamine-induced stereotypy (8.0 mg/kg, 3-way RM ANOVA; drug x genotype interaction, F (1, 38) = 13.89, $P = 0.0006$; genotype, F (1, 38) = 9.839, $P = 0.0033$, n = 11-10) and hyperlocomotion (3.0 mg/kg, curve-fit; F (4, 370) = 16.33, $P < 0.0001$, n = 11-10). Single-unit recordings demonstrated that Slc1a1 overexpression was associated with increased firing of dopaminergic neurons (spikes fired in bursts, unpaired t (10) = 4.684, $P = 0.0009$, firing rate, unpaired t (10) = 3.808, $P = 0.0034$, n = 6-6). Furthermore, dLight1.1 fiber photometry showed that these behavioral abnormalities were associated with increased dorsal striatum dopamine release both at baseline (unpaired t (9) = 3.379, $P = 0.0081$, n = 5-6) and following amphetamine (3.0 mg/kg) (unpaired t (8) = 5.065, $P = 0.0010$, n = 5-5). In contrast, no impact of overexpression was observed on anxiety-like behaviors or SKF-38393-induced grooming. Importantly, overexpression solely in adulthood failed to recapitulate these behavioral phenotypes, suggesting that overexpression during development is necessary to generate pathology. However, doxycycline-induced reversal of Slc1a1/EAAT3 overexpression in adulthood normalized both the increased dopaminergic firing and AMPH-induced responses.

Conclusions: Our data suggest that alterations in dopaminergic transmission during development may be a mechanism underlying the effects of Slc1a1/EAAT3 overexpression, warranting additional work to understand the dynamics of glutamatergic and dopaminergic signaling in basal ganglia circuits during early developmental periods. Our electrophysiological and behavioral data following reversal of EAAT3 overexpression suggest that EAAT3 inhibitors should be evaluated as a potential treatment for basal ganglia-mediated repetitive behavior.

Keywords: Glutamate Transporter (EAAT3), Dopamine, Sensitive Period, Fiber Photometry, Amphetamine

Disclosure: Nothing to disclose.

P447. EFP-Neurofeedback in Adolescents With Borderline Personality Disorder: A Proof-Of-Concept Trial

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Background: Neurofeedback can teach patients to regulate brain activity. Targeting the amygdala for neurofeedback training to improve emotion regulation could be an effective adjuvant treatment for patients suffering from Borderline Personality Disorder (BPD). The scalability of functional magnetic resonance imaging (fMRI) amygdala-neurofeedback could be significantly increased by advancing to fMRI-informed electroencephalography (EEG) signatures such as the amygdala Electrical Fingerprint (EFP). The aim of this study is to assess the feasibility and validity of amygdala-EFP neurofeedback-training in adolescent BPD patients undergoing therapy.

Methods: 28 adolescents with BPD (14 in the treatment group) undergoing residential Dialectical-Behavior-Therapy (DBT) were assessed in this open-label neurofeedback-study. EFP provides an EEG surrogate of deep brain activation optimized to predict the amygdala Blood Oxygenation Level dependent (BOLD) signal. The treatment group received 10 EFP-neurofeedback training sessions over 5 weeks, while the treatment-as-usual control group did not receive any training. An acoustic brain-computer interface was used for training, where the subject voluntarily decreased the volume of a melody representing EFP activation. The study began and ended with an MR-Scan that included brief amygdala-BOLD-neurofeedback to analyze improvement of amygdala regulation. In MR sessions, feedback was given visually via a thermometer-like display. Simultaneous EEG-fMRI data were recorded in the last MR-session to determine whether the amygdala-EFP predicts the amygdala-BOLD signal. FMRI data from the pre- and post-training session were used to quantify transfer of learned EFP-regulation to

veridical amygdala-BOLD regulation (i.e., target engagement test). The simultaneous EEG-fMRI data from the last session were used to quantify correlation of the EFP with the fMRI BOLD signal (i.e., validation of EFP). FMRI data were preprocessed with the open software fMRIPrep. Statistical parametric modeling analysis was applied to analyze amygdala down-regulation in the a-priorily defined amygdala region of interest. The fMRI analysis to validate the EFP was done with family-wise error correction with $p < 0.05$ at the voxel-level in the whole brain. Self-report measures (Toronto Alexithymia Scale, Beck Depression Inventory, Borderline Symptom List short version, Affect Lability Scale) and participant drop-out were assessed for clinical effects and feasibility assessment. The study is registered at clinicaltrials.gov, NCT03964545.

Results: Significant improvements in both groups were found for the Beck Depression Inventory ($F = 8.99$, $p = 0.006$) and Affect Lability Scale ($F = 7.08$, $p = 0.014$), but not for the Toronto Alexithymia Scale and the Borderline Symptom List ($p > 0.05$). Study drop-out was 40%, of which 60% were due to leaving therapy early. Final MRI- and EEG-analysis results were on the way at abstract submission and will be presented on the conference poster.

Conclusions: Attrition due to premature discharge from residential treatment is important to consider for future neurofeedback trials in this setting. As DBT is an effective treatment, clinical improvements exceeding the therapy effect are difficult to achieve with adjuvant interventions. Ideally, outcomes for adjuvant neurofeedback will be defined on a mechanistic level rather than as psychopathology measures, as the latter do not leave much space for improvement on top of DBT. The verification of amygdala-targeting with EFP in BPD is critical to advance to a randomized clinical trial.

Keywords: Amygdala, Neurofeedback, Borderline Personality Disorder

Disclosure: Nothing to disclose.

P448. Prefrontal Cortex Engagement During an fMRI Task of Emotion Regulation as a Potential Predictor of Antidepressant Treatment Response in Borderline Personality Disorder

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Background: Borderline personality disorder (BPD) is a severe mental illness affecting over four million individuals in the United States. Emotion dysregulation is a core feature underlying BPD. While prior work has examined the neural underpinnings of emotion dysregulation in BPD, little is known about how this relates to treatment response. The present study examines how baseline blood oxygen level dependent (BOLD) activation during a functional magnetic resonance imaging (fMRI) task of emotion regulation is related to antidepressant response following six months of Dialectical Behavior Therapy (DBT) or Selective Serotonin Reuptake Inhibitor (SSRI) treatment.

Methods: Individuals with BPD reporting a history of suicidal behavior or suicidal ideation ($N = 37$) underwent an fMRI task on a 3T GE scanner in which they were asked to either distance (i.e., downregulate their emotional response) or immerse (i.e., freely experience emotions) when recalling aversive personal memories. Patients were then randomized to six months of either DBT ($N = 17$) or SSRI ($N = 20$) treatment. Baseline and post-treatment measures of depression severity, affect lability and suicidal ideation were obtained. BOLD signal was analyzed using FSL and analyses of brain-wide voxel effects were thresholded at voxel ($p < 0.001$) and cluster ($p < 0.05$).

Results: When including treatment type as a covariate and controlling for baseline depression scores, neural activation during distancing was associated with treatment change in depression severity. In the SSRI group, greater activation in a cluster containing the orbital frontal cortex (OFC), frontal pole, and inferior frontal gyrus was associated with lower BDI scores at 6 months, indicating more improvement, while less activation during distancing was associated with higher BDI scores, indicating poorer outcomes. The opposite was seen in the DBT group, with greater activation in this cluster associated with less improvement.

Conclusions: Prior research suggests engagement of prefrontal regions during emotion regulation is associated with more effective regulation and greater cognitive control. The findings of this study indicate that individuals with greater prefrontal engagement at baseline experience a greater reduction in depressive symptoms when receiving SSRI treatment but the opposite for DBT. Further research is needed to understand how neural activation during emotion regulation may predict whether fluoxetine or DBT is a more effective antidepressant treatment for individual patients with BPD. The opposite direction of the relationships suggest that this effect is related to treatment response and not a nonspecific correlate of improvement independent of treatment type.

Keywords: Functional MRI (fMRI), Borderline Personality Disorder, Psychotherapy, Antidepressants, Prefrontal Cortex

Disclosure: Nothing to disclose.

P449. Protein Aggregation in a Subset of Patients With Schizophrenia

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Background: Schizophrenia (SCZ) is a complex disorder, which complicates the understanding of the mechanisms underlying SCZ. Genetic mutations and environmental stressors can lead to abnormal proteins. One way to conceptualize a disorder as complex as SCZ is to identify a cellular process that could have broad implication in neuronal functioning that is tied to the way neurons handle abnormal proteins in general. Proteostasis and the ubiquitin proteasome system (UPS) are central to proper cellular functioning via control of protein synthesis, folding, protein interactions, trafficking and degradation. Abnormalities in this process would lead to improperly regulated proteins, which could be diverse. This could help explain the multitude of cellular dysfunction and symptoms seem in SCZ. The failure of the cell to handle abnormal proteins results in protein insolubility. Ubiquitin is a marker for protein insolubility, and is found in the insoluble fraction of brain disorders with protein insolubility. Recently, attention has focused on the role ubiquitin and the UPS has in SCZ, with studies suggesting dysregulation at the genetic and protein level. The UPS and protein insolubility may provide the potential opportunity to understand how environmental stressors and genetic mutations relate to SCZ. Furthermore, sequestration of critical proteins that are prone to aggregation can lead to neuronal dysfunction.

Methods: Prefrontal cortex or superior temporal gyrus from autopsy brains obtained from the University of Pittsburgh, University of Texas, and Harvard Brain Banks, and olfactory neurons obtained from living subjects from the Johns Hopkins Schizophrenia Center were processed using a fractionation protocol designed to extract the proteins into insoluble and

soluble fractions. Levels of protein insolubility and ubiquitin reactivity, markers for protein aggregation, were quantified after SDS-PAGE separation followed by Coomassie staining and Western blot analysis and normalized to total homogenate protein. Mass spectrometry was performed in order to identify the protein composition in the insoluble fraction. Gene Ontology Enrichment Analysis and Ingenuity Pathway Analysis were used to assess the potential biological relevance of the detected proteins in the insoluble fraction.

Results: A subset of patients with schizophrenia showed an increase in markers for protein

aggregation, specifically protein insolubility and ubiquitination. Mass spectrometry of the insoluble fraction revealed that cases with increased insolubility and ubiquitination showed similar pattern of peptide clustering by principal component analysis. The proteins that were significantly altered in the insoluble pellet were enriched for proteins related to the UPS at every step in the process, as well as relating to axon target recognition and nervous system development and function. Furthermore, protein insolubility was demonstrated in a subset of patient's olfactory neurons, providing the potential for clinical correlations.

Conclusions: This study demonstrates the pathological process of protein aggregation in a subset of patients with schizophrenia. Understanding the mechanisms related to protein aggregation in schizophrenia could lead to a better understanding of the disease process and novel therapeutic targets.

Keywords: Schizophrenia, Protein Aggregation, Ubiquitination, Proteostasis, Ubiquitin Proteasome System

Disclosure: Nothing to disclose.

P450. Genetic Moderators of Cognitive Predictors of Employment Status in Patients With Schizophrenia

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Background: Among a number of factors that influence functional outcomes in patients with schizophrenia, the degree of cognitive impairment an individual manifests has been shown to be a key predictor. Prior work has demonstrated that IQ, a general measure of cognitive ability, is related to poor functional outcomes (including employment) in schizophrenia, but it remains unresolved whether this relationship might be driven by deficits in specific cognitive domains. Six cognitive domains (verbal memory, NBack working memory, visual memory, processing speed, card sorting/executive function, and span working memory) have been found to be separable, but correlated, factors that characterize somewhat distinct aspects of cognitive deficits in patients. Here, we examine whether and which of these factors predict employment outcomes. Additionally, as schizophrenia is highly heritable, and genetic factors play a role in heterogeneity of the disorder, we also investigated whether differences in polygenic risk levels moderate the relationship between cognition and employment outcomes. Understanding reliable and more specific predictors of functional outcome in the patient population is a necessary step toward developing priorities for cognitive treatment targets, and if genetic markers are influential then these may be an important step towards personalized medicine approaches.

Methods: Patients with schizophrenia spectrum disorders ($n = 129$; 98 with schizophrenia, 19 with schizoaffective disorder, and 12 with a psychotic disorder not otherwise specified; 41 female, 88 male; mean age 33.1 +/- 10.6 years) participated in an extensive research visit at the National Institute of Mental Health Intramural Research Program that included neuropsychological and genetic testing. Individuals were recontacted an average 8.8 (+/-4.3) years later to obtain information on functional outcome, including employment status (whether employed or unemployed). Logistic regressions using Wald chi-square testing (excluding retired individuals, $n = 4$) were used to test whether cognitive measures, including 'g' (a measure of general cognition), IQ (as measured by Wechsler Adult Intelligence Scale), and 6 cognitive domains (listed above; each factor being calculated based on patients' performance on a battery of cognitive tests at their initial visit), were predictors of patients' employment status at follow-up. Additionally, in a subset of patients ($n = 104$), we derived polygenic scores for schizophrenia risk (PGSscz) and cognitive ability (PGScog) based on summary statistics from published genome-wide association studies. Logistic regressions were repeated after including polygenic scores in the models, which identified whether genetic predisposition moderated each cognitive predictors' effects on employment status.

Results: At follow-up, 43.8% of patients were employed. Employment at follow-up was associated positively with g ($n = 111$, $B = 0.693$, SE = 0.320, Wald = 4.709, $p = 0.030$), IQ ($n = 112$, $B = 0.061$, SE = 0.022, Wald = 7.492, $p = 0.006$), and verbal memory ($n = 111$, $B = 0.475$, SE = 0.214, Wald = 4.925, $p = 0.026$) such that individuals with higher scores on these cognitive measures were more likely to be employed. Other cognitive subdomains were not significantly associated with employment status. When considering whether PGSscz is a potential moderator of cognitive performance effects, we found an NBack working memory-by-PGSscz interaction effect ($n = 81$, $p = 0.044$) on employment status as well as a processing speed-by-PGSscz interaction effect ($n = 92$, $p = 0.038$) on employment status. The processing speed-by-PGSscz interaction effect indicated that, in patients with higher PGSscz, higher processing speed scores were associated with a higher likelihood of employment. This was not the case in the patients with lower PGSscz where there was no meaningful difference between processing speed scores and whether or not someone was employed at follow-up. The NBack working memory-by-PGSscz interaction effect was similar in magnitude, but harder to interpret. However, the PGScog was not found to moderate the relationship of cognitive measures and employment status.

Conclusions: Overall, we found direct relationships between g, IQ, verbal memory and employment status in patients with schizophrenia spectrum disorders. While genetic risk for schizophrenia (PGSscz) moderated some cognitive predictors of employment outcomes, this was unexpectedly not the case for cognitive genetics (PGScog). These results suggest that therapies targeting broad cognitive ability may influence employment outcomes and that the specific domain of verbal memory might also impact real-life functional outcomes and constitute a secondary therapeutic target. Furthermore, the possibility that schizophrenia genetic risk background may play a role in moderating cognitive influences on employment status merits further investigation.

Keywords: Cognitive Impairment Associated With Schizophrenia, Functional Outcomes, Polygenetic Risk Score

Disclosure: Nothing to disclose.

P451. The Schizophrenia-Associated Variant in SLC39A8 Alters N-Glycosylation in the Mouse Brain

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Background: Schizophrenia is a severe mental illness with strong heritability, and advances in genetics have started to unravel the complex molecular underpinnings of this disorder. GWAS have identified over 250 loci linked to schizophrenia, though most are in the non-coding region of the genome. The most significantly

associated coding variant in schizophrenia GWAS is rs13107325 in SLC39A8, resulting in a missense mutation (A391T) in the eponymous manganese (Mn2+) transporter. Mn2+ transport by SLC39A8 is critical for glycosylation, the enzymatic attachment of carbohydrates to proteins and lipids, and we have previously shown that human carriers of A391T have reduced serum manganese, altered plasma glycosylation, and brain MRI changes consistent with altered metal transport. In this study, we employed a knock-in mouse model homozygous for A391T to investigate molecular changes in the brain resulting from the schizophrenia associated mutation, focusing specifically on the protein glycosylation pathway.

Methods: Homozygous knock-in A391T mice and controls from both sex were included in our study. Brain tissue was harvested from four regions (cortex, hippocampus, striatum, and cerebellum) at 12-weeks of age for analysis. MALDI-TOF MS glycomics of Asn (*N*-) and Ser/Thr (O-) linked glycans was performed on all four brain regions in groups of $N \geq 4$ for both sexes, with results presented as the percent abundance of different glycan categories between regions from each genotype. Confirmatory glycan quantifications were performed using two separate techniques following derivatization using F-MAPA and sialic acid (NANA) kits. Full RNA sequencing was performed from cortex and cerebellum of A391T and control mice ($N = 4$/group) at the MGH NextGen Sequencing Core using standard protocols, resulting in quantification of over 14,000 transcripts from each region. Quantitative *N*-glycoproteomics of cortex was performed from A391T and controls ($N = 5$/group) following multi-lectin affinity purification, removal and tagging of N-glycopeptides using PNGase F and TMT, and analysis using a Thermo Orbitrap nano-electrospray MS.

Results: N-Glycosylation was most impaired in A391T cortex, with nearly half of the glycan categories showing significant differences ($p < 0.05$). Quantitative analyses confirmed a reduction of glycan concentrations in the cortex of A391T mice (Control 3.24 vs A391T 2.45 nmol N-glycans/ µg protein, $p = 0.043$). RNAseq analysis showed negligible variation between genotypes, consistent with changes in the activity of glycosylation enzymes rather than gene expression. Quantitative glycoproteomics showed that nearly one third of detected glycopeptides were expressed at different levels in the cortex, including members of several pathways previously implicated in schizophrenia such as cell adhesion molecules and neurotransmitter receptors. Single cell expression data of transcripts from the altered glycopeptides suggests that the proteins originate from multiple cell types across the cortex.

Conclusions: The A391T mutation associated with increased risk for schizophrenia causes small but significant changes in protein N-glycosylation in the mouse cortex, consistent with the predicted effect size of a common variant. These findings provide a mechanistic link between a risk allele and potentially reversible biochemical changes in the brain, furthering our molecular understanding of the pathophysiology of schizophrenia and representing a novel opportunity for therapeutic development targeting the glycosylation pathway.

Keywords: Glycosylation, Schizophrenia (SCZ), SLC39A8

Disclosure: Nothing to disclose.

P453. Adiposity in Schizophrenia: A Systematic Review and Meta-Analysis

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Background: Although a relationship between schizophrenia (SCZ), antipsychotic (AP) medication, and metabolic dysregulation is now well established, the effect of adiposity is less well understood. By synthesizing findings from imaging techniques that measure adiposity, our systematic review and meta-analysis (PROSPERO CRD42020192977) aims to determine the adiposity-related effects of illness and treatment in this patient population.

Methods: We searched MEDLINE, EMBASE, PsychINFO and Scopus for all relevant case-control and prospective longitudinal studies from inception until February 2021. Measures of adiposity including percent body fat (%BF), subcutaneous adipose tissue (SAT), and visceral adipose tissue (VAT) were analyzed as primary outcomes.

Results: Our search identified 29 articles that used imaging methods to quantify adiposity among patients with SCZ spectrum disorders. Analyses revealed that patients have greater %BF (mean difference (MD) = 3.09%; 95%CI: 0.75 to 5.44), SAT (MD = 24.29 cm^2; 95%CI: 2.97 to 45.61) and VAT (MD = 33.73 cm^2, 95%CI: 4.19 to 63.27) compared to healthy controls. AP treatment was found to increase SAT (MD = 31.98 cm^2; 95%CI: 11.33 to 52.64) and VAT (MD = 16.30 cm^2; 95%CI: 8.17 to 24.44) with no effect on %BF. However, change in %BF was higher for AP-free/AP-naïve patients compared to treated patients.

Conclusions: Our findings indicate that patients with SCZ spectrum disorders have greater adiposity than healthy controls which is increased by AP treatment. Young, AP-naïve patients may be particularly susceptible to this effect. Future studies should explore the effect of specific APs on adiposity and its relation to overall metabolic health.

Keywords: Schizophrenia Spectrum Disorders, Adiposity, Antipsychotic Agents, Subcutaneous Fat, Visceral Fat

Disclosure: Nothing to disclose.

P454. A Pilot Study of Brain Activity Associated With the Rubber Hand Illusion in Youth With Schizophrenia Spectrum Disorders

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Background: Self-disturbance, referring to subjective alterations in the sense of ownership of one's body, thoughts, and actions, along with deficits in self-monitoring, is hypothesized to be a core disturbance in schizophrenia [1]. It is notable for being present during the early stages of the disorder and may be associated with increased risk for non-affective psychosis [2]. The neurophysiologic mechanisms underlying self-disturbance are not yet well understood. The rubber hand illusion (RHI) experiment has been used extensively in studies of visuo-tactile integration in the multisensory literature, and more recently to examine the sense of body ownership (feeling that one's own body belongs to oneself). Susceptibility to the RHI has been associated with impaired body ownership mechanisms in patients with schizophrenia relative to controls, but there is very little data regarding brain activity during the RHI in individuals with schizophrenia spectrum disorders (SSD), and none in early psychosis. We therefore carried out a pilot study to examine pathophysiology underlying self-disturbance in schizophrenia by using magnetoencephalography (MEG) to compare brain activity in youth with SSD compared to healthy controls during the experience of self-disturbance induced by the RHI.

Methods: Participants included youth aged 14-25 with SSD with symptom onset within the past 5 years and age matched HC. The RHI task included three conditions in randomized order, each occurring for one 3.5minute block, based on protocol of Rao and Keyser [3]. In the first condition (Illusion), meant to induce the RHI, the left hand was placed in a box on the table in front of the subject with a tactile stimulus attached to the left index finger. A rubber hand was placed next to the box in view of the subject with an

identical tactile stimulus device and a red LED which blinked synchronously with the tactile stimulus. The tactile stimulus was presented using a weak 0.5ms duration electrical current to the left index finger. In the Incongruent control condition, the rubber hand was placed in an unnatural position relative to the body such that the hand could not be mistaken for the participants own hand. In the "Real" control condition a rubber hand was not used; the left hand instead was visible while receiving tactile stimulation. The time of the onset and end points of the illusion was recorded by the participant through a button press by the right hand. The Rubber Hand Illusion Post-task Questionnaire [3] was used to measure the subjective strength of the illusion after each condition. MEG data was obtained using a 306-channel Neuromag Vector View system using standard procedures for head localization, eye blink and heart beat artifacts. Structural MRI data for co-registration with MEG was obtained using an MPRAGE sequence (1x1x1mm resolution, TR/TE/FA 2500/2.8/8; Parallel imaging 2x, 176 slices, FOV 256x256). Spectral data was processed and source localized similar to Stone et al. [4]. Participants first underwent the RHI task outside of the scanner for training and to screen for susceptibility to the RHI; HC who did not experience the illusion did not have neuroimaging. Analysis: We first compared average power during each condition (Illusion, Incongruent, Real) between SSD and HC. We then measured the difference in average spectral power between the epochs in which a participant reported experiencing the RHI and control conditions and compared these differences between groups. We focused on alpha and beta bands in right and left frontal (RF, LF) and central (RC, LC) regions, based on the findings reported by Rao and Keyser.

Results: The final sample included 14 SSD (12m, 21.6 ± 4.5yrs) and 11 HC (3m, 24.3 ± 5.4yrs). We did not find significant effects of condition (Illusion, Incongruent, Real) between groups. However, we found significant effects of diagnosis on the magnitude of the difference in spectral power between Illusion and control conditions: HC had significantly greater differences in alpha-band power between Illusion and Incongruent conditions than SSD in LF (SSD = -.089, HC = .07, $p < .0001$) and LC (SSD = -.068, HC = .005,$p < .046$), and beta-band power in LC (SSD = -.003, HC = .011, $p < .013$). For HC power was greater in the Illusion than in Incongruent condition, while for SSD power was slightly less in the Incongruent than Control conditions. There were not group effects in the Illusion-Real condition. The participant ratings of subjective strength of the RHI were not different between groups.

Conclusions: While self-disturbance may be a key early feature in the development of schizophrenia spectrum disorders, there is little known about associated differences in brain function. We studied youth with early psychosis, and found that while HC had significant differences in brain activity during the RHI, individuals with SSD did not, despite similar subjective reports of the strength of the illusion. While this is the first MEG study of the RHI in early psychosis, our results appear consistent with a previous EEG study in chronic schizophrenia[5] Limitations include the small sample size and lack of objective measurements of strength of the RHI. Future directions include replication in a larger sample, and relationship of findings to clinical characteristics.

References: 1. Schiz Res 152(1):1-4,2014. 2. Schiz Bull 38 (6):1277-87,2012. 3. Fr Hum Neuro 11:377, 2017. 4. Acta Psychol 142(2):177-83,2013. 4. Fr Hum Neuro 8:788, 2006. 5. Schiz Res 64(2-3):157-63,2003.

Keywords: Early Psychosis, Magnetoencephalography, Rubber Hand Illusion

Disclosure: Nothing to disclose.

P455. Optimizing Cognitive Control Activity in Med-Naïve First Episode Psychosis Patients and Healthy Controls

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Background: Cognitive impairment is a core feature of First Episode Psychosis (FEP). While cognitive control paradigms commonly identify dysfunction in several brain circuits, they fail to identify known sources of trial-to-trial variability in cognitive control. Trial Specific Costs (TSCs) include Block Restart (BR), Previous Trial Interference (PTI), Response Switching (RS), and Stimulus Item (SI) effects, each of which elicit robust behavioral costs. The current study examined whether modeling TSCs improves detection of brain dysfunction in FEP.

Methods: 22 FEP patients (50% male, age = 24.2 (4.4) years) and 22 healthy controls (46% male, age = 27.9 (4.6) years) were scanned on a Siemens 3T Magnetom Prisma scanner with a 64-channel head/neck receiver coil. This included a T1-weighted scan (TR = 2400 msec, TE = 2.22 msec, voxel size = 0.8 mm3, scan length = 6 min, 38 sec) and four Simultaneous Multi-Slice Echo Planar Imaging scans (multiband acceleration factor = 8, TR = 720 msec, TE = 33.00 msec, voxel size = 2.2x2.2x2.0 mm) in which the Multi-Source Interference Task (MSIT) was performed. Two runs were composed of mostly congruent (75% congruent:25% interference) trials and two runs were composed of mostly interference (25% congruent:75% interference) trials.

First-level models included regressors for congruent, interference, and error trials, in addition to TSCs. The BR regressor consisted of a separate condition for the first trial of each block. The PTI regressor was a parametric modulation regressor which indicated if the previous trial was congruent or not. The RS regressor was a parametric modulation regressor which indicated if the previous trial was a response switch. The SI regressor was a parametric modulation regressor which indicated if the current stimulus was a 1, 2, or 3. First-level models also included the cardiac-BOLD regressor, reflecting fluctuations in BOLD that track heart rate, in addition to nuisance (motion and aCompCor physiology) regressors. Group models examined interference-related (interference-congruent) activity, while controlling for group, sex, age, and Framewise Displacement. Group differences were thresholded at a voxelwise and clusterwise $p = 0.05$. To compare model fit, subject models with and without TSCs were compared across seven functional networks (i.e. the Yeo functional parcellation networks) for average *T*-values for the interference control (i.e. incongruent-congruent) contrast and model Mean Squared Error (MSE) adjusted for the number of model parameters (i.e. MSE/df).

Results: The models revealed group differences in distinct networks for interference control (interference-congruent) and for each of the TSCs. While the healthy control group had greater dorsolateral prefrontal cortex and anterior cingulate cortex activation than FEP patients during interference control, different regions were identified for PTI, BR, RS, and SI effects. Paired *T*-tests between models including TSCs and models including all other task and nuisance regressors, but no TSCs, revealed that mean *T*-values were significantly greater for interference control in the Ventral Attention ($t(43) = 4.5, p = 4.9\text{x}10\text{-}5$), Frontoparietal ($t(43) = 3.5, p = 0.0011$), Dorsal Attention ($t(43) = 3.1, p = 0.0028$), and Visual Networks ($t(43) = 2.4, p = 0.018$) for the TSC models. Model fit (MSE/df) was also improved for the TSC compared to the no TSC models across the whole brain and for all networks except the Visual Network.

Conclusions: Models of cognitive control function are improved by modeling known behavioral costs reflecting within condition trial variability. Cognitive control dysfunction in First Episode Psychosis can be better characterized by examining TSCs in addition to primary condition effects.

Keywords: First Episode Psychosis, Cognition, Functional Brain Network, Task fMRI

Disclosure: Nothing to disclose.

P456. Linking Lifetime Stress to Abnormal Functional Activation and Current Stress Processing During an Anticipated Threat Task

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Background: Stress across the lifetime plays a major role in increasing the incidence and severity of numerous psychiatric diseases, but exactly how stress translates into impact on brain circuitry remains an area of major interest. Brain areas responsible for the processing of and response to stress have been implicated, including limbic areas like the hippocampus which itself is sensitive to damage by stress, and the insula which is a key region in psychosis symptomatology. We hypothesized that lifetime stress-induced perturbation of stress-sensitive cortico-limbic brain areas leads to altered functional activation in response to subsequent stressful situations during adulthood, underlying an altered stress responsivity in schizophrenia spectrum disorder (SSD).

Methods: We have developed a highly translational stress induction paradigm that is human MRI compatible and ethical. The ankle shock threat task (AST) paradigm is analogous to the classic approach of inducing stress in animal studies using foot shock. For this small pilot study, we recruited fifteen individuals (11 SSD patients, 8/11 male) and 4 adult community controls (CC, 2/4 male) were included in this pilot study. Structured Clinical Interview for DSM-IV was completed to verify psychiatric diagnoses. Major early life stress was recorded using the Childhood Trauma Questionnaire, while recent stress (past 30 days) was measured with the Perceived Stress Scale. Symptoms were measured with the Peters Delusional Index. fMRI data was collected using a 3-T Siemens Prisma scanner and 64-channel coil, with an electrode attached to one ankle of each participant. A pre-determined voltage was applied for 0.1s during the task (similar to the experience of touching a surface with static electricity, using the Transcutaneous Aversive Stimulator, Coulborne Instruments). There are 3 conditions in this paradigm: (1) a shock condition in which a few random shocks are delivered while a color sign on the screen indicates shocks are possible; (2) a threat condition in which the same color is present but no shock is given; and (3) a safe condition in which no shock is given and the color indicates safety. All image preprocessing includes slice timing corrections and were volume co-registered. Images were linearly detrended, normalized into MNI standard space, and spatially smoothed (FWHM = 8mm) using SPM12. First level models were developed for each subject by entering all the volumes into a single analysis regressing the "shock", "threat" and "safe" conditions. The contrast of interest was the threat - safe condition to study how the brain is processing the threat of the unpleasant shock but without the interference of the actual shock. Multiple regression analyses were completed in SPSS (IBM). Non-significant predictors including interaction effects were removed in step-wise fashion. All experimental protocols were approved by the University of Maryland Baltimore IRB.

Results: Nominally significant group differences were found in multiple regions from the threat - safe contrast ($p < 0.05$) in a previous larger sample analysis. Two regions with significant patient-control activation differences were selected from this list to examine in this smaller pilot study ($n = 15$) due to their roles in stress processing and symptomatology: right hippocampus and right insula. In multiple regression analysis, a significant model ($F(3,12) = 7.8$, $p = 0.002$) for right insula activation included predictor variables of CTQ, PSS and PDI. In second regression model exploring the R hippocampus activation, CTQ and PSS were predictors in a significant model for right hippocampus activation ($F(3,12) = 7.5$, $p = 0.003$).

Conclusions: In this pilot study, we observed a link from past and recent stress measures to highly stress- and disease-relevant functional brain activations using an anticipated threat task. These findings suggest that more severe early life stress as well as recent stress combine to lead to altered activations revealed during an active functional imaging task, which may in part contribute to altered stress reactivity as well as symptoms in SSD.

Keywords: Lifetime Stress, Functional Neuroimaging, Hippocampus, Insula, Schizophrenia (SCZ)

Disclosure: Nothing to disclose.

P457. Diffusion Measures of Extracellular Free Water Predict Response to Treatment in Individuals With Schizophrenia-Spectrum Disorders

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Background: One of the more recent developments in the care of individuals with schizophrenia and other psychotic disorders is the introduction of coordinated specialty care (CSC). Despite the advantages of this approach, clinical outcomes continue to vary widely. Consequently, there is a need to leverage precision medicine tools to optimize treatment at the individual level and maximize the impact of CSC in these individuals. We propose that neuroimmune perturbation is a developmental factor that may represent considerable heterogeneity in clinical outcomes. Evidence has been accumulating for an immune-based component of schizophrenia based on several decades of epidemiological research showing a strong link between increased risk for psychosis and maternal infections, genetic models implicating MHC-related risk genes in increased synaptic pruning during adolescence, and post mortem studies showing altered gene expression of immune related proteins in the brain in psychotic disorders. One recently developed diffusion imaging measure, extracellular free water, is a putative marker of neuroinflammation that may offer insight into these neuroimmune processes in the brain. Free water has been shown to be reliably increased in first episode psychosis, including our own early psychosis samples. Furthermore, we have identified significant relationships between gray matter free water and BPRS scores, linking this measure to clinical state. However, in many studies of free water, there is significant overlap between groups in free water values and consequently only a subset of patients may evidence a strong immune component to their illness. This heterogeneity may partly explain why several clinical trials evaluating add-on treatment using anti-inflammatory agents have shown relatively modest benefits in relieving symptomatology, with one additional study showing no benefit. Thus, the goal of this study is to evaluate the potential of baseline extracellular free water as a predictor of treatment response over a one year period in CSC in a sample of patients with first episode schizophrenia-spectrum disorders.

Methods: A total of 46 individuals with schizophrenia-spectrum diagnoses were recruited, assessed using the SCID-IV and BPRS, and enrolled in our CSC program. Participants were re-assessed after one year and those whose BPRS score improved by twenty percent or greater were considered treatment responders, while the remainder of the group were considered non-responders. All participants underwent a diffusion MRI scan on a 3 Tesla Siemens Trio scanner in which multiple *b*-value shells were acquired to improve estimation of extracellular free water. Diffusion images were aligned to individual subject MPRAGE scans, and existing

segmented MPRAGE masks were used to define whole-brain gray- and white-matter free water estimates. Logistic regression was implemented to test the prediction that higher free water at baseline would be associated with positive response to treatment. Pearson correlations (with follow-up nonparametric analyses for non-normal data) were computed between free water values and clinical symptomatology in SPSS.

Results: After one year in treatment, 26 individuals were classified as treatment responders and 20 as non-responders, based on the twenty percent BPRS improvement threshold. In line with our predictions, treatment responders showed a trend for higher whole-brain gray matter free water values at the baseline assessment ($t = 1.91$, $p = .06$, OR = 1.9). Additionally, higher gray matter free water values were negatively associated with BPRS scores at 12 month follow-up ($r = -.384$, $p = .008$), suggesting that those who initially presented with high free water values tended to show low symptomatology at follow-up. Whole-brain white matter free water was not significantly different between responders and non-responders ($p = .73$) and showed a similar but non-significant relationship to symptoms at follow-up ($r = -.18$, $p = .23$).

Conclusions: These data suggest that even with gold-standard CSC a substantial proportion of patients did not show a clinically meaningful response to treatment. However, whole-brain gray matter free water showed promise as a predictor of treatment response, given that individuals showing high free water levels at baseline were more likely to be classified as treatment responders one year later, albeit at a trend level of significance. These data were consistent with significant inverse correlations between gray matter free water and BPRS scores at one year, which suggest that those who began the study with a more prominent inflammatory profile showed lower symptoms at follow-up. These relationships were largely unique to free water present in the gray matter, showing some specificity to tissue type. While preliminary, these data suggest that the use of MRI-based markers, such as free water, may offer the potential of a more personalized approach to future clinical trials of inflammation-related therapies, in which patients are stratified based upon diffusion MRI data and offered targeted intervention based upon these measures. Finally, additional participants are being continually recruited and new participants will be added to the sample for the poster presentation.

Keywords: Free Water Imaging, Schizophrenia (SCZ), Treatment Response, Psychotic Disorders

Disclosure: Nothing to disclose.

P458. Reducing Cardiovascular Effects in Ketamine PharmacoBOLD

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Background: Schizophrenia is associated with psychotic symptoms that remain partially or fully refractory to standard antipsychotic medications for ~2/3 of patients. Alternative, glutamatergic approaches for treatment development have been proposed but have not yet led to FDA-approved medications. A major barrier to effective glutamatergic treatment development is the absence of validated measures for target engagement that can identify effective compounds and guide dose selection. As part of the recently completed NIMH multicenter FAST-PS initiative, we evaluated ketamine-induced pharmacoBOLD (phBOLD) in healthy volunteers (HV) as a potential target engagement biomarker for development of metabotropic glutamate (mGluR2/3) agonists. In our initial HV study, we demonstrated that ketamine induces a robust increase ($p < 0.0001$, Cohen's $d = 5.4$) in phBOLD response. In FAST-PS, we selected a high dose of ketamine (0.23 mg/kg) in order to produce robust pharmacological effects. However, at this dose, ketamine-induced tachycardia can influence cerebrovascular volume and oxygenation, and thus phBOLD, which may add "noise" to phBOLD comparisons. In the present study, we titrated this dose downward in HV in order to identify doses that produce reduced psychotomimetic effects, but nevertheless sufficiently robust ($d = \sim1.5$) phBOLD effects to permit detection of mGluR2/3 agonist effect while at the same time minimizing cardiovascular effects.

Methods: HV participated in 2 identical phBOLD sessions at least 7 days apart in 10 person cohorts for a total of 20 subjects. On both days, clinical assessments were performed following removal of the subject from the scanner. The first cohort received 0.08 mg/kg, which has previously been shown to produce a large effect size ($d = 1.97$). The second cohort received 0.06 mg/kg. We regressed out the effects of cardiorespiratory variance and assessed the correlation between phBOLD magnitude in dorsal ACC and symptoms. This approach allowed us to identify the lowest ketamine dose at which reliable phBOLD effects are observed, in order to minimize psychotomimetic/non-specific perfusion effects in future studies.

Results: At 0.08 mg/kg ketamine, the phBOLD response peaked at ~3 min with a ~1% signal change. The peak responses during the first and second scan were not significantly different from each other (*t*-test, $p = 0.49$). A full reliability analysis will be conducted once data from all cohorts are finished being collected. The preliminary effect size for scan 1 is 3.7, which shows the expected drop off from our previous study using a 0.23 mg/kg bolus ($d = 5.4$). The behavioral results from the 1st and 2nd cohorts shows the expected dose response. The BPRS increase in the present study remains significant ($p = 0.02$, $d = 0.61$). No significant cardiovascular changes were seen.

Conclusions: This preliminary data confirms that phBOLD can still produce robust effects and be used for reliable testing of novel glutamatergic compounds while also avoiding the most common fMRI confound from ketamine administration. The robust phBOLD and BPRS response suggests that further downward titration is possible.

Keywords: Schizophrenia, Antipsychotics, Ketamine, PharmacoBOLD

Disclosure: Nothing to disclose.

P459. In Vivo Plasticity Between Ventral Hippocampal Inputs and Medial Prefrontal Cortex Microcircuits in a Mouse Model of 22q11.2 Deletion Syndrome

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Background: Functional connectivity between rodent ventral hippocampus (vHPC) and medial prefrontal cortex (mPFC) supports cognitive functions such as spatial working memory (SWM), and is disrupted in Df(16)A + /– mice with a microdeletion syntenic to the schizophrenia-predisposing 22q11.2 microdeletion. Optogenetic inhibition of direct vHPC-mPFC neural projections or somatostatin-positive (SST+) interneurons in mPFC of wildtype (WT) mice impairs vHPC-mPFC connectivity and SWM performance, mimicking the effects of the microdeletion. To examine how functional interactions between these circuit elements may contribute to vHPC-mPFC dysconnectivity in Df(16)A +/- mice, and how these interactions may be altered to promote vHPC-mPFC connectivity, we tested the ability of vHPC-

mPFC inputs to modulate in vivo activity of discrete mPFC neuronal populations in WT and Df(16)A +/- mice using an all-optical approach.

Methods: The red light-activated channelrhodopsin ChrimsonR was expressed in vHPC neurons of adult male and female WT and Df(16)A +/- mice. The calcium indicator GCaMP6f was expressed in mPFC SST + interneurons, parvalbumin-positive (PV+) interneurons, and CaMKII+ pyramidal neurons in separate cohorts (n = 5-13). Red light pulses were delivered to the mPFC to excite vHPC terminals, and postsynaptic activation of GCaMP6f was simultaneously monitored using fiber photometry.

Results: Stimulation-evoked photometric responses in SST + interneurons varied systematically with pulse number (1-40) and frequency (1-40Hz), with maximal responses achieved by 40 pulses at 40Hz. SST + interneuron responses in Df(16)A +/- mice were weaker than those in WT mice ($p = 0.03$, main effect of Genotype, 3-way ANOVA). Repeated daily high-frequency stimulation over 12 days increased evoked SST + interneuron responses for up to 50 days in both genotypes ($p = 0.006$, Day x Stimulation, 3-way ANOVA). High-frequency stimulation in Df(16)A +/- mice potentiated SST + interneuron responses to levels observed in WT mice without such stimulation. PV + interneuron responses to high-frequency stimulation in WT mice were weak and biphasic; the negative component of this response was non-significantly enhanced across stimulation days ($p = 0.064$, one-way ANOVA). CaMKII+ pyramidal neuron responses to the same high-frequency stimulation in WT mice were unchanged across stimulation days ($p = 0.235$, one-way ANOVA). Behavioral tracking of all mice revealed no evidence of differences in locomotion or position within the stimulation chamber that explain the group differences or time-dependent changes in neural responses.

Conclusions: These findings suggest that the capacity of vHPC inputs to engage mPFC SST + interneurons may be impaired in Df(16)A +/- mice, and that repeated high-frequency stimulation of vHPC-mPFC inputs induces in vivo plasticity that may involve recruitment of SST + interneurons. Future work will assess how this plasticity influences SWM performance. Together, these data reveal properties of cell-type-specific functional connectivity within intact vHPC-mPFC circuits that inform how discrete circuit elements may interact to mediate normal and disordered cognitive function.

Keywords: Hippocampal-prefrontal, GABAergic Interneurons, Neural Plasticity, 22q11 Deletion Syndrome, Functional Connectivity

Disclosure: Nothing to disclose.

P460. AUT00206, a First-In-Class Kv3 Modulator, Improves Auditory Processing in Patients With Schizophrenia

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Background: Evidence suggests that patients with psychotic illness show deficits in primary auditory function [Javitt et al. 2015, Am.J.Psych 172, 17-31], including real-world difficulties, such as understanding speech in a background of noise [Illiadou et al. 2013, Am.J.Aud. 22, 201-208]. Auditory deficits likely impact social and cognitive function, and contribute to or exacerbate other symptom domains. Early treatment of sensory deficits could reduce or prevent psychotic episodes. Kv3.1 potassium channels are critical for the function of neurons in auditory circuits [Kaczmarek 2012, PLoS Comp. Biol. 8, e1002424]. Positive modulation of Kv3.1 channels can rescue deficits in auditory temporal processing in rodents [Chambers et al, 2017, Sci. Rep. 7, 17496]. AUT00206 is a novel Kv3.1 modulator that reduced the impact of ketamine on BOLD responses in healthy volunteers [Deakin et al. 2019, Schiz. Bull. 45, S245-S246]. Here, we investigated the ability of AUT00206 to improve auditory function in patients with schizophrenia.

Methods: In a randomised, double-blind, placebo-controlled study, 24 subjects with schizophrenia (< 5 yrs since diagnosis) were randomised 2:1 to AUT00206 or placebo for 28 days (NCT03164876). Clinical and exploratory biomarker assessments were completed by all subjects. Audiological assessments were completed by 11 subjects on AUT00206 and 4 on placebo. At Baseline, Day 1, and three days over a 28-day period, subjects completed a comprehensive test battery that probed different levels of auditory information processing including otoacoustic emissions (OAEs), pure-tone audiometry, mismatch negativity (MMN), and a Words-in-Noise test (WIN).

Results: All patients had clinically normal hearing thresholds and performed within normal ranges on tests of basic auditory function. Tests that indicated abnormal auditory processing at baseline (MMN, WIN), showed an improvement with treatment. A consistent increase in OAEs and contralateral suppression of OAEs was observed in the AUT00206 group that reached statistical significance at several frequencies. Increased amplitude of pitch MMN was observed over the course of the dosing period in the AUT00206 group compared to baseline and placebo. Performance of the WIN test at 40dB HL showed a significant treatment difference between AUT00206 (-2.6dB improvement) and placebo (+0.8dB worsening) ($p = 0.016$). Seventy percent (7/10) of the AUT00206 subjects showed an improvement in WIN performance by Day 21, with only twenty five percent (1/4) showing an improvement on placebo. The degree of improvement in OAEs and WIN performance in AUT00206 group at Day 21 is considered highly clinically relevant.

Conclusions: Treatment with AUT00206 improved measures of auditory information processing at different levels of the auditory system in patients with schizophrenia, consistent with a critical role for Kv3.1 channels in high fidelity information processing and transfer. Treatment with Kv3.1 modulators may not only correct information processing at higher cortical levels, but also ameliorate sensory deficits across all modalities – a novel, but long overdue approach that may help improve symptoms and quality of life through a bottom-up effect in patients with schizophrenia. These results provide the first evidence that targeting PV + interneuron through modulation of their intrinsic ion channels is an important new approach to the treatment of information processing disorders, such as schizophrenia. Future studies will have to establish if Kv3.1 modulators also improve other dysfunctions such as cognitive deficits believed to be due to PV + interneuron dysfunction.

Keywords: Kv3 Channels, Parvalbumin Neurons, Auditory Processing, Schizophrenia

Disclosure: Autifony Therapeutics Limited: Stock / Equity, Board Member, Employee (Self)

P461. Acetylated Chromatin Domains Link Chromosomal Organization to Cell and Circuit Level Dysfunction in Schizophrenia

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Background: The three-dimensional (3D) genome organization is crucial for the interplay between cell specific gene expression, chromatin structure, and other factors (e.g., histone modification) underneath our brain functions. Dysregulation of transcription and chromatin structure leads to debilitating disorders such as autism and schizophrenia (SCZ). To explore the dysregulation in chromatin structure in SCZ and bipolar disorder (BD), we capitalized on histone modification landscapes as they are tightly linked to chromatin structures.

Methods: In the first part of the study, we mapped active promoter- and enhancer-associated histone methylation (H3K4me3) and acetylation (H3K27ac) profiles in PFC of 564 brain donors, providing to date the largest histone modification dataset for SCZ and BD.

In the second part of this study, we built chromatin structure by leveraging the inter-individual correlations between histone peaks to identify domains of physically interacting regulatory elements called cis-regulatory domains (CRDs). Our in-silico biological validation of CRDs from both assays H3K4me3 and H3K27ac showed tight link to the structures of self-interacting domains, with enrichments of CTCF structural proteins at CRD domain boundaries, which is in line with CTCF enrichment of PFC NeuN+ Hi-C defined TAD boundaries.

Results: Dysregulation of enhancer-associated histone modified peaks (H3K27ac) across SCZ (BD) cases and controls showed stronger enrichment of SCZ (BD) GWAS risk variants in neurons than in tissue. Additionally, we noticed enrichment in SCZ variants was coming from hyper-acetylated peaks but not from hypo-acetylated peaks.

Next, we investigated dysregulation in chromatin structure using CRDs expression as a metric. Interestingly, SCZ and BD sensitive CRDs which were hyper-acetylated were strongly enriched for SCZ (BD) GWAS risk variants than hypo-acetylated CRDs. To further explore the role of disease sensitive CRDs, we created another layer of chromatin by taking the inter-individual correlation of diseased CRDs which manifested A/B compartments of chromatin organization. K-means clustering of correlation of disease-sensitive H3K27ac CRDs revealed strong, cluster-specific convergences defined by A/B compartment chromatin organization, dysregulation (hypo vs. hyper-acetylation), cell types and specificity towards neurodevelopment. Cluster with majority of hyper-acetylated CRDs were strongly enriched for glutamatergic specific H3K27ac peaks and chromHMM active chromatin states in fetal brain linking the connection of SCZ and BD with neurodevelopment.

Finally, the projection of hyper-acetylated CRDs from A-compartment cluster in three-dimensional genome constructed using chrom3D utilizing PFC-NeuN+ HiC showed localization of disease sensitive TADs in 3D nuclear 3D space linking neuronal 3D genome, or chromosomal organization in psychiatric disorders.

Conclusions: We showed that hyperacetylated CRDs were strongly enriched for excitatory projection neuron-specific peaks with regulatory sequences indexed by chromHMM as active chromatin showing significant associations with SCZ and BD genetic risk architectures for the adult, and in case of SCZ, also the fetal PFC. Therefore, this cluster-specific fingerprint could signal that many chromatin domains important for PFC projection neurons play a critical role early in the disease process.

Taken together with the finding that chromatin states of the fetal brain are disproportionally over-represented among the set of common risk variants linked to schizophrenia, a finding that was replicated for the group of hyperacetylated enhancers in SCZ groups, it is plausible to hypothesize that a subset of hyper-acetylated chromatin domains in diseased PFC neurons are vestiges of an early occurring neurodevelopmental disease process.

In summary, the work presented here describes some of the first fine-mappings of chromosomal domains in large series of disease and control brains, primarily defined by the correlational structure of nucleosomal histone modifications and then integrated into the Hi-C chromosomal conformation landscape. We expect that the findings and resources presented here, which were highly reproducible across various brain collections, will provide a unique roadmap for future studies aimed at gaining a deeper understanding of this emerging link between the neuronal 3D genome, or chromosomal organization, and cell-circuit-specific neuronal dysfunction in psychosis including underlying genetic risk architectures.

Keywords: Neuropsychiatric Disorders [Schizophrenia, Parkinson's Disease, Major Depressive Disorder], Computational Methods, Histone Acetylation, Histone Methylation

Disclosure: Nothing to disclose.

P462. Pre-Clinical Evaluation of Hippocampal Long-Term Potentiation After Acute and Sub-Chronic Oral Dosing With Luvadaxistat in Mice

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Background: Luvadaxistat (also known as TAK-831, NBI-1065844) is a highly selective and potent inhibitor of the D-amino acid oxidase (DAAO) enzyme (Howley et al., 2017) being studied in the clinic for cognitive impairment associated with schizophrenia (CIAS). Inhibition of DAAO results in an increase in D-serine, a co-ago¬nist of the NMDA receptor (Hondo et al., 2013; Howley et al., 2017). Luvadaxistat has been studied pre-clinically in a variety of assays relevant to assess cognitive performance in rodents (i.e., novel object recognition task, attention set-shifting task, and eye-blink conditioning). Notably, a sensitization in the behavioral response in novel object recognition task (a decrease in the minimum effective dose with a loss of efficacy at higher doses) was demonstrated after sub-chronic dosing of luvadaxistat (14 d) when compared to responses after acute dosing (Fradley et al., in preparation). The observation of sensitization suggested that luvadaxistat may be influencing hippocampal synaptic plasticity after sub-chronic dosing. We studied hippocampal synaptic plasticity using a well-established long-term potentiation (LTP) paradigm after acute and sub-chronic (14 d) dosing with luvadaxistat.

Methods: Mice (total of 97; $n = 5$–8 per condition) were given an acute or chronic dose of luvadaxistat (from 0.001 mg/kg to 10 mg/kg, p.o.) and hippocampal slices were harvested after 5 hours. Cerebellum and blood plasma were also obtained to measure D-serine levels achieved in the experimental animals. Electrophysiological recordings were obtained from a single slice placed on the chamber and perfused with aCSF at a constant rate. Extracellular fEPSPs were recorded in the CA1 stratum radiatum using a glass micropipette filled with aCSF. fEPSPs were evoked by electrical stimulation of Schaffer collaterals/commissural pathway at 0.1 Hz with a glass stimulating electrode placed in the stratum radiatum. To test the effect of luvadaxistat on basal synaptic transmission, Input/Output (I/V) curves were constructed at the beginning of the experiment. The slope of fEPSPs was measured and plotted against different intensities of stimulation (from 0 to 100 uA). Long-term potentiation was induced by a theta burst stimulation protocol (10 bursts of 4 pulses at 100 Hz, 200 ms between bursts) at baseline stimulation intensity. Following this conditioning stimulus, a 1 hr test period was recorded where responses were again elicited by a single stimulation every 10 s (0.1 Hz) at the same stimulus intensity

Results: Luvadaxistat produced a dose-dependent increase in D-serine levels in mouse brain (from 8.7 nmol/g to 34 nmol/g) and

mouse plasma (from 213 ng/ml to 490 ng/ml). Acute dosing of luvadaxistat did not induce a measurable change in LTP. However, when luvadaxistat was dosed sub-chronically (14 d), there was a statistically significant increase (1-way ANOVA post-hoc multiple comparison analysis) in LTP after 0.001 mg/kg (*, $p < 0.05$ vs vehicle) and 0.01 mg/kg dose (**, $p < 0.01$ vs vehicle). Higher doses (0.1 mg/kg and 10 mg/kg) induced decreases in LTP.

Conclusions: Luvadaxistat induced dose-dependent increases in plasma and brain D-serine as demonstrated previously (Howley et al. 2017). Sub-chronic dosing with luvadaxistat significantly increased LTP suggesting increases in synaptic plasticity. Higher doses (0.1 to 10 mg/kg) decreased LTP, suggesting an inverted U-shape dose response. This phenomenon may underlie the leftward shift in behavioral responses (i.e., novel object recognition task) observed after acute vs chronic dosing (Fradley et al., in preparation). The leftward shift of the dose-response relationship reported in this study may suggest low doses may be efficacious in clinical trials of DAAO inhibitors.

Keywords: Long Term Potentiation, Synaptic Plasticity, D-serine, Cognitive Impairment Associated with Schizophrenia, D-Amino Acid Oxidase

Disclosure: Engrail Therapeutics: Employee (Self)

P463. Context-Dependent Effects of Haloperidol on Rat Brain Volumes and Structural Covariance Networks: An Ex Vivo MRI Study in a Rat Maternal Immune Activation Model

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Background: Apparent changes in regional brain volumes and structural covariance are commonly reported in Magnetic Resonance Imaging (MRI) studies of individuals with schizophrenia (SZ). An unresolved question however is whether these apparent changes are driven by the underlying illness or represent iatrogenic effects of antipsychotic medication. We previously reported that that chronic exposure to haloperidol or olanzapine decreased rat whole brain and cortical volume (Vernon et al. Biol. Psych. 2014). A limitation of this work however is the use of naïve animals, which lack any pathology relevant to SZ. To address this, we investigated the impact of adult haloperidol (HAL) exposure on rat brain volumes and networks of structural covariance in a maternal immune activation (MIA) model of relevance for SZ, using high resolution ex vivo structural MRI. We hypothesised HAL will induce differential (i.e context-dependent) changes in these measures in MIA-exposed offspring as compared to controls.

Methods: Male Sprague-Dawley rat offspring from control (CON; saline, i.v.; GD15; $n = 5$ dams) and MIA exposed dams (POL; poly I:C, 4 mg/kg i.v. GD15; $n = 5$ dams) were randomly allocated to one of four groups: CON/vehicle; CON/haloperidol; POL/vehicle and POL/haloperidol, comprising $n = 10$ offspring per group. Each group contained a maximum of $n = 2$ offspring from the same litter. Vehicle (VEH) or the D2-receptor antagonist haloperidol (HAL; 0.5 mg/kg/d s.c.) were administered for 28 days via osmotic mini-pumps in adulthood starting on postnatal day (P)80. At the end of treatment (P108) rats were culled and perfused with 4% PFA, with the brain left in the cranium. T2-weighted ex vivo 3D MR images were acquired using a 7T small bore MRI scanner (Agilent technologies) and a quadrature volume radiofrequency (RF) coil (39 mm inner diameter, RAPID Biomedical) using the following parameters: TE/TR = 60/2000, echo train length = 8, matrix size = 192x128x192 and field of view (FOV) = 28.8x19.2x28.8 mm, yielding isotropic voxels of 150 µm3. Total scan-time per brain was 1hr 44min. MR images were converted to NIFTI format and processed using a combination of FSL, ANTs and in-house Python software. Regional brain volumes were extracted following registration to a publicly available rat brain MRI atlas (Papp et al. Neuroimage, 2014). Group differences were assessed for each atlas brain region of interest (ROI; $n = 61$) using linear models with between-group effects of "prenatal treatment" (CON or POL) and within-group effects of "postnatal treatment" (VEH or HAL), and their interaction, including total brain volume as a covariate. Multiple comparisons were controlled for using the False Discovery Rate (5%). To assess structural covariance by region within the dataset, the volumes of $n = 44$ atlas-segmented grey matter brain ROIs were subjected to correlational analysis using Pearson's r as the readout. Correlational data were grouped into five anatomical clusters defined by agglomerative hierarchical clustering. Mean r-values for each group correlation matrix were compared using a Kruskal-Wallis 1-way ANOVA. All statistical analysis used custom scripts written in R-studio (version 1.4.1106).

Results: Statistically significant effects of prenatal treatment (CON vs. POL) were found for 5% (3/61) of rat brain atlas ROIs. Specifically, substantia nigra volumes were decreased, whilst the volumes of the cingulate and frontal association cortex were increased (all $\eta p2 > 0.35$). There were no statistically significant effects of postnatal haloperidol (VEH vs. HAL) exposure. Consistent with context-dependent drug effects, statistically significant interaction effects (prenatal * postnatal treatment) were however found for 7% (4/61) of rat brain atlas ROIs. These included the inferior colliculus, fornix, bed nucleus of the stria terminalis (BNST) and white matter tracts (all $\eta p2 > 0.25$). Illustrating these data, BNST volumes were reduced in POL/VEH rats, relative to all other treatment groups. By contrast, there were no differences between POL/HAL rats and CON/VEH or CON/HAL rats, suggestive of a normalising effect of HAL exposure in POL offspring on the volume of the BNST. Mean r-values derived from structural covariance matrices were significantly different across the groups (Kruskal-Wallis $p < 0.01$; eta2 [H] > 0.1), with increased overall structural covariance in POL/VEH rats relative to all other groups (Bonferroni post-hoc $p < 0.05$). Visual inspection of cluster-level changes in POL/VEH rats suggests this is driven by increased structural covariance between brainstem, hippocampal and frontal cortical ROIs. These effects were not observed in POL/HAL rats, which no longer differed from CON/VEH or CON/HAL rats, suggestive of a normalising effect.

Conclusions: We found that prenatal POL exposure leads to regional brain volume and structural covariance changes in the adult rat brain, consistent with our prior work (Crum et al. Brain Behaviour and Immunity, 2017). Furthermore, we provide preliminary evidence that adult HAL exposure has context-dependent effects on regional brain volumes and structural covariance networks in the rat brain. In particular, HAL exposure appeared to normalise putative pathological changes observed in the POL-exposed rats. Longitudinal in vivo MRI studies and behavioural assessment in offspring of both sexes are now required to confirm these data and establish the underlying cellular correlates.

Keywords: Antipsychotic, Schizophrenia (SCZ), Animal Models, Maternal Immune Activation

Disclosure: Nothing to disclose.

P464. Long-Range Synchronization in the Alpha-Band Differentially Engages VIP and SST Interneurons in Visual Cortex to Support Novelty Detection During an Oddball Paradigm

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Background: People with schizophrenia exhibit difficulties in the processing of basic sensory information in a broader spatiotemporal and behavioral context. Such abnormalities undermine how affected individuals perceive and cognitively relate to their world. Impaired

sensory context processing is reflected in reduced early cortical registration of contextually novel stimuli, as recorded with EEG and indexed via the "mismatch negativity" biomarker during visual or auditory "oddball" paradigms. Despite this observation, the basic cellular and circuit basis of mismatch negativity and underlying contextual processing functions remain unclear, both in terms of basic neurobiology and as it relates to pathology. We recently translated a classic visual oddball paradigm to mice to understand the basic neural mechanisms of how preceding context modulates neural processing of incident stimuli. This work identified large neural responses to rare stimuli in the "oddball" paradigm (i.e. "deviance detection", a neural correlate of mismatch negativity) that were localized to superficial layers of primary visual cortex (V1). Optogenetically suppressing distal inputs to V1 from prefrontal cortex (ACa) eliminated this effect. Here we sought to understand how these top-down inputs interact with superficial layers of V1 at the circuit level, and how this relates to neuro-oscillatory signatures of mismatch negativity commonly measured in clinical settings.

Methods: We recorded electrophysiological and calcium signals in awake head-fixed mice (male and female; VGlut-cre, VIP-cre, SST-cre) during classic visual oddball and "many-standards control" sequences, which allowed the separation of genuine deviance detection signals to novel stimuli from the simple absence of stimulus specific adaptation. Local field potentials (LFPs) were recorded with bipolar electrodes placed in V1 and ACa ($n = 7$ mice). Two-photon calcium imaging of vasoactive intestinal peptide positive interneurons (VIP), somatostatin postive interneurons (SSTs), and pyramidal neurons (PYRs) from layers 1-4 of V1 were recorded while prefrontal inputs to V1 were ontogenetically driven at paradigm-relevant frequencies (ChR2; 2 to 40-Hz; $n = 6$ mice). Finally, chemicogenetic silencing of VIP interneurons (DREADDs; hM4Di vs CNO-only controls) was carried out during LFP recordings to determine their relevance as a local mediator of ACa-V1 synchrony and "deviance detection" signals ($n = 14$ mice).

Results: During the oddball paradigm, ACa and V1 displayed strong phase-locking (Rayleigh statistic>0.4; $p < .001$) at theta to alpha frequencies (6-10hz). Rhythmically activating ACa axons in V1 in this frequency range (but not e.g. gamma or delta) activated both VIPs and PYRs but suppressed SSTs in V1 (rmANOVA for each cell type). Chemicogenetically suppressing V1 VIP cells during the oddball paradigm selectively eliminated ACa-V1 coherence in the 6-10-Hz range ($d = 0.92$; hM4Di mice) and disrupted V1 deviance detection signals in the 1-10Hz LFP signal ($d = 0.41$; hM4Di mice; mixed-ANOVA with hM4Di $n = 6$ mice vs CNO-only controls ($n = 8$ mice); pre-vs-post CNO).

Conclusions: Our results suggest that top-down inputs to V1 engage a canonical VIP to SST inhibitory motif in V1, specifically in the theta-alpha band. This VIP-SST motif may effectively disinhibit local sup-populations of PYR neurons in V1, giving rise to augmented "deviance detection" signals (i.e. the neurobiological basis of mismatch negativity) to rare stimuli in the oddball paradigm.

Keywords: Visual Cortex, Mismatch Negativity, GABAergic Interneurons, Neuronal Oscillations, Prefrontal Cortex

Disclosure: Nothing to disclose.

P465. Automated Language Analysis of Emotional Expression in Schizophrenia: Development of a Novel Method and Preliminary Findings

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Background: Social cognitive deficits associated with schizophrenia are core features of the illness and strong predictors of functional outcomes. A major barrier to targeting these deficits is quantifying them effectively and efficiently. Standard social cognition assessments often rely on subjective judgements, have limited resolution, and are expensive to administer. Recent studies examining these assessments have demonstrated that most widely used measures have suboptimal psychometric characteristics. There is a critical need for objective, precise, and low-cost measurement approaches for application in research and clinical care. Recent advances in computational methods from artificial intelligence and natural language processing (NLP) have transformed our ability to gain clinical insights from speech, a rich source of data, and several studies have examined their application in schizophrenia. Thus far, however, NLP tools have mostly been used to examine semantic coherence as an indicator of thought disorganization. Whether similar automated methods can be used to estimate social cognitive function in schizophrenia is unclear. In this study, we aimed to address this gap by developing a novel method of quantifying free responses to naturalistic, accessible, emotionally evocative content and tested this approach in a pilot sample.

Methods: As part of a clinical trial, 39 men with schizophrenia and 51 neurotypical control men completed an experimental task during which they viewed a series of brief, emotionally evocative video stimuli that are publicly available. After viewing each stimulus, participants had 30 seconds to respond verbally to an on-screen prompt: "How did the video make you feel?" We audio recorded and transcribed these responses. Then we used a neural network trained to identify expression of emotion in text to derive a measure of each participant's emotional alignment relative to average neurotypical responses. For each transcript, the neural network provided a probabilistic estimate for expression of 27 distinct emotions--a vector of emotion probabilities. The emotional alignment score (ranging from 0-1) reflects the cosine similarity between this emotion vector and the average vector computed from the neurotypical sample. We also computed an emotional alignment score for each neurotypical control participant using leave-one-out cross-validation (i.e., comparing to the average vector from every other control). To assess the specific contribution of our emotion alignment metric, we computed sentence-level coherence--a common metric used in language analysis in schizophrenia--and compared relationships to clinical characteristics between both metrics: coherence and emotional alignment.

Results: We found that patients showed impaired emotional alignment ($M = 0.447$, $SD = 0.148$) relative to controls ($M = 0.534$, $SD = 0.139$), $t(72.644) = 2.770$, $p = 0.007$. Better emotional alignment was associated with better performance on a mentalizing task, $r(80) = 0.328$, $p = 0.003$; less severe negative symptoms, $r(82) = -0.364$, $p = 0.001$; and a higher level of functioning, $r(82) = 0.319$, $p = 0.003$. It was not associated with positive symptom severity, $r(34) = 0.093$, $p = 0.590$. Coherence was also impaired among patients ($M = 0.797$, $SD = 0.060$) relative to controls ($M = 0.825$, $SD = 0.053$), $t(69.835) = 2.299$, $p = 0.024$. However, coherence was not associated with negative symptoms, $r(82) = -0.053$, $p = 0.633$; level of functioning, $r(82) = 0.131$, $p = 0.235$; mentalizing performance, $r(80) = 0.128$, $p = 0.251$; or positive symptom severity, $r(34) = 0.159$, $p = 0.354$. Comparing each metric's ability to classify participants (patient vs. control), we found that emotional alignment performed slightly better than coherence (AUROC of 0.675 vs. 0.625), and that combining the two resulted in improved performance (AUROC of 0.700).

Conclusions: An automatically computed measure of emotional alignment may provide an easily obtained estimate of social cognitive function in schizophrenia that complements existing NLP metrics used for clinical assessment of other symptom domains. Additional work is needed to validate this approach and to replicate our preliminary findings in larger and more diverse samples.

Keywords: Schizophrenia (SCZ), Natural Language Processing (NLP), Emotion

Disclosure: Nothing to disclose.

P466. RNA Editing in the Hippocampus Mitigates Behavioral Deficits Associated With Prenatal Stress

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Background: Psychological stress during pregnancy has been linked with developmental delay in addition to increased risk for disorders associated with impaired socialization in male offspring but not in females (Hodes and Epperson 2019). An epitranscriptomic process known as RNA editing plays an important role in brain development (Hwang, Park et al. 2016) and altered levels of RNA editing have been detected in the hippocampus of individuals with neurodevelopmental disorders (Tran, Jun et al. 2019).

Methods: In this study, we exposed pregnant mice to prenatal restraint stress three times daily during weeks 2 and 3 of gestation. Male offspring that were exposed to prenatal stress ($n = 20$) and a group of male controls ($n = 20$) received treatment with haloperidol (1mg/kg), clozapine (5mg/kg) or saline twice daily for 5 days in adulthood before we measured social behavior using the three-chamber test for social interaction, and locomotor activity. After euthanasia, we used quantitative PCR and/ or next-generation sequencing to measure RNA editing in the prefrontal cortex and hippocampus of the mice. We also tested female mice ($n = 20$) for social behavior and locomotor activity after exposure to prenatal stress. This sample size of 20 mice provides 80% power to detect a linear relationship between social interaction and the abundance of an RNA editing isoform with an R square value of 0.3 and probability level = 0.05.

Results: Prenatally stressed male mice had lower social interaction relative to non-stressed male mice ($F = 17.0$, df = 1, 18, $p = 0.001$). Prenatally stressed mice treated with clozapine had higher social interaction levels than the prenatally stressed group treated with vehicle ($F = 11.5$, df = 1,18, $p = 0.009$). Social interaction behavior correlated with hippocampal RNA editing of: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunits GluA2 ($R = 0.57$, $F = 8.80$, df = 1, 18, $p = 0.008$), GluA3 ($R = 0.49$, $F = 5.59$, df = 1, 18, $p = 0.030$) and GluA4 ($R = 0.48$, $F = 5.43$, df = 1, 18, $p = 0.032$), the potassium channel Kv1.1 ($R = 0.766$, $F = 25.5$, df = 1, 18, $p < 0.0001$), the calcium channel subunit Cav1.3 ($R = 0.49$, $F = 5.70$, df = 1, 18, $p = 0.028$), calcium-dependent secretion activator (CAPS-1) ($R = -0.45$, $F = 4.62$, df = 1,18, $p = 0.046$) and the calcium-dependent cell adhesion protein, cadherin 22 (CDH22) ($R = 0.51$, $F = 6.22$, df = 1, 18, $p = 0.023$). Treatment with clozapine, but not haloperidol, reversed the deficits in social interaction and GluA2 RNA editing in prenatally stressed mice ($F = 26.3$, df = 1, 8, $p = 0.001$). GluA2 RNA editing was not altered in the prefrontal cortex of prenatally stressed mice. We did not observe these effects in female mice.

Conclusions: Reduced RNA editing of molecules regulating calcium homeostasis may contribute to impaired hippocampal function after exposure to prenatal stress in male mice. Clozapine may improve social interaction through an indirect mechanism that includes the upregulation of GluA2 RNA editing in the hippocampus but not the prefrontal cortex. These data indicate that prenatal stress modifies an epitranscriptomic pathway leading to lifelong deficits in social behavior. These findings may lead to the development of therapies that modulate glutamatergic signaling using a genetic approach, which may have greater target specificity and avoid the side effects of seizures and/ or cognitive deficits that are produced by conventional glutamatergic drugs. Our ongoing studies aim to improve our understanding of why male mice have a higher level of vulnerability to the long-term effects of prenatal stress.

Keywords: Glutamate Receptor, Gene Expression, Sex Differences

Disclosure: Nothing to disclose.

P467. Real-Time fMRI Neurofeedback for Auditory Hallucinations in Schizophrenia Reduces Aberrant Auditory Cortex Activity and Connectivity With the Default Mode Network

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Background: Auditory hallucinations (AH) are a core symptom of schizophrenia, can be highly distressing, and do not respond to treatment with antipsychotic medications in 25-30% of schizophrenia patients. Non-pharmacological interventions targeted specifically to AH pathophysiology are critically needed to give AH sufferers greater control over their experiences of AH. According to the resting state hypothesis of AH (Northoff and Qin, 2011), AH may arise from abnormally elevated functional connectivity between the auditory cortex (located in the superior temporal gyrus, STG) and the anterior hub of the default mode network (i.e., medial prefrontal cortex, mPFC), resulting in inappropriately elevated endogenous activity in the auditory cortex as well as heightened attention to, and increase in self-relatedness of internally generated voices. We have previously demonstrated AH reduction following real-time functional magnetic resonance imaging (rt-fMRI) neurofeedback targeting the STG, a technique in which individuals use real-time feedback about STG activity to volitionally modulate activity in that area (Okano et al., 2020). In this randomized sham-controlled study, we sought to investigate the effect of real rt-fMRI neurofeedback (from STG) vs. sham rt-fMRI neurofeedback (from primary motor cortex) on STG activity and STG-mPFC connectivity in schizophrenia patients with AH. We predicted that real, STG neurofeedback but not sham neurofeedback would reduce STG activity and STG-mPFC functional connectivity.

Methods: We acquired data from 25 male and female schizophrenia patients with frequent AH (Positive and Negative Syndrome Scale P3 item ≥ 4). Participants were randomized to receive a single session of either real ($n = 12$ NFreal) or sham ($n = 13$ NFsham) rt-fMRI neurofeedback. In the NFreal condition, patients were shown a visual display of real time blood oxygen level dependent (BOLD) activity delivered from their individually localized STG while engaging in a voice recognition task. In the task, pre-recorded voice recordings were played back in the scanner and patients were instructed to upregulate their STG activity by attending to sentences in their own voice and downregulate STG activity by ignoring sentences spoken in a stranger's voice. Patients in the NFsham condition were given the same instructions (attend to own voice, ignore stranger's voice) but shown BOLD activity delivered from their motor cortex rather than STG. Immediately before (transfer 1) and after NF (transfer 2), we scanned patients while they engaged in the voice recognition task in the absence of NF. We also acquired resting state fMRI scans before and after NF. We analyzed data from the voice recognition task to investigate within subject pre-post NF changes in STG activity, and the resting state fMRI scans to investigate pre-post NF changes in STG-mPFC functional connectivity.

Results: The paired, pre-post NF t-test in the 12 NFreal patients with neurofeedback delivered from the subject's individually defined STG showed reduced STG BOLD activation during the voice recognition task in the post-NF (transfer 2) scan relative to the pre-NF (transfer 1) scan ($p < 0.002$). Paired, pre-post NF t-test in the 13 NFsham patients did not show this effect in STG activity ($p < 0.19$). In addition, STG-directed NFreal resulted in a reduction in resting state mPFC-STG functional connectivity, computed with mPFC as a seed region, in the NFreal [($t(11) = 5.57$, $p < 0.001$)], but not in the NFsham group [$t(12) = 1.41$, $p < 0.183$]. The reduction in mPFC-STG connectivity in the post-NF scan relative to the pre-NF scan was observed in each of the 12 patients who underwent STG-directed NFreal.

Conclusions: These results provide preliminary evidence in support of the resting state hypothesis of AH and suggest that STG-directed real neurofeedback may be effective in decreasing the abnormally elevated STG activity and mPFC-STG connectivity that have been proposed to underlie the experience of AH in individuals with schizophrenia.

Keywords: Real-Time fMRI Neurofeedback, Schizophrenia (SCZ), Auditory Hallucinations, Auditory Cortex, Default Mode Network

Disclosure: Nothing to disclose.

P468. Cell-Type Specific Alterations in Cannabinoid Receptor 1 (CB1) in Patients With Schizophrenia

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Background: Alterations in cannabinoid receptor 1 (CB1) are implicated in schizophrenia. However, previous CB1 receptor ligand binding and immunoreactivity studies showed discrepant results in the prefrontal cortex of schizophrenia. These studies utilized antibodies that preferentially target CB1 at inhibitory boutons, and the exclusion of CB1 measurement in excitatory boutons may underlie the discordant findings. CB1 participates in both depolarization-induced suppression of inhibition and excitation, and understanding cell-type specific CB1 alterations in schizophrenia may increase our insight into its influences on excitatory-inhibitory balance disruptions within the pathology. Here, we investigate CB1 distribution in both inhibitory and excitatory boutons in schizophrenia using quantitative fluorescent microscopy.

Methods: Postmortem tissues containing the dorsolateral prefrontal cortex (DLPFC) from 10 pairs of subjects with schizophrenia and nonpsychiatric comparisons were used. These subject pairs, matched for sex, age, and postmortem interval, were previously found to demonstrate reciprocal alterations between CB1 ligand binding and immunoreactivity study results. Sections were labeled with antibodies for CB1 (validated to target both excitatory and inhibitory boutons), vGlut1 (labeling intracortical excitatory boutons), and vGat (labeling intracortical inhibitory boutons). Fluorescent intensities of CB1 within vGlut1- and vGat-positive boutons were analyzed across all cortical layers using quantitative fluorescence microscopy.

Results: CB1 co-expression with both vGlut1 and vGat were visualized in axons and boutons across all cortical layers. CB1 fluorescent intensity was lower in excitatory boutons compared to inhibitory boutons and exhibited distinct patterns within each cortical layer.

Conclusions: Previous studies examining CB1 alterations in postmortem cortex of subjects with schizophrenia utilized antibodies preferentially targeting CB1 at inhibitory boutons. Using a CB1 antibody that targets both excitatory and inhibitory boutons, we demonstrated distinct cell-type specific distributions of CB1 in the DLPFC of subjects with schizophrenia and nonpsychiatric comparisons. This suggests a possible mechanism by which CB1 dysregulation alters excitatory-inhibitory balance within schizophrenia. Expanding upon these findings, we plan to evaluate cell-type specific CB1 synaptic proteomics in schizophrenia as a potential contributor to the functional disturbances seen in this disorder.

Keywords: Endocannabinoids, Cell Type Specific, Postmortem Brain Tissue

Disclosure: Nothing to disclose.

P469. Multivariate Relationships Between Functional Connectivity and Social Cognitive Performance Across Schizophrenia Spectrum Disorders and Healthy Controls

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Background: Schizophrenia spectrum disorders (SSDs) often feature social cognitive deficits, associated with functional outcome. Social cognition can be divided into lower-level (e.g., emotion recognition) and higher-level (e.g., theory of mind) processes, subserved by partially dissociable neural networks. Recent work suggests that neural activation patterns during social processing may relate to cognitive performance rather than diagnosis across SSDs and healthy controls. Our objective was to identify multivariate relationships between functional connectivity during a social processing task and social and non-social cognitive performance across individuals with SSDs and healthy controls. We hypothesized that functional connectivity in relevant regions would covary with specific behavioral domains, perhaps reflecting the delineation of lower- and higher-level social cognitive and non-social cognitive constructs.

Methods: Data come from the Social Processes Initiative in the Neurobiology of the Schizophrenia(s) (SPINS) study. Across three sites, 197 people with SSDs and 157 healthy controls (216 males, 138 females) completed the Empathic Accuracy task during functional magnetic resonance imaging, a naturalistic social processing task. Participants also completed measures of lower- and higher-level social cognition and non-social cognition outside the scanner. Partial least squares correlation (PLSC) was used to identify latent variables capturing multivariate brain-behavior relationships with maximal covariance from a 'brain set' of functional connectivity metrics (between 392 regions of interest) and a 'behavior set' of cognitive performance measures (9 social cognitive and 6 non-social cognitive metrics). Permutation testing (1000 iterations) and bootstrap resampling (1000 iterations) were used to evaluate the significance of identified latent variables, and the reliability of contributing brain and behavior measures, respectively.

Results: PLSC followed by permutation testing identified two significant latent variables ($p < .05$), explaining 74.0% and 10.3% of the variance, respectively. The first latent variable was characterized by an association between connectivity across much of the brain, including frontal, occipital, temporal, parietal, and subcortical regions, and better performance across both lower- and higher-level social and non-social cognitive measures. The second latent variable was characterized by an association between frontal-parietal and temporal-parietal connectivity, among other regions, and worse social cognitive performance on a subset of lower-level social cognitive measures.

Conclusions: The data-driven delineation of social cognitive constructs is of particular interest given its relationship with

functioning and the need to identify treatment targets in SSDs. Our results suggest that patterns of functional connectivity during social processing are associated with both social and non-social cognitive performance across people with SSDs and healthy controls. A general pattern of increased connectivity may be related to better overall cognitive performance across groups. Interestingly, lower-level social cognitive performance also appears to be negatively associated with increased connectivity in a subset of regions, indicative of functional connectivity patterns that may delineate this aspect of social cognitive processing.

Keywords: Schizophrenia Spectrum Disorders, Social Cognition, Functional Connectivity, Neurocognition, Multivariate Analysis

Disclosure: Nothing to disclose.

P470. Mismatch Negativity and Theta Band Oscillation Deficits During Auditory Deviance Processing in Early Illness Schizophrenia

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Background: Amplitude reduction of mismatch negativity (MMN), an event-related potential (ERP) component thought to index NMDA receptor-dependent auditory echoic memory and predictive coding, is a widely-replicated finding in schizophrenia. MMN is pre-attentively and automatically elicited when a sequence of repeated standard sounds is interrupted by an infrequent discriminable deviant sound. Traditionally, the response to auditory deviance has been studied with the MMN ERP component; however, recent research has begun to use time-frequency analysis of single trial EEG epochs time-locked to standard and deviant stimuli to better understand event-related oscillatory activity underlying MMN, including measures of event-related spectral perturbation (ERSP; aka total power) and inter-trial phase coherence (ITC; aka cross-trial phase synchrony). Research has identified increases in theta band activity in response to deviant stimuli, relative to standard stimuli, as the principle neuro-oscillatory response to auditory deviance, with a number of studies showing ERSP and/or ITC theta deficits during auditory deviance processing in chronic schizophrenia. However, the status of theta oscillatory responses to deviance in the early course of schizophrenia (ESZ) has received less attention. Accordingly, we examined both the MMN ERP component and ERSP and ITC theta oscillations elicited during a multi-deviant MMN paradigm in ESZ patients within five years of illness onset and compared them to healthy controls (HC).

Methods: ESZ ($n = 105$) and HC ($n = 89$) male and female participants underwent EEG recording during two-deviant (duration, frequency) and single-deviant (duration + frequency "double-deviant") MMN paradigms, yielding three MMN variants: duration-deviant (DUR), frequency-deviant (FREQ), and double-deviant (DBL). In the two-deviant paradigm, 80% of the stimuli were standard tones (50 ms, 633 Hz), 10% were DUR (100 ms, 633 Hz), and 10% were FREQ (50 ms, 1000 Hz). In the single-deviant paradigm, 90% of the stimuli were standard tones (50 ms, 633 Hz) and 10% were DBL (100 ms, 1000 Hz). As MMN activity is maximal over frontocentral electrodes, analyses were focused on six frontocentral electrodes. ERP amplitude for MMN was calculated by subtracting standard tone ERP waves from deviant tone ERP waves, and then identifying the most negative peak between 90-290 ms in these difference waveforms. Group ERP amplitude differences between HC and ESZ groups were assessed using a repeated measures analysis of variance (rmANOVA) with Group (HC and ESZ) as the between-subjects factor, Deviant Type (DUR, FREQ, DBL) and Lead (frontal and central) as within-subjects factors, and age as a covariate. For ERSP and ITC, single-trial EEG epochs time-locked to auditory standards and deviants were calculated using a Morlet wavelet decomposition. Theta ERSP and ITC for deviants were calculated by extracting and averaging values from windows of 100-200 ms and 4-6 Hz (DUR) and 75-175 ms 4-6 Hz (FREQ and DBL). Theta ERSP and ITC for standards were calculated by extracting and averaging values from a window of 75-200 ms and 4-6 Hz. Group theta ERSP and ITC differences between HC and ESZ groups were each assessed using a rmANOVA with Group (HC and ESZ) as the between-subjects factor, Stimulus Type (standard and deviant), Deviant Type (DUR, FREQ, DBL), and Lead (frontal and central) as within-subjects factors, and age as a covariate. Additionally, we assessed if ERP amplitude and theta ERSP and ITC measures from deviant stimuli were associated with antipsychotic dosage and current symptom severity in the ESZ group.

Results: Relative to HC, the ESZ group showed ERP MMN amplitude deficits across all deviant types ($p = .006$). For theta ITC, there was a significant Stimulus Type x Deviant Type x Lead x Group interaction ($p = .005$). Parsing this interaction, HC had greater theta ITC than ESZ for both DUR deviants ($p = .005$) and standards ($p = .023$) at central electrodes. HC also had greater theta ITC than ESZ across both standards and deviants ($p = .015$) at frontal electrodes. For ERSP, there was a significant Stimulus Type x Lead x Group interaction ($p = .050$). Parsing this interaction, HC had greater ERSP than ESZ across deviants ($p = .014$) at central electrodes, but groups did not show theta ERSP differences for standards. Additionally, greater MMN amplitude deficit was associated with greater antipsychotic dosage ($r = .35$, $p = .001$), but not symptom severity, in the ESZ group. Theta ERSP was not significantly correlated with clinical measures.

Conclusions: Current study findings suggest that ERP and EEG-time frequency measures are sensitive to deficits in response to auditory deviance in schizophrenia. In a replication of our previous work, we did not find a differential effect of MMN deviant type amplitude deficits in ESZ. Similarly, we found an overall reduction in theta power across deviants in ESZ. In contrast, consistent with previous research, ESZ showed a deficit in theta ITC for DUR, but not FREQ and DBL. These ITC results suggest that theta phase synchronization deficits in ESZ are specific to duration deviants, whereas ESZ show deficits in MMN amplitude, as well as theta power responses to standards and to deviants, irrespective of deviant type, suggesting that these measures reflect distinct underlying mechanisms. Overall, findings provide novel evidence for theta neuro-oscillatory deficits as a potential illness biomarker during early stages of psychosis.

Keywords: Auditory Mismatch Negativity, Schizophrenia (SCZ), Event-Related Potential, Time-Frequency, Theta Band Oscillatory Measures

Disclosure: Nothing to disclose.

P471. NMDAR Hypofunction via D-Serine Availability Disrupts Excitation/Inhibition Balance and Causes Social Deficits in Adolescent Mice

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Background: Alterations in the ratio of activity in the cortical excitatory and inhibitory (E/I) balance is increasingly evidenced to be central to the etiology of multiple neurodevelopmental disorders including schizophrenia (SZ). SZ patients experience deficits in social cognition, which are linked to reduced levels of inhibition. Although these social abnormalities in SZ are

associated with altered cortical circuitry in adulthood, our understanding of the timing and mechanisms involved in normative cortical inhibitory interneuron (CIN) circuit maturation that subserve these behaviors are limited. Large-scale genetic studies have identified numerous SZ risk genes that encode proteins important for glutamatergic neurotransmission. We and others have established aberrant social and cognitive deficits in glutamate N-methyl-D-aspartate receptor (NMDAR) hypofunction rodent models, however a remaining question is whether the onset of phenotypic changes has a developmental time course that reflects symptom onset. NMDARs are unique compared to other glutamate receptors, because in addition to the binding of glutamate, NMDAR activation requires the binding of a co-agonist, either glycine or D-serine. D-serine is racemized from L-serine by the enzyme serine racemase (SR). Mice lacking D-serine due to genetic deletion of SR (SR knockout; SR-/-) display forebrain NMDAR hypofunction. We previously demonstrated that SR-/- mice have reduced GABA immunostaining embryonically and fewer CINs in the medial prefrontal cortex (mPFC) at postnatal day 16. Here, we investigate how constitutive NMDAR hypofunction affects mPFC E/I balance at juvenile and adolescent timepoints. We further determine whether aberrant behavioral phenotypes were present in adolescent SR-/- mice which could inform our understanding of maturational events in NMDA receptor signaling relevant to schizophrenia onset.

Methods: Wild-type and SR knockout (SR-/-) mice were generated from heterozygous SR +/- breeding pairs, and group-housed male mice were used for all experiments. For the three-chambered social interaction test, time that mice voluntarily spend proximal to an unfamiliar social stimulus mouse vs a novel object or a familiar mouse were compared between genotypes. For western blot experiments, brains were collected at postnatal days (PND) 16 and 30. For mPFC electrophysiology recordings, to measure Layer 4-evoked postsynaptic potentials (PSPs), a bipolar, nichrome wire stimulating electrode was placed in Layer 4 and current clamp recordings at holding potential of -60 mV were made from Layer 2/3 pyramidal neurons in the absence of synaptic blockers. E/I ratio was calculated from averaged baseline subtracted traces as the maximum depolarization amplitude (in mV) divided by the maximum hyperpolarization amplitude in the 600 ms after the stimulus. The McLean Hospital and The University of California Davis IACUC approved all animal care and experimental procedures. Two-way ANOVA and unpaired t-test were used to determine significance. $N = 8$-19/genotype for all experiments.

Results: At PND 16, there was a significant reduction in the IPSP, but not EPSP component of the compound EPSP/IPSP, resulting in an increased E/I ratio in Layer 2/3 of the mPFC. At PND30, SR-/- mice have significantly reduced levels of GABA neurotransmission protein markers, including the GABA transporter GAT1, in the mPFC. At PND27-31, SR-/- mice display abnormalities in social novelty, but not sociability, in the three-chamber social interaction test, which engages the prefrontal circuitry.

Conclusions: Our in vitro electrophysiological results suggest that the reduced number of mPFC INs we previously observed at PND16 in SR-/- mice alters the E/I balance, and provides evidence that NMDA hypofunction disrupts prefrontal inhibitory circuits during or before adolescence. These findings provide critical information on how the availability of NMDAR co-agonist D-serine, which is required for channel opening, controls aspects of prefrontal cortex neural circuits development that regulates the social behaviors disturbed in psychiatric disorders.

Keywords: NMDA Receptor, Excitation-Inhibition Balance, Schizophrenia-Like Behavior, Early Adolescence, Cortical Interneurons

Disclosure: Nothing to disclose.

P472. Discovery of Potentiators of the T-type Calcium Channel Cav3.3 to Potentially Treat the Sleep Spindle Deficits Observed in Schizophrenia Patients

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Background: Schizophrenia is a debilitating disorder that lacks effective treatments for the negative symptoms (e.g., social withdrawal) and cognitive disruptions (e.g., working memory deficits) in patients. Recently, large scale human genetics studies including genome wide association studies (GWAS) and exome sequencing studies have identified many replicable genomic loci for schizophrenia risk. CACNA1I was implicated in schizophrenia risk by GWAS and rare variations and it encodes the functional core, α1 subunit, of CaV3.3 voltage-gated calcium channels. CaV3.3 channels are expressed in a subset of neurons including GABAergic neurons of the thalamic reticular nucleus (TRN) where they regulate neuronal excitability. The TRN has emerged as a crucial brain nucleus in the generation of sleep spindles, sleep dependent memory, focused attention, and cognitive flexibility, all of which are impaired in patients with schizophrenia. Our group and others have demonstrated that loss of CaV3.3 dramatically impairs TRN neuronal firing and reduces sleep spindles occurrences in mice, and reduction of sleep spindles is a highly reproducible trait of schizophrenia patients. Based on these genetic and biological evidence, we hypothesize that CaV3.3 potentiators could benefit patients with schizophrenia by rescuing sleep spindle deficits and improving sleep dependent cognitive function. However, there is a lack of selective and potent CaV3.3 small molecule potentiators, therefore, we set out to identify highly selective and potent Cav3.3 potentiators.

Methods: We first established a novel primary high-throughput screening (HTS) fluorescence-based assay utilizing a customized membrane tethered GCaMP6s to capture the increased calcium influx mediated by CaV3.3 in HEK cells. The novel FLIPR (Fluorescent Imaging Plate Reader) assay enabled an HTS campaign of ~80,000 compounds, and we subsequently identified 5 validated CaV3.3 potentiator hit compounds in the HTS. We then selected two hit compounds to optimize for potency and selectivity against Cav3.2 and Cav3.1 channels using iterative medicinal chemistry. High throughput in vitro electrophysiology using automated patch clamp (Nanion Technologies) was then used to evaluate the compound mechanism of action on channel properties. Counter screens against Cav3.1 and Cav3.2 were performed with both FLIPR and electrophysiology assays. Additional compound selectivity was evaluated against a broad selectivity/safety panels using functional assays (SafetyScreen Functional Panel, Cerep). The effects of selective compounds were then evaluated on the known Cav3.3-dependent hyperpolarization induced rebound bursting of TRN neurons using whole-cell patch clamp electrophysiology in acute 270um horizontal mouse brain slices containing TRN obtained from 3-4 week old C57bl6 mice. Patched TRN neurons in current clamp configuration were hyperpolarized by a 500ms hyperpolarizing pulse and rebound burst firing was observed at every 2mV membrane potential ranging from -80 mV to -56 mV. Hyperpolarization induced bursts were defined as events containing 2 or more action potentials with a maximum of 70 ms interspike interval. Data are expressed as mean ± SEM.

Results: Utilizing cell based molecular pharmacology, high throughput automated patch clamp electrophysiology and a Cav3.3 dependent native tissue assay in which we performed

whole cell patch clamp of TRN neurons, we identified a set of potent CaV3.3 potentiators that selectively enhance CaV3.3 function. Here we highlight Compound A, which displays an EC50 of 0.54 μM in our cell based Cav3.3 FLIPR assay and displays an V1/2 activation EC50 of 5.5 μM and a V1/2 inactivation EC50 of 5.5 μM in our high throughput Cav3.3 electrophysiology assay. Importantly, Compound A displayed no activity at Cav3.2 and Cav3.1 in our high throughput electrophysiology assays. Furthermore, in ex vivo whole cell patch clamp electrophysiology experiments on mouse TRN neurons, we report that application of Compound A significantly increased the Cav3.3-dependent hyperpolarization induced rebound bursting of TRN neurons (Δ in burst number = 1.39 ± 0.98, $n = 13$ cells/N = 7 animals) compared to DMSO treated neurons (Δ in burst number = -1.59 ± 0.71, $n = 13$ cells/N = 7 animals) $p = 0.029$, Mann Whitney test. Additionally, when evaluating the effect of compound on the threshold membrane potential in which hyperpolarization induced rebound bursting occurs, cells treated with Compound A fired hyperpolarization induced rebound bursts at significantly more hyperpolarized membrane potentials (-1.62 mV ± 0.52, $n = 13$ cells/N = 7 animals; $p = 0.0089$ paired t-test) compared to baseline whereas 0.1% DMSO treated cells had no significant change in the threshold bursting membrane potential compared to baseline (-1.34 mV ± 1.15, $n = 13$ cells/N = 7 animals; $p = 0.25$ paired t-test).

Conclusions: Taken together, we present novel and unpublished results that identified Compound A as a potent and selective CaV3.3 potentiator that modulated CaV3.3 channel activities in ex vivo in brain slices. Given the critical role of Cav3.3 function of TRN in generating sleep spindles, Compound A may represent an exciting step towards the development of a novel class of potential therapeutics for the treatment of sleep disturbances in patients with schizophrenia.

Keywords: Schizophrenia Novel Treatment, T-Type Calcium Channel, Drug Discovery - New Approaches, Sleep Spindles, Electrophysiology

Disclosure: Nothing to disclose.

P473. Decoding Auditory Verbal Hallucinations Based on Patterns of Neural Activity in the Temporal Cortex in Schizophrenia

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Background: Auditory verbal hallucinations (AVH) are a core symptom of schizophrenia occurring in 60–80% of patients. Of these, about 30% are unresponsive to available treatments, indicating the need for novel therapeutic approaches. One possible approach that leverages recent advances in MRI and machine learning consists of real-time fMRI neurofeedback targeting multivoxel patterns of brain activity within a region, rather than average levels of activity in a region, also known as decoded neurofeedback (DecNef). This non-invasive approach may allow biasing brain states towards or away from specific target patterns (e.g., such as that associated with a phobic stimulus). However, this first requires developing a multivoxel decoder that can differentiate the relevant brain states with sufficient accuracy at the individual level, a step that is missing for AVH. Therefore, here we aimed to develop a multivoxel decoder of AVH in a sample of unmedicated patients with schizophrenia who actively hallucinated during an fMRI speech-discrimination paradigm, and tested its decoding accuracy for individual subjects.

Methods: Unmedicated patients with schizophrenia ($n = 32$; medication-free for at least 3 weeks) were scanned during a speech-discrimination fMRI task. They were presented with speech stimuli (68 trials), non-speech auditory stimuli (68 trials) or no stimulus (68 trials), and were prompted to respond on each trial whether they heard speech or not using a two-alternative forced-choice design. "AVH trials" were defined as no-stimulus trials in which patients reported hearing speech (false alarm) and "blank trials" were defined as no-stimulus trials in which they reported not hearing speech (correct rejection). Multi-band fMRI data were pre-processed using fMRIPrep and normalized to MNI space with 2x2x2 mm resolution. Of the patients, 8 had sufficient AVH trials (at least 10) to perform classification. A searchlight analysis was performed for each subject for every voxel within the temporal cortex (searchlight radius of 4 mm; 27 voxels total) using The Decoding Toolbox with linear support vector machines (SVM) trained to classify between AVH trials and blank trials via leave-one-trial-out cross-validation with balanced training classes. Individual-subject statistical significance was determined by permutation testing where the trial labels were shuffled 1,000 times (cluster-generating threshold of $p = 0.01$). Group-level permutation tests randomly selected the reshuffled first-level accuracy maps for each of the 8 subjects 10,000 times. Results were considered statistically significant at the cluster level for family-wise-error corrected p-values of 0.05. Follow-up analyses used sparse logistic regression using all voxels within an identified cluster as features to classify speech stimuli ("speech trials") versus non-speech auditory stimuli trials ("non-speech trials") and AVH versus blank trials. Here, again individual-subject significance was determined by permutation testing where the trial labels were shuffled 100 times ($p < 0.05$ considered significant). Family-wise permutation tests were performed to determine the chance level of obtaining the number of subjects with significant classification by randomly sampling the first level permutation results 10,000 times ($p < 0.05$ considered significant).

Results: The searchlight analysis identified a cluster of 62 voxels located in the right superior temporal gyrus (STG) where the pattern of activity within the searchlights could significantly distinguish AVH and blank trials (cluster-level corrected $p = 0.0462$; permutation test). The average classification accuracy across patients over each of the 62 searchlights was 57.05 +/- 4.20%. Individual-subject decoders using sparse logistic regression found that the activity patterns in this STG cluster could also classify speech versus non-speech trials (over 58% accuracy in 6 subjects, family-wise-error corrected $p = 0.0003$; permutation test), confirming this cluster's functional selectivity for speech. To explore the potential utility of the AVH-related activity patterns for DecNef in the STG cluster, we further evaluated classification accuracy at the individual level. We trained individual-subject decoders using sparse logistic regression to classify AVH and blank trials using all 62 voxels in the STG cluster. The average accuracy across subjects was 57.24 +/- 5.28%, with 5 out of 8 subjects showing significant classification accuracy (over 56% classification accuracy in 5 subjects, family-wise-error corrected $p = 0.0001$; permutation test) suggesting that subject-specific patterns may be viable targets for intervention.

Conclusions: These results provide initial evidence that AVH can be decoded from the pattern of neural activity in speech-selective regions within the temporal cortex. The pattern of activity in these voxels showed individual classification accuracies in a range that may permit pattern-based interventions such as DecNef, at least in some individuals or with sufficient training data for a given individual. Ongoing work is evaluating the generalizability of STG AVH decoders in an independent sample. If this finding generalizes, it may represent a first step in developing novel approaches to modulate AVH.

Keywords: MVPA, Auditory Hallucinations, Machine Learning Classification, Neurofeedback, Psychosis

Disclosure: Nothing to disclose.

P474. Exploring the Influences of Functional Connectivity Architecture on Cortical Thickness Networks in Patients With Early Psychosis

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Background: Cortical thickness and functional connectivity are two parallel approaches that have been widely used to gain insights into psychotic disorders. Significant abnormalities in these modalities, even at the early stage of psychosis, have been shown in the literature. However, few have studied them together or explored the influences of functional connectivity architecture on cortical thickness networks. Thus, it is not well understood whether cortical thickness reductions are seen in particularly vulnerable in regions with functional connectivity abnormalities. Prior studies reported that cortical thickness regions susceptible to thickness reductions are strongly interconnected [1] and that brain tissue volume loss in schizophrenia is conditioned by structural and functional connectivity [2]. Neufeld et al used data-driven co-variance methods to derive structural brain networks based on cortical thickness and showed that patients with remitted psychotic depression had significantly thinner cortex in five networks [3]. However, these previous studies used a gyral-based atlas, so whether the formation and organization of cortical thickness networks are functionally-shaped is not well understood. In this study, we applied a novel, data-driven approach by deriving cortical thickness networks using a functional network parcellation to both determine how cortical thickness is organized and identify regions that are key/hubs in each network in a cohort of early psychosis patients (ESP). In addition, we compared cortical thickness networks differences between ESP patients and controls to determine which networks are especially vulnerable to reductions. Given that cortical thickness reductions have been observed to be more severe in non-affective psychosis patients relative to affective psychosis patients [4], we further explored whether diagnosis specificity (affective and non-affective psychosis) would affect cortical thickness networks.

Methods: Data were collected from 237 participants (82 controls, 155 ESP patients) of both sexes. Structural magnetic resonance imaging (MRI) data were acquired on a 3T Siemens scanner. T1-weighted images were converted to NIFTI files, and analyzed with FreeSurfer 6.0 using a functional network parcellation for grey matter. Cortical thickness measures were calculated for 114 regions from both hemispheres that were assigned to 17 functional networks [5]. The measurements from all subjects were jointly analyzed using an orthogonal Non-negative Matrix Factorization (NMF) approach to determine regions that covary with thickness and formed features. The optimal number of features to describe the 237 patient by 114 region matrix was determined using cophenetic correlation. The regions associated to each feature were then organized into networks using Bayesian Belief Networks (BBN) to find the dependency structure between the highly correlated regions based on thickness. BBN analysis organized features into networks and identified the most interconnected regions that serve as a key role in the structure of the network. We also assessed the differences in the distribution between diagnosis (controls, affective psychosis, non-affective psychosis) for each feature in the NMF embedding space through a Kolmogorov-Smirnov test.

Results: The analysis of the NMF embeddings identified that cortical thickness regions were optimally organized and reduced into 8 features. Each feature and the regions that determined each feature appeared to be more spatially organized compared to functionally organized by functional networks. For example, features 1-3 were found to consist of mostly fronto-parietal, prefrontal cortex, and temporal regions, respectively. Thus, our results suggest that cortical thickness networks are spatially organized. BBN analysis organized the regions of each feature into a dependency structure and identified highly-connected network drivers. For example, in network 1, the superior parietal lobule in the right hemisphere was determined to be the key region. In testing the cortical thickness distribution, the Kolmogorov-Smirnov test revealed that networks 2 (prefrontal), 5 (parietal-temporal) and 6 (insula-prefrontal) were significantly different between ESP patients and controls, suggesting that regions in these cortical thickness networks are especially vulnerable to cortical thickness reductions as an effect of psychosis. A majority of cortical thickness networks also differed between affective and non-affective psychosis (5 out of 8) including: 1 (fronto-parietal) 2 (prefrontal) 3 (temporal) 6 (insula-prefrontal) 8 (cingulate-temporal), suggesting diagnosis specificity.

Conclusions: We used a novel approach to explore the influences of functional connectivity architecture on cortical thickness networks in patients with early psychosis. Our results show that cortical networks appear to be spatially organized and that several cortical thickness networks are particularly vulnerable in ESP patients with diagnosis specificity. These regions are likely to be involved in functions that are affected in illness, such as the prefrontal cortex regions that are linked to cognition.

Keywords: First Episode Psychosis, Structural Mri, Machine Learning, Early Psychosis

Disclosure: Nothing to disclose.

P475. Evidence for Schizophrenia-Specific Pathophysiology of Nicotine Dependence

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Background: Tobacco use is the top preventable cause of early mortality in schizophrenia, leading to a 25-year decreased life expectancy compared to the general population. Prior imaging studies of candidate brain circuits have not converged on a biological basis for schizophrenia's link to nicotine addiction. We therefore employed an entirely data-driven analysis of the connectome to identify both shared and schizophrenia-specific circuits of nicotine addiction.

Methods: We reanalyzed existing data from two neuroimaging studies using a data-driven approach. In the first cohort, thirty-five smokers (18 schizophrenia, 17 controls) underwent resting-state fMRI imaging and clinical characterization. We conducted a transdiagnostic assessment to identify shared and diagnosis-specific circuits of nicotine use. We used a multi-variate pattern analysis of whole-connectome data to identify the strongest links between daily cigarette consumption and functional connectivity. In the second cohort, twelve participants with schizophrenia and 12 controls were enrolled in a randomized, controlled crossover study of transdermal nicotine patch with resting-state fMRI. We calculated mean change in default mode network (DMN) connectivity (FCnicotine – FCplacebo) and correlated change in whole-network functional connectivity with nicotine dose.

Results: In cohort 1, the strongest ($p < .001$) correlate between functional connectivity and daily cigarette consumption was driven by individual variation in the topography of the DMN. This effect was entirely driven by participants with schizophrenia despite the fact that groups were matched for severity of nicotine dependence. Schizophrenia individuals with higher daily cigarette use had expanded DMN topography in a parieto-occipital region.

In cohort 2, we observed a linear relationship between nicotine dose and reduction in DMN connectivity (R = -0.50; 95% CI -0.75 to -0.12, $p < .05$). There was a significant effect of diagnosis on DMN connectivity. Schizophrenia subjects had DMN hyperconnectivity compared to controls in the placebo condition ($p < .05$), which was normalized following nicotine administration.

Conclusions: It has been hypothesized that the biological basis of nicotine dependence is different in schizophrenia and in non-schizophrenia populations. We here provide direct evidence in support of this hypothesis by demonstrating that tobacco use is strongly linked to brain network organization only in participants with schizophrenia. Our results suggest that the high prevalence of nicotine use in schizophrenia may be a product of both a hyperconnected DMN that interferes with cognitive performance and is more sensitive to nicotine in schizophrenia compared to controls. Future experiments will directly test the acute effect of nicotine on this network and on measures of cognitive performance in schizophrenia and control populations.

Keywords: Schizophrenia (SCZ), Nicotine Dependence, Resting State Functional Connectivity, Default Mode Network (DMN)

Disclosure: Nothing to disclose.

P476. Test-Retest Reliability of the Aperiodic 1/f Slope: A Novel Biomarker of Cortical Function for Therapeutic Development in Schizophrenia

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Background: Schizophrenia (SZ) is a debilitating neuropsychiatric disorder with pervasive neurocognitive impairment and poor functional outcomes. While several promising pharmacologic and non-pharmacologic interventions targeting the cognitive impairment in SZ exist, the variability of individual treatment responses remains problematic. Neurophysiologic biomarkers may help parse the heterogeneity of individual treatment responses for pro-cognitive therapeutics in SZ. We previously reported that acute doses of the NMDA receptor antagonist, memantine (20 mg p.o.), transiently 'normalized' abnormalities in cortical excitation and inhibition (E/I) balance as indexed by dynamic shifts in the aperiodic 1/f component of the neural power spectrum. In this secondary analysis, we assess the test-retest reliability of this novel biomarker.

Methods: Thirty-five subjects, including subjects with a diagnosis of schizophrenia (SZ, $n = 16$; M:$F = 12$:4; age = 36.3 (1.6), mean (sem)) and nonpsychiatric comparison subjects (NCS, $n = 19$; M:$F = 16$:3; age = 29.1 (2.1), mean (sem)) underwent electroencephalography (EEG) recordings using a passive auditory oddball task. Subjects were tested on two days about a week apart, after ingesting either placebo or 10 or 20 mg MEM p.o., in a double-blind, within-subject cross-over randomized design. For the present analyses, only data from the placebo and MEM (10 mg) conditions— which our laboratory has found to have no detectable impact on E/I balance or other encephalographic measures—are reported. The aperiodic component of the EEG power spectrum was estimated from individual power spectral densities using a robust linear regression algorithm and was averaged across trials. Test-retest correlations were calculated from the composite aperiodic index (i.e., slopes collapsed across channels) using Pearson's r. Complementary analyses of aperiodic slope correlations as a function of scalp distribution are provided. Fisher's r-to-Z transformation was used to assess differences in correlation coefficients between groups.

Results: Analyses revealed strong test-retest correlations for the composite aperiodic index ($r = 0.94$, $p < 0.0001$). The magnitude of these correlations were similar across diagnostic groups and were not significantly affected by test order, age, sex, or chlorpromazine equivalents (SZ only). Follow up analyses assessing the reliability of the aperiodic slopes across scalp topographies revealed 'good' to 'excellent' test-retest correlations ($r = 0.63 - 0.96$, p's < 0.0001) across all subjects. Correlations were similar between diagnostic groups and were unaffected by covariates.

Conclusions: Findings suggest that aperiodic slopes are reliable indices of cortical function in SZ patients and nonpsychiatric comparison subjects. While these results are preliminary, as they were obtained after the ingestion of placebo as well as a 'biologically inert' dose of memantine (10 mg), they suggest that aperiodic slopes may be useful biomarkers for the assessment of therapeutic sensitivity in pro-cognitive clinical trials for SZ and other neuropsychiatric disorders. Current studies are being conducted to confirm the reliability of this novel biomarker-setting the stage for its application in future personalized medicine trials and pro-cognitive therapeutic development.

Keywords: EEG Biomarkers, Experimental Therapeutics, Test-Retest Reliability, Psychosis

Disclosure: Nothing to disclose.

P477. Joint Influences of Common Genomic Variants and Anticholinergic Medication Burden on Cognitive Performance in a Diverse Cohort of US Veterans With Schizophrenia or Bipolar I Disorder

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Background: Cognitive impairments are a hallmark of schizophrenia, with the most severe deficits tending to be in processing speed, working and episodic memory, and executive functioning. Our group and others have shown previously that polygenic risk scores (PRS) constructed from genome-wide association studies (GWAS) in the general population are generalizable to patients with schizophrenia or bipolar I disorder, albeit explaining only a small fraction of the variance in cognitive performance. Following a recent report demonstrating the robust association of anticholinergic medication burden (ACB) across multiple cognitive domains, we extend these findings to VA Cooperative Studies Program (CSP) #572.

Methods: Leveraging the VA's extensive electronic health record (EHR) for 9,378 individuals with confirmed diagnoses of schizophrenia or bipolar I disorder, we constructed individual-level ACB scores from prescription records and clinical procedure terms (e.g. for LAIs) within 90 days of participants' in-person assessments. For >8,000 participants genotyped on a custom Affymetrix Axiom Biobank array, we constructed PRS for schizophrenia and intelligence. We jointly modeled the effects of ordinally-coded ACB (0-2 => "low"; 3-4 => "intermediate"; 5 or greater => "high") and selected PRS on a univariate latent factor ("g") derived from multiple tests of neurocognitive and everyday functioning, covarying for age, gender, ancestry, and diagnosis.

Results: Overall, age explained the greatest amount of variance in cognitive functioning (13-18%), followed by differential diagnosis of schizophrenia or bipolar disorder (~5%). Among European Americans (EA), ACB and PRS explained 2.5% and ~1% of variance, respectively, compared to 1% and 0.32% in African Americans (AA). Compared to low ACB, intermediate ACB

conferred a significant negative effect on cognition in EA (β = -0.070; 95% CI:[-0.107,-0.033]; $P = 0.0002$) and AA (β = -0.059; 95% CI:[-0.110,-0.007]; $P = 0.00255$), with markedly larger effects observed for high ACB in both EA (β = -0.200; 95% CI:[-0.238,-0.162]; $P < 10\text{-}23$) and AA (β = -0.149; 95% CI:[-0.202,-0.096]; $P < 10\text{-}7$). In the combined model, PRS for intelligence remained significant in both EA (β = 0.0635, 95% CI:[0.046,0.081]; $P < 10\text{-}12$) and AA (β = 0.035, 95% CI:[0.011,0.059]; $P < 0.01$). Exploratory analyses indicated a significant interaction effect between age and high ACB (β = -0.086, 95% CI:[-0.126,-0.045]; $P < 10\text{-}4$).

Conclusions: We replicated prior findings demonstrating robust effects of ACB on cognitive performance among schizophrenia patients, extending these findings to bipolar I disorder and further demonstrating that relevant influences of ACB and PRS are largely independent. Ongoing analyses will explore associations between ACB and treatment outcomes and comorbidities across the lifespan, and evaluate whether jointly modeling ACB in GWAS can empower so-called "gene-finding" efforts.

Keywords: Anticholinergic Medication Burden, Polygenetic Risk Score, Cognitive Functioning, Veterans, Schizophrenia and Bipolar Disorders

Disclosure: Nothing to disclose.

P478. Psychopathology and D1 Receptor Availability in Schizophrenia

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Background: A central role for dopamine systems dysfunction in schizophrenia pathophysiology has been an unequivocal foundation of our understanding of this debilitating disorder. Along with the facts that all first-line antipsychotic medications target dopamine receptors and pro-dopaminergic agents may induce psychosis, this role has found ample support from molecular neuroimaging studies that have permitted direct, in vivo assessments of central dopamine systems in patients. In this literature, both presynaptic dopamine function in the basal ganglia and dopamine-dependent cortical circuitry have been frequently implicated, though a full mechanistic understanding of observed deficits remains elusive. Given long-standing hypotheses about D1 dopamine receptor related mechanisms at play in the prefrontal cortex in schizophrenia, several prior case-control studies have used [11C]-NNC112 positron emission tomography (PET) to quantify cortical D1 receptor availability in the cortex and have yielded a complex collection of findings. Relative to controls, both decreases, as seen in a study of treated patients, and increases, as seen in studies of medication-free and medication-naïve patients, have been observed. Schizophrenia is a highly heterogeneous condition, with substantial interindividual variability in symptom profiles and severity, and it is likely that such variability is undergirded by differences across patients at the neural level, which has relevance for biomarker and therapeutic development. We have recently found that presynaptic dopamine systems may vary as a function of clinical presentation, yet is unclear whether postsynaptic D1 receptor availability is meaningfully linked to the characteristics of individual patient's symptom burdens.

Methods: Thirty individuals (mean age 28.7 +/-8.6 years; eight female) with schizophrenia spectrum disorder underwent clinical assessments and PET scans in a medication-free state (at least four weeks) in a closely-monitored inpatient research setting at the NIH Clinical Center in Bethesda, MD. Positive and Negative Syndrome Scale (PANSS) ratings quantified total symptoms and five-factor subscale ratings as previously established. D1 receptor availability was estimated using [11C]-NNC112 PET. A 20 mCi bolus was administered to participants, with a 90 minute, dynamically binned series of emission frames collected immediately thereafter. All scans were preceded by a transmission scan for attenuation correction, and participants refrained from caffeine or nicotine for four hours prior to scanning. PET frames were aligned to separately collected T1 weighted structural MRI volumes and then warped to a common space. A native-space derived cerebellar time-activity curve served as the reference input to a voxelwise simplified reference tissue model calculation performed with PMOD software. The resulting binding potential (BPnd) parameter was then subjected to regression analyses with total, positive, and negative symptom scores, controlling for age and sex. Findings meeting an uncorrected voxelwise threshold of $p < 0.005$ were reported.

Results: Positive relationships between BPnd and total symptoms were observed in both subcortical (bilateral caudate, right putamen) and, less prominently, cortical (right inferior frontal gyrus) regions. When positive symptoms were examined separately, a more pronounced array of cortical regions (bilateral dorsolateral prefrontal cortex and anterior cingulate cortex) showing a positive relationship with BPnd was evident, but no subcortical regions met threshold. When negative symptoms were examined separately, a positive relationship with BPnd was observed predominantly in the bilateral caudate and right putamen.

Conclusions: In individuals with schizophrenia spectrum illness, both cortical and subcortical D1 dopamine receptor systems may show correspondence to clinical differences in a symptom-specific manner. If replicated, these observations suggest upregulation of D1 receptors may characterize individuals with more symptom burden during the medication-free state. As D1 dopamine systems have been forwarded as a possible treatment target in schizophrenia, further work is needed to determine to what degree these findings are stable traits over time or fluctuate with illness course and treatment.

Keywords: Schizophrenia (SCZ), D1 Dopamine Receptors, Psychosis

Disclosure: Nothing to disclose.

P479. Analysis of Dynamic Methylation Changes in Relationship to Psychotic Symptoms in Subjects With Schizophrenia

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Background: Genes play a rather important role in mediating the influence of stressful life experiences on psychosis. Furthermore, there is also increasing evidence of epigenetic mechanisms influencing psychotic symptoms. Among the epigenetic modifications, DNA methylation is the most studied and easily detectable when surveying the entire genome.

The proposed project will investigate genome-wide methylation changes as predictors of worsening of psychotic symptoms in patients with schizophrenia.

Methods: We recruited 138 subjects with schizophrenia from the Centre for Addiction and Mental Health (CAMH) in Toronto assessed at study entry. DNA samples were collected for MethylationEPIC array profiling to determine genome-wide methylation. Brief Psychiatric Rating Scale (BPRS) severity was the primary outcome variable in the model, with methylation changes as predictor. Genome-wide methylation patterns were measured. Furthermore, 17 individuals were reassessed after three months at follow-up for changes in stress, psychosis severity and methylation patterns.

Results: There were no significant CpG sites associated with baseline psychosis severity or worsening at follow-up.

Conclusions: This study is one of the first to investigate dynamic methylation changes across the genome as predictor of psychosis severity in subjects with schizophrenia. We did not find genome-wide significant methylation sites associated with psychosis severity in this study. However, the investigation of dynamic changes in the epigenome can help to monitor symptom and treatment outcome in schizophrenia.

Keywords: Epigenetics, DNA Methylation, Schizophrenia (SCZ)

Disclosure: Nothing to disclose.

P480. Pimavanserin Ameliorates Negative Symptomsin Combined PCP and Stressed Mouse Model

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Background: Negative symptoms are a core, pervasive, and often treatment-refractory phenotype of schizophrenia, one which contributes to poor functional outcome, ability to work, pursue educational goals, and quality of life, as well as a caretaker burden. Improvement of negative symptoms in some patients with schizophrenia has been reported with some atypical antipsychotic drugs [AAPDs], but improvement is absent in many patients and partial in others

Methods: Treatment refractory mouse model 1: Sub-chronic [sc] treatment of mice with phencyclidine [PCP], 10 mg/kg; IP; bid for 7 days, followed by 7 days withdrawal. Following withdrawal, a subset of these sc-PCP mice will be given 2 hours acute restraint stress [2h-ARS]. 1-hour post-stress, behavioral tasks will be conducted. Sample size: 10-12/group.

Treatment refractory mouse model 2: Sub-chronic [sc] treatment of mice with phencyclidine [PCP], 10 mg/kg; ip; bid for 7 days, followed by 7 days withdrawal. Following withdrawal, a subset of these sc-PCP mice will be given 21 days of chronic unpredictable stress [21-days CUS]. One week post 21-days CUS, behavioral tasks will be conducted. Sample size: 10-12/group.

Behavioral tests: Novel object recognition task [NOR], locomotor activity [LMA], and social interaction [SI] to gauge object recognition domain of cognitive functioning, psychosis, and negative symptoms respectively.

Results: a. By themselves, in sc-PCP mice, the AAPDs, risperidone, olanzapine, and aripiprazole, but not pimavanserin [PIM], rescued the SI deficit, as did the combination of PIM with sub-effective doses of each of these AAPDs.

b. In both sc-PCP + 2h-ARS and sc-PCP + 21-days CUS mouse models, the AAPDs tested, alone, did not rescue SI deficit, indicating these stress mice were treatment refractory.

c. Co-administration of PIM with any of the AAPDs significantly restored SI in the mice which had received sc-PCP, sc-PCP + 2h-ARS, as well as sc-PCP + CUS mouse models. d. Clozapine alone, unlike the other AAPDs mentioned above, was effective to restore SI in the sc-PCP + 2h-ARS as well as sc-PCP + CUS treatment-refractory mouse models.

e. The combination of clozapine + PIM rescued cognitive and negative symptom deficits,

Conclusions: The combination of AAPDs and PIM rescued cognitive and negative symptom deficits, indicating that it may be an effective adjunctive therapy for treating cognitive impairment and negative symptoms in some patients who have failed to respond to AAPDs or PIM alone. Further study is required to determine whether other AAPDs and/or ligands, in combination with PIM may also be effective for refractory psychopathology.

Keywords: Atypical Antipsychotics, Treatment Refractory, Negative Symptoms

Disclosure: ACADIA Pharmaceutical: Stock / Equity (Self & Other Immediate Family Member)

P481. Geographical Variation in Hospitalization for Psychosis Associated With Cannabis Use and Cannabis Legalization in the United States

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Background: Prior studies have established an association between cannabis use and psychotic disorders. There is a lack of research on the impact of cannabis legalization on the risk of psychosis in the United States.

Methods: The 2017 National Inpatient Sample database was utilized to investigate the association between cannabis legalization in the United States and hospitalizations for psychosis associated with cannabis use. In patients ages 18-64 years, hospital discharges for psychosis were defined using ICD-10-CM diagnosis codes for cannabis-induced psychotic disorder (F12.15x, F12.25x, F12.95x) OR a combination of cannabis use disorder/poisoning (F12.x, T40.7x) and psychosis. Psychosis was defined as unspecified psychosis (F28, F29), brief psychotic disorder (F23), delusional disorder (F22), schizophrenia spectrum disorders (F20), schizoaffective disorder (F25), hallucinations (R44.0-3) and major depressive disorder or bipolar disorder with psychotic features (F32.3, F33.3, F30.2, F31.2, F31.5, F31.64). We compared the odds of hospital discharges for psychosis associated with cannabis use in the Pacific census division, where most states legalized medical and recreational cannabis use, with other divisions. Multivariable logistic regression estimated odds ratios (OR) and 95% confidence intervals (CI) adjusting for confounders. In a secondary analysis, we calculated a score for each census division representing cannabis legality as the population-weighted sum of state scores: 1=illegal or cannabidiol/low potency cannabis; 2=standard medical marijuana; and 3=recreational and medical marijuana legalized. Pearson's correlation coefficients (r) quantified the relationship between these scores and the proportion of hospitalizations with psychosis associated with cannabis.

Results: In 2017, there were an estimated 129,070 hospital discharges for psychosis associated with cannabis use. The Pacific census division had significantly higher odds of discharges for psychosis associated with cannabis compared with other divisions (adjusted OR 1.55; 95% CI 1.25 – 1.93). There was a significant positive correlation between the cannabis legality score and proportion of hospital discharges for psychosis associated with cannabis use in each census division ($r = 0.67$, $p < 0.05$).

Conclusions: In this cross-sectional population-based study of hospital discharges in the United States, we observed geographic variation in hospitalizations for psychosis with cannabis use associated with implementation of cannabis legalization. Given the rapid changes in legislative landscape, there is a need for further longitudinal research to better understand the nature of the relationship between cannabis legalization and the risk of psychosis.

Keywords: Cannabis, Psychosis, Marijuana Policy

Disclosure: Nothing to disclose.

P482. Anti-Viral and Pro-Inflammatory Genomic Transcription in Schizophrenia: Using a Pathway-Informed Bioinformatics Approach to Compare People With Schizophrenia With Non-Psychiatric Comparison Subjects

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Background: Prenatal viral infection and inflammation are plausible biological mechanisms underlying development of schizophrenia, supported by epidemiological and basic research findings. In utero exposure to viral infections has been linked to increased rates of schizophrenia in humans, and to differential brain gene expression, neurodevelopment, and neurocircuitry in animal models. Pro-inflammatory cytokines affect neurotransmission and are associated with severity of psychopathology. In people with schizophrenia (PwS) who show elevated inflammatory marker levels and early-stage psychosis, anti-inflammatory treatments may improve psychopathology.

Upregulated expression of antiviral and pro-inflammatory proteins has been observed in postmortem brain samples from PwS, but it is unclear whether similar differences occur in peripheral tissues. This study examined genome-wide RNA profiles in circulating immune cells using pathway-informed bioinformatics analyses to determine whether PwS show alterations in antiviral and/or pro-inflammatory gene regulation.

We hypothesized that 1) PwS would have upregulated expression of antiviral and pro-inflammatory genes compared to normal controls (NCs), and 2) bioinformatics analyses of empirical differences in gene expression would identify increased activity of antiviral transcription control pathways (e.g., Interferon Response Factor; IRF) and/or pro-inflammatory pathways (e.g., NF-kB, AP-1) in immune cells from PwS.

Methods: The cohort included 89 PwS (based on DSM-IV-TR criteria) and 95 age- and sex-comparable NCs (mean age 50.8, SD 11.5, range 28 to 71 years). The sample included equal proportions of men and women.

Peripheral blood mononuclear cell RNA was profiled by RNA sequencing (cDNA: Lexogen QuantSeq 3′ FWD; sequencing: Illumina HiSeq 4000 yielding average 13 million reads/sample; read mapping: STAR aligner; normalization: Transcripts Per Million mapped reads, with subsequent normalization to 11 standard housekeeping genes).

Mixed effect linear models analyzed the association between gene expression and study group (PwS vs NC), both unadjusted and controlling for age, sex, educational attainment, lifetime history of smoking, lifetime history of heavy alcohol consumption, BMI, and race/ethnicity. Primary analyses examined average expression of pre-specified sets of 32 gene transcripts involved in innate antiviral responses (Type I interferon response genes) and 19 pro-inflammatory genes. Secondary bioinformatics analyses tested for differential prevalence of transcription factor-binding motifs (TFBMs) for IRF, NF-kB, and AP-1-family transcription factors in the promoters of all genes found to differ by >2-fold in average expression in PwS relative to HC. Parallel Transcript Origin Analyses (TOA) was conducted on the same set of differentially expressed genes to identify specific cell types mediating empirical transcriptome differences.

Results: Primary analyses indicated elevated expression of antiviral genes in circulating immune cells from PwS (.135 ± .038, $t(9148) = 3.58$, $p = .0003$), which persisted when controlling for demographic and lifestyle factors (.115 ± .053, $t(7348) = 2.20$, $p = .0281$). In contrast, no differences in inflammatory gene expression were observed in unadjusted ($p = .3404$) or adjusted analyses ($p = .2085$). In secondary bioinformatics analyses of all 85 genes showing > 2-fold differential expression in PwS vs HC (79 up-regulated and 6 down-regulated), promoter-based bioinformatics indicated increased activity of the antiviral IRF transcription factor family (3.01-fold, 1.592 ± .361 log2, TFBM ratio in up- vs. down-regulated genes; $z = 4.41$, $p < .0001$), which persisted with adjustment for covariates (1.72-fold, .784 ± .405 log2, $z = 1.94$, $p = .0542$). Parallel analyses of pro-inflammatory transcription factors found no significant up-regulation of either AP-1 activity (.63-fold, $p = .037$) or NF-κB activity (.85-fold, $p = .379$). Parallel TOA bioinformatics implicated B lymphocytes as primary carriers of transcripts up-regulated in PwS (mean cell type diagnosticity score = .788 ± .222, $z = 3.56$, $p = .0003$) and NK cells as primary carriers of transcripts down-regulated in PwS (1.472 ± .221, $z = 6.67$, $p = .0005$).

Conclusions: Collectively, these results indicate a selective up-regulation of innate antiviral activity in peripheral immune cells from PwS. Our findings are consistent with hypotheses of viral involvement in the pathogenesis of schizophrenia, but might also arise from increased exposure to viral infections as a consequence of schizophrenia. Longitudinal analyses and novel assessments of inflammation (microbiome, acute immune challenges) and viral activity (metagenomic RNA sequencing) are warranted to better understand how antiviral and immune pathophysiology is linked to schizophrenia and how to develop targeted interventions. This study is continuing, and updated results will be presented at the meeting.

Keywords: Psychosis, Gene Expression, Immune Cells

Disclosure: Nothing to disclose.

P483. Impaired Contrast Gain Control in Psychosis

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Background: Contrast perception is a fundamental function of the human visual system. Previous work suggests impaired visual contrast perception among people with psychosis spectrum disorders (e.g., schizophrenia), but the neural basis of this anomaly remains unclear. One possibility may be a difference in gain control, which describes how neural responses change with increasing stimulus intensity. Effective gain control maintains the dynamic response range and prevents responses from becoming too large. If gain control is impaired in psychosis, this might account for abnormalities in visual contrast perception.

Methods: We recruited female and male participants from three groups: people with psychosis ($n = 52$), their first-degree biological relatives ($n = 43$), and healthy controls ($n = 43$). Studying relatives permitted us to examine the role of genetic liability for psychosis in visual perception. We measured visual contrast perception in a behavioral threshold versus contrast paradigm, with seven stimulus intensities between 0-20% contrast. Threshold versus contrast data were fit using standard (i.e., Naka-Rushton) equations, in order to obtain predicted contrast response functions from our behavioral data. We also acquired 7 tesla functional MRI data during visual contrast perception in the same participants, which allowed us to quantify neural contrast response functions in visual cortex. Finally, we obtained 7 tesla MR spectroscopy data from these participants, in order to quantify neural metabolite concentrations in early visual cortex.

Results: Contrast discrimination thresholds were overall higher (worse) among people with psychosis compared with relatives and controls ($X^2(2) = 6.80$, $p = 0.033$). Poorer contrast discrimination was correlated with higher psychiatric symptoms (BPRS; $r(105) = 0.29$, $p = 0.002$). Computational modeling of behavioral data revealed significant differences in contrast-response function parameters between groups (group differences for jackknifed model fits, X^2(2) values > 73.5, p-values < 1 x 10^-16). In particular, people with psychosis and relatives showed higher contrast response function slopes, which may be consistent with impaired contrast gain control. This was offset by a lower inflection point (both groups) and lower overall response scaling (especially in psychosis), such that predicted neural responses were lower for the psychosis group but not relatives, compared to controls. 7 T fMRI responses in primary visual cortex (V1) were

similar across groups ($F(2,95) = 1.66$, $p = 0.19$), indicating a mismatch between predicted and observed neural responses in people with psychosis. Glutamate and GABA levels in visual cortex from 7 T MR spectroscopy did not differ between groups (X^2(2) values > 2.58, *p*-values > 0.3), but higher GABA was correlated with poorer behavioral contrast discrimination across all subjects (r (100) = 0.23, $p = 0.02$).

Conclusions: Our results may be explained by a combination of two effects: 1) an overall deficit in task performance across all conditions in people with psychosis, yielding worse discrimination for all contrast levels, and 2) impaired contrast gain control in both the psychosis and relative groups, which leads to differences in the pattern of behavioral responses at certain stimulus intensities. If an overall impairment in discrimination task performance depends on a higher-level cognitive deficit, rather than lower neural activity overall in visual cortex (as predicted by the model), then this might be sufficient to explain the mismatch we observed between model predictions and V1 fMRI responses in people with psychosis. Our findings highlight the potential value of studying biological relatives of people with psychosis. Like affected individuals, behavioral data from relatives also appeared consistent with impaired gain control, suggesting that abnormal contrast gain control might be linked to genetic liability for psychosis. But unlike the psychosis group, relatives showed a good match between model predictions and V1 fMRI results, which may help us to differentiate the effects of a generalized deficit and impaired contrast gain control in this data set. Finally, although we found no group differences in glutamate or GABA, our spectroscopy results suggest that higher GABA levels may be associated with reduced contrast sensitivity across all subjects.

Keywords: Psychosis, 7 Tesla fMRI, Visual Perception, MR Spectroscopy

Disclosure: Nothing to disclose.

P484. Lower Somatostatin mRNA Levels Without Deficits in Somatostatin Neuron Density in the Dorsolateral Prefrontal Cortex of Schizophrenia

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Background: Cognitive dysfunction in schizophrenia is associated with altered GABA signaling markers in the dorsolateral prefrontal cortex (DLPFC). For example, levels of somatostatin (SST) mRNA, expressed in a subset of GABA neurons, are markedly lower in DLPFC gray matter in schizophrenia. The density of neurons with detectable SST mRNA is also lower in schizophrenia. However, whether schizophrenia is associated with fewer SST neurons or a failure of certain SST neurons to express detectable levels of SST mRNA remains unknown.

Methods: To identify all SST neurons, we used multiplex fluorescent in situ hybridization to simultaneously label neurons containing SOX6 (expressed in both SST and parvalbumin (PV) neurons) and vesicular GABA transporter (VGAT) mRNAs, neither of which appear altered in schizophrenia, and SST or PV mRNA. SOX6 + /VGAT + /SST + and Sox6 + /VGAT + /PV- neurons were considered SST neurons. The density of labeled neurons was quantified in 30 pairs of schizophrenia and unaffected comparison subjects. We focused on layer 2 and layer 4, given the enrichment for SST and PV neurons, respectively, in these layers. Subject pairs were matched perfectly for sex (22 M/8 F) and as close as possible for age. To provide the greatest likelihood of detecting a true deficit in SST neuron density, we selected subjects who had previously been reported to exhibit marked deficits in SST mRNA levels within total DLPFC gray matter homogenates. All procedures were approved by the University of Pittsburgh Committee for Oversight of Research and Clinical Training Involving Decedents and the Institutional Review Board for Biomedical Research.

Results: In DLPFC layer 2, mean density of SOX6 + /VGAT + /SST + neurons was significantly 31.3% lower in schizophrenia subjects (F1,51 = 7.6, $p = 0.008$), but the density of SOX6 + /VGAT + /PV- neurons (which includes all SST neurons regardless of SST mRNA level) did not significantly differ between subject groups (F1,51 = 1.4, $p = 0.25$; Bayes' Factor in favor of null hypothesis = 3.01). In DLPFC layer 4, the mean density of SOX6 + /VGAT + /SST + neurons was significantly 33.1% lower in schizophrenia subjects (F1,51 = 15.0, $p = 0.0003$), but the density of SOX6 + /VGAT + /PV- neurons did not significantly differ between subject groups (F1,51 = 0.43, $p = 0.51$; Bayes Factor in favor of null hypothesis = 2.0). Additionally, consistent with prior reports, the density of SOX6 + /VGAT + /PV + neurons did not significantly differ between subject groups in DLPFC layer 4 (F1,51 = 2.0, $p = 0.16$; Bayes' factor in favor of null hypothesis = 1.6). Studies in the same cohort using high resolution imaging to quantify levels of SST per neuron are in progress.

Conclusions: These findings suggest that the density of SST neurons is not altered in the DLPFC of schizophrenia but that a subset of these neurons fails to express detectable levels of SST mRNA. These findings, together with prior findings of a normal complement of PV neurons and no deficit in total neuron number in the DLPFC of individuals with schizophrenia, strongly suggest that schizophrenia is not characterized by abnormalities in cortical neuronal migration or neuronal death. The presence of a normal complement of SST neurons suggests that these neurons can be considered as potential targets for novel therapeutic interventions to improve cognitive dysfunction in schizophrenia.

Keywords: Somatostatin, Dorsolateral Prefrontal Cortex, Schizophrenia (SCZ), RNAscope Fluorescence in Situ Hybridization, Parvalbumin Neurons

Disclosure: Nothing to disclose.

P486. Incomplete Hippocampal Inversion Determines Hippocampal Shape in Schizophrenia

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Background: Shape analyses have revealed bilateral deformations of the antero-lateral hippocampus in schizophrenia. Hippocampal shape differences in schizophrenia are most prominent in the CA1 subfield. A recent study revealed that incomplete hippocampal inversion (IHI), an anatomical variant of the human hippocampus resulting from an arrest during neurodevelopment, is more prevalent and severe in patients with schizophrenia. We hypothesized that IHI contributes to hippocampal shape differences in schizophrenia.

Methods: We studied 199 schizophrenia patients and 161 healthy control participants with 3-Tesla MRI and measured the prevalence and severity of IHI in each hippocampus bilaterally using established, quantitative criteria. Both sexes were included in this study. Hippocampal surface reconstructions with 40,962 vertices per hemisphere were completed using the SPHARM-PDM toolkit. The local shape displacement of each participant's hippocampal surface was calculated from the cohort average of all participants by quantifying the perpendicular distance between surfaces at a vertex-to-vertex level. Vertex displacement was regressed onto IHI score to test the effect of IHI on hippocampal shape variation using SurfStat. We conducted a sensitivity analysis to test for group shape differences with and without IHI included as a main effect. All models included age, gender, and intracranial volume as covariates. Random Field Theory (RFT) was applied in SurfStat to account for multiple comparisons.

Results: IHI is associated with inward displacements of the lateral and medial surfaces and outward displacements of the superior and inferior surfaces of the hippocampus. Linear models not including IHI as a main effect replicate well-known hippocampal shape differences in schizophrenia patients localized to the CA1 region of the antero-lateral hippocampus (left hemisphere cluster: peak t358 = 3.73, cluster size = 533 vertices, RFT corrected $P = 1.34 \times 10^{-6}$; right hemisphere: peak t358 = 3.15, cluster size = 346 vertices, RFT corrected $P = 2.01 \times 10^{-5}$). Clusters of significance are also present in the uncus and hippocampal tail. Including IHI as a main effect in the model eliminates the bilateral significant shape differences in the CA1 subfield.

Conclusions: IHI impacts hippocampal shape and contributes to morphological differences observed in schizophrenia. Our results suggest that the well-known shape differences, particularly in the CA1 subfield, are due to an abnormal development of the hippocampus.

Keywords: Schizophrenia (SCZ), Hippocampus, Subcortical Shape Analysis, Neurodevelopment, CA1

Disclosure: Nothing to disclose.

P487. Elucidating the Relationship Between White Matter and Cognition in Schizophrenia

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Background: Previous research demonstrated that many individuals with schizophrenia experience cognitive deficits. Additionally, magnetic resonance imaging (MRI) studies suggest that cognitive deficits might be linked to white matter (WM) connectivity. Specifically, slower processing speed appears to be a core deficit in schizophrenia that is directly related to disrupted WM. However, several studies fail to connect cognitive deficits and WM microstructure, which suggests that the relationship could be changing depending on age range, chronicity and medication status. Here, we harmonize and combine multimodal data from over 900 individuals to explore cognitive deficits and their association with clinical variables and WM microstructure across the illness trajectory.

Methods: Data were collected from 13 independent, international sites and combined. Cognitive data was harmonized following a two-step approach. First, we calculated T scores for all cognitive tests the single sites had administered. Next, we sorted all cognitive tests into eight domains: language, processing speed, vigilance, working memory, verbal memory, non-verbal memory, motor function, and executive function. Finally, we combined cognitive data with already harmonized average positive and negative symptom scores, chlorpromazine equivalent dosages (CPZ), and whole-brain fractional anisotropy (FA, a measure for WM integrity).

We utilized ANCOVAs to compare the cognitive domains between individuals with schizophrenia and healthy individuals. We used Spearman's rank correlation coefficient to describe the association between cognitive performance and averaged positive and negative symptom severity. To investigate the influence of medication dosage on cognitive performance, we calculated eight ANCOVAs with the dependent variable cognitive performance, the independent variable CPZ group, and covariates age and sex. To investigate whether WM abnormalities can explain cognitive deficits, we then applied regression-mediation analyses to model the association between the group, whole-brain FA, and cognitive performance.

Results: Individuals with schizophrenia demonstrated worse cognitive performance than healthy individuals for all domains ($p < .006$, Bonferroni-corrected for eight domains, Cohen's d between .48 and 1.17). Poorer processing speed (rs(449) = -.17, $p < .0001$), vigilance (rs(272) = -.19, $p < .001$), working memory (rs(445) = -.14, $p = .0040$), and verbal memory (rs(442) = -.15, $p = .0020$) performance were associated with more severe positive symptoms. Individuals with schizophrenia who took higher dosages of daily medication presented with better processing speed ($F(3,4040) = 6.55$, $p < .0001$, partial Eta2 = .046), working memory ($F(3,399) = 13.08$, $p < .0001$, partial Eta2 = .090), executive function ($F(3,344) = 4.38$, $p = .0050$, partial Eta2 = .037), and worse language performance ($F(3,378) = 6.31$, $p < .0001$, partial Eta2 = .048).

Regression analyses demonstrated a significant association between whole-brain FA and language (standardized $B = .16$, $T = 3.24$, $p < .001$, adjusted R2 = .018), processing speed (standardized $B = .13$, $T = 2.65$, $p = .0080$, adjusted R2 = .011), working memory (standardized $B = .17$, $T = 3.55$, $p < .0001$, adjusted R2 = .067), verbal memory (standardized $B = .13$, $T = 2.78$, $p = .0060$, adjusted R2 = .016), and non-verbal memory (standardized $B = .25$, $T = 4.52$, $p < .0001$, adjusted R2 = .053) in individuals with schizophrenia. Mediation analyses highlighted that whole-brain FA partially mediates the association between language, processing speed, working memory, and non-verbal memory performance. Processing speed partially mediates the relationship between whole-brain FA and the other cognitive domains.

Conclusions: The present study is the first to investigate the association between cognitive deficits, clinical data, and WM microstructure in a well-powered, large-scale, multi-site retrospective, and harmonized dataset. We observe widespread cognitive deficits in individuals diagnosed with schizophrenia, most pronounced for processing speed and working memory. Our results suggest that individuals with schizophrenia who show more cognitive deficits experience more positive symptoms and that medication has a mixed effect. WM deficits partially explained cognitive deficits, demonstrating the central role of WM pathology for the disorder and supporting the disconnection theory. Our exploratory analyses imply that processing speed partially explains the association between a diagnosis of schizophrenia, WM microstructure, and cognitive deficits. This finding supports the idea that processing speed is a core deficit contributing to other cognitive deficits and might be a key marker for functioning and treatment response.

Keywords: Cognition, Diffusion Weighted Imaging, Schizophrenia (SCZ), Processing Speed, Scanner Harmonization

Disclosure: Nothing to disclose.

P489. Characterizing Distinct Pyramidal Cell Populations in the Ventral Hippocampus

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Background: The ventral hippocampus (vHipp) regulates a diverse set of behaviors associated with motivation and emotion. Hyperactivity in this region has been observed in schizophrenia and we have previously shown that normalizing the activity of specific vHipp projections can alleviate behavioral and physiological deficits in a neurodevelopmental disruption model. Specifically, reducing activity in the vHipp-medial prefrontal cortex (mPFC) pathway improves correlates of negative and cognitive symptoms while inhibition of the vHipp-nucleus accumbens (NAc) pathway improves correlates of

positive symptoms. In the current experiments, we aimed to further characterize these distinct projection pathways.

Methods: We used a combination of techniques to characterize vHipp projections. To determine the anatomical location of vHipp projection cells, we used retrograde viral tracing. Specifically, AAV2 retro viruses expressing GFP or myrScarlet were injected into either the mPFC or NAc. In the next set of experiments to characterize the transcriptional profile of vHipp projections, retrogradely labeled cells were sorted by flow cytometry, then subjected to RNASeq. RNA Scope was used to confirm the expression of differentially expressed genes. Next, to determine the anatomical connectivity of Hipp projections by local interneuron populations, eGRASP was used to label synaptic connections between pyramidal cells and specific interneuron subtypes. Immunogold labeling and electron microscopy were used to confirm these results. Finally, to determine if these anatomical differences result in changes in functional regulation, we used fiber photometry, in vivo electrophysiology, and optogenetics. Specifically, optogenetics were used to inhibit interneuron activity while recording the firing rate of pyramidal cells that project to either the NAc or mPFC.

Results: First, retrograde tracing demonstrated that vHipp-mPFC and vHipp-NAc projections are made up of distinct and anatomically segregated cell populations. NAc projections made up approximately 43% of labeled cells while mPFC-projections accounted for ~56%. Only ~1% of cells projected to both regions. Next, RNASeq identified 99 differentially regulated genes between the two pyramidal cell populations, including Nedd and Grn, two genes involved in synaptic plasticity. The differential expression of these two genes was confirmed by RNAScope.

We have shown previously that unique interneuron subtypes (parvalbumin (PV) or somatostatin (SST)) within the vHipp also differentially regulate behavior. Therefore, the next set of experiments aimed to determine the anatomical connectivity and functional regulation of vHipp projections by local interneuron populations. First, using eGRASP and electron microscopy, we found that PV interneurons form a similar number of synaptic connections on pyramidal cells that project to both mPFC and NAc, while SST-positive interneurons form far fewer synapses on mPFC projections. Then to determine if these anatomical differences result in functional changes, we used fiber photometry to show that inhibiting PV cells increased pyramidal cell activity regardless of projection target while SST cell inhibition has a much smaller effect on the activity of mPFC-projecting cells.

Conclusions: Together, these results suggest that vHipp projections are made up of discrete cell populations with unique gene expression and patterns of connectivity. Characterization of these cell populations may lead to the identification of new molecular targets for the treatment of specific symptoms of schizophrenia and other psychiatric disorders associated with vHipp dysfunction.

Keywords: Ventral Hippocampus, GABAergic Interneurons, Brain Circuitry, Projection Targets

Disclosure: Nothing to disclose.

P491. Understanding Negative Symptoms in Clinical Trials of Acute Schizophrenia

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Background: Drug trials assessing patients with schizophrenia rely on the PANSS and other scales with interconnected symptom domains. In studies focused on negative symptoms, patients are often selected based on the severity and stability of their negative symptoms relative to their positive symptoms, using criteria which are not suitable for trials of acute schizophrenia. Based on the observation that the structure of schizophrenia symptoms at baseline appear related to the structure of symptom change over time (Hopkins et al., 2018), we hypothesized that information on a prespecified factor of interest could be contained within the PANSS assessments derived from single individual subjects prior to randomization.

Methods: Here we present a method to enrich for subjects having a specific predefined factor structure based on PANSS assessments which we apply to the measurement of negative symptom change in trials of acute schizophrenia. In acute schizophrenia trials at baseline, the amount of variance explained by the Marder negative factor in a 5-factor model of PANSS is typically 10-20%. Here we sought to develop an a priori method to identify individual subjects, prior to randomization, that when taken as a subpopulation, present with a high level of variance explained by the Marder Negative Symptom Factor. A vector of 1335 PANSS elements based on between- and within-item variances, covariances and differences between items was created to calculate an index of heterogeneity and to enrich for the Marder PANSS Negative Symptom (MPNS) construct prior to randomization. We analyzed drug-placebo differences of 2 compounds from 2 different pharmacological classes, ulotaront (TAAR1 agonist) and lurasidone (D2-antipsychotic) to evaluate the improvements in patients identified with the MPNS construct.

Results: Using PANSS scores obtained at screening and baseline across $N = 4{,}873$ subjects enrolled in acute schizophrenia trials ($N = 13$), we demonstrate the ability to select for a subpopulation having the greatest amount of variance explained across the 7-items of the MPNS construct. As expected for D2 antagonist antipsychotics, improvements on negative symptoms with lurasidone lacked specificity for the subpopulation having the maximum MPNS construct. In subjects having the MPNS construct treated with lurasidone ($N = 218$), the magnitude of improvement in negative symptoms was similar to that seen in subjects without the MPNS construct ($N = 1{,}314$) prior to treatment. In contrast, the novel TAAR1 agonist ulotaront demonstrated significant improvements in subjects with the MPNS construct ($N = 63$), that were substantially greater than was seen in subjects without the MPNS construct ($N = 182$) prior to treatment.

Conclusions: These results demonstrate that in acute schizophrenia trials a subpopulation of patients have substantially more symptom variance explained by their negative symptoms, compared to the other symptom domains in PANSS. We propose a novel prognostic enrichment strategy to select patients on the basis of a greater likelihood of having a specified negative symptom construct (MPNS) in order to provide the basis to demonstrate specific treatment benefit on negative symptoms. The analytical approaches piloted here with already-conducted clinical trials, can be prospectively defined as analyses in clinical trials of acute exacerbation of schizophrenia, to facilitate the characterization of compounds with non-D2 mechanisms of action.

References:

Hopkins SC, Ogirala A, Loebel A, Koblan KS Transformed PANSS Factors Intended to Reduce Pseudospecificity Among Symptom Domains and Enhance Understanding of Symptom Change in Antipsychotic-Treated Patients with Schizophrenia. Schizophr Bull. 2018 Apr 6;44(3):593-602.

Keywords: Schizophrenia (SCZ), Schizophrenia Negative Symptoms, TAAR1, Atypical Antipsychotics

Disclosure: Sunovion Pharmaceuticals: Employee (Self)

P493. CVL-231 as a Novel Positive Allosteric Modulator of Cholinergic M4 Receptors for the Treatment of Schizophrenia: Results From an Early Proof-Of-Concept Study in Patients With Schizophrenia

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Background: CVL-231 is a novel, brain-penetrant, highly selective M4 muscarinic positive allosteric modulator in development for the treatment of schizophrenia. Previous clinical observations support the role of muscarinic agonists in modulating symptoms of psychosis in both schizophrenia and Alzheimer's disease.1,2 Preclinical characterization of CVL-231 shows favorable brain penetration, direct target engagement, and robust in vivo activity in preclinical psychosis models, including reversal of amphetamine-stimulated locomotor activity and prepulse inhibition. Here, we present data from a two-part, phase 1b, multiple-ascending-dose trial in patients with schizophrenia (NCT04136873). Part A of the trial was a multiple-ascending-dose study focused primarily on safety, tolerability, and pharmacokinetics (PK) in a stable schizophrenia population. This presentation will focus primarily on Part B, the second part of the trial in patients with acute psychosis, including pharmacodynamic (PD) assessments of the compound's antipsychotic potential at the highest exposures examined in the Part A, which were generally well tolerated up to 21 days. Based on the preclinical in vitro binding and in vivo data, these exposures are expected to achieve efficacious levels of target engagement in the brain.

Methods: Part B was a double-blind, randomized, placebo-controlled, parallel-arm design to evaluate the safety, tolerability, PK, and PD of CVL-231 in patients with acute psychosis. Patients with a primary diagnosis of schizophrenia and a current episode of acute exacerbation or relapse of psychotic symptoms with a baseline Positive and Negative Syndrome Scale (PANSS) total score of at least 80 points at the time of screening were enrolled into the study. Patients in Part B had a history of relapse and/or exacerbation of symptoms when not receiving antipsychotic treatment and had acute exacerbation of symptoms within the 2 months prior to screening. 81 patients were randomized to 1 of 2 doses of CVL-231 (30 mg once daily [QD] or 20 mg twice daily [BID]) or placebo for 6 weeks of inpatient treatment. Pharmacodynamic measures to assess antipsychotic effects included PANSS and the respective subscales. Standard safety and tolerability measures along with limited PK sampling were also assessed in the study.

Results: Safety: CVL-231 was safe and generally well tolerated in this clinical trial. The incidence of treatment-emergent adverse events for both dose groups was similar to placebo (CVL-231 30 mg QD, 52%; CVL-231 20 mg BID, 56%; placebo, 52%). Headache was the most frequently reported adverse event (incidence of 26% to 30% across placebo and CVL-231 treatment groups). CVL-231 was not associated with a greater incidence of weight gain than placebo and no adverse events related to extrapyramidal symptoms or akathisia were reported.

Pharmacokinetics: Steady-state maximum concentration (Cmax) and area under the curve (AUC) values for CVL-231 were similar across both dose groups.

Pharmacodynamics: Clinically meaningful improvements of psychosis symptoms, as measured by PANSS, were observed in both the 30-mg QD and the 20-mg BID groups compared with placebo. The 30-mg QD group demonstrated a mean reduction from baseline of 19.5 points in the PANSS total score, with a placebo-corrected change of 12.7 points at week 6 (nominal $P = 0.023$). The-20 mg BID group demonstrated a mean reduction from baseline of 17.9 points in PANSS total score, with a placebo-corrected change of 11.1 points at week 6 (nominal $P = 0.047$). These results were further supported by clinically meaningful reductions in the PANSS Positive, Negative, and General Psychopathology subscales.

Conclusions: These data suggest the potential for CVL-231 to be a novel, once-daily antipsychotic with reduced burden of side effects compared with currently approved medications to treat schizophrenia, without need for titration. CVL-231 is progressing into additional clinical trials for further evaluation in patients with schizophrenia.

Keywords: Muscarinic M4, Schizophrenia, Antipsychotic, Modulator

Disclosure: Nothing to disclose.

P494. Predicting Long-Term Outcome in Schizophrenia

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Background: Schizophrenia is characterized by progressive cognitive and psychosocial deficits that lead to grave personal, familial and psychosocial consequences in a majority of cases. However, disease trajectories can vary considerably between affected individuals, hence, raising a need for early clinical or neurobiological predictors of long-term outcome as a major step towards personalized treatment strategies.

Methods: We here relied upon 381 patients from the Iowa Longitudinal Study (ILS) cohort that received an extensive characterization, including repeated magnetic resonance imaging (MRI) scans, over a mean surveillance period of 11.07 years. We explored whether pre-diagnostic markers, clinical markers at the first psychotic episode, or magnetic resonance imaging (MRI) measures at the onset of the disease were predictive of relapse or remission of specific symptom patterns later in life.

Results: A set of clinical parameters were significantly correlated with long-term outcome. Premorbid adjustment during adolescence, negative symptoms at disease onset and three major neuropsychological domains – IQ scores, verbal memory and attention – stood out as predictors for time in future relapse. In contrast, regional brain volumes at baseline did not correlate with outcome, while progressive volume loss was highly correlated with symptom severity.

Conclusions: These findings suggest a set of robust and easily acquirable predictors for the long-term course of the disease in schizophrenia patients to improve prospective care and treatment planning for schizophrenia patients.

Keywords: Schizophrenia (SCZ), Patient Outcomes, Systems Neurobiology, Clinical Predictors, Neural Predictors

Disclosure: Nothing to disclose.

P495. Using Next-Generation Facial Analysis to Elucidate Neurodevelopmental Patterns Associated With Craniofacial Abnormalities in 22q11.2 Deletion Syndrome and in Patients Along the Psychosis-Spectrum

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Background: Minor physical anomalies (MPAs) of aberrant facial development are common in 22q11.2 deletion syndrome (22q11DS) and in psychosis-spectrum patients (PS). Given the embryological basis of facial development, comparing discrete measurements of facial MPAs in individuals with and without specific genetic abnormalities may help elucidate risk for overlapping features such as psychosis. Here we use 2D photographs and the Facial Dysmorphology Novel Analysis (Face2Gene (FDNA Inc.) technology, which combines facial recognition software with clinical knowledge enabling detection of dysmorphic features and recognizable patterns of human malformations.

Methods: 2D photographs from patients with 22q11DS ($n = 171$), schizophrenia (SZ; $n = 37$), clinical high risk for psychosis (CHR; $n = 16$) and healthy individuals (HC; $n = 94$) were collected and submitted to FDNA. The top 30 syndromes to which an individual's photograph resembled were reported along with a "gestalt" score as measurement of similarity. A series of Receiver Operating Curve (ROC) analyses, Principal Component Analysis (PCA), and follow-up t-tests were performed. In addition, images were analyzed using the Emotrics, a newly developed, machine-learning algorithm that provides automated, objective facial measurements using 68 facial coordinates that are automatically placed. Facial landmarking in Emotrics outlines the superior border of the brow, the free margin of the upper and lower eyelids, the nasal midline, the nasal base, the mucosal edge and vermillion-cutaneous junction of the upper and lower lips, and the lower two-thirds of the face. The outcome measures from Emotrics included eyebrow height, marginal reflex distance (MRD), and palpebral fissure (PFH).

Results: In 22q11DS, 98.2% of facial images submitted to FNDA had positive hits for 22q11DS with an average mean gestalt score (0.51). ROC analysis correctly classified 22q11DS individuals 88% of the time using only the 22q11DS gestalt score. In SZ, 65.5% of images submitted to F2G were positive hits for 22q11DS but with a lower gestalt score (0.21), but, expectedly, no one syndrome could correctly identify SZ, CHR, or HC. PCA analysis of the whole sample found that PC1 distinguished 22q from other groups and PC2 distinguished psychosis-spectrum (CHR + SZ) from HC. Gestalt scores of 22q11DS explained the most variance of PC1 while gestalt scores for Fragile X syndrome explained the most variance for psychosis-spectrum (CHR + SZ) with other syndromes also contributing. When additional syndromes were included in the ROC analysis the AUC for 22q11DS increased to over 90% and was significant for psychosis spectrum (AUC = 68%). Average gestalt scores were significantly different between non-deleted groups: HC > SZ $t(83.67) = 3.75$, $p = 0.0003$ and HC > CHR $t(33.03) = 3.87$. $p = 0.0005$. Specificity analysis using Emotrics indicated that as compared to HC, 22q11DS and SZ showed smaller MRD ($p < 0.001$) and PFH ($p < 0.001$), while CHR only showed lower MRD ($p < 0.008$). Specificity analysis indicated that MRD differences were most prominent from the pupil to top eyelid. There were no differences in brow height.

Conclusions: These preliminary results suggest overlap in facial dysmorphogenesis between 22q11DS and SZ patients. Future work linking measures of facial dysmorphology to clinical and neurobiological phenotypes will help identify those features most directly linked to psychosis risk. Also, to the extent that these developmental markers are evident before sub-psychotic symptoms are evident, they may allow more reliable identification of psychosis risk.

Keywords: Psychosis, Schizophrenia, 22q11 Deletion Syndrome, Automated Facial Affect Recognition

Disclosure: Nothing to disclose.

P496. Associative Dysregulation and Impaired Expressivity in Schizophrenia: Latent Structures of Clinical and Automated Speech Analysis Features

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Background: Recent computational advances have enabled objective, automated measurements of speech biomarkers which have accurately predicted clinical phenotypes such as schizophrenia spectrum disorder (SSD) diagnosis and conversion to psychotic disorder from clinical high risk. However, if we are to use features from acoustic analysis and natural language processing to understand brain dysfunction, it is important to understand the underlying structure at the appropriate level of granularity. Here, we extracted latent factors representing associative dysregulation and impaired expressivity in speech, and sought to explore their relationships to clinical status and features from automated speech analysis.

Methods: Cohort 1 included 83 prospectively evaluated participants (34 SSD, 11 other psychiatric disorders, 38 healthy control). Cohort 2 included 47 speech samples derived from publicly available psychiatric interviews on YouTube (28 psychotic disorders, 19 other psychiatric disorders). Both cohorts were rated on the Scale for the Assessment of Thought Language and Communication (TLC) and two items from the Scale for the Assessment of Negative Symptoms (SANS; decreased vocal inflection and increased latency).

Cohort 1 was additionally rated on the full SANS for negative symptoms, the Brief Psychotic Rating Scale (BPRS) for overall psychosis symptoms, and role and interpersonal functioning items from the Quality of Life Scale (QLS). Cohort 2 speech underwent transcription and processing for acoustic (e.g. pauses, pitch), lexical (e.g. parts of speech), coherence (e.g. cosine distance of sentence embeddings, and graph features where edges connect consecutive words, represented as nodes (e.g. density, degree).

Exploratory factor analysis (eFA, promax rotation) was used to identify latent factors for clinical ratings of speech disturbance across both cohorts. We excluded items with poor sampling adequacy, based on low frequency of non-zero ratings and the Kayser-Meyer-Olkin test. In Cohort 1, factor scores were evaluated against participant characteristics and clinical ratings using multiple linear and logistic regressions. In Cohort 2, principal component analysis (PCA, promax rotation) and correlational analyses were used to examine relationships between the clinical factors and features from automated speech analysis. All analyses were completed using R.

Results: Two latent factors were found for the clinical ratings of speech disturbance across Cohort 1 and Cohort 2, explaining 41% and 12% of variance, respectively: F1) "Associative Dysregulation" – derailment, loss of goal, circumstantiality, tangentiality, poverty of content of speech, incoherence, illogicality, pressured speech, perseveration, and distractibility; F2) "Impaired Expressivity" – poverty of speech, decreased vocal inflections, and increased response latency.

Factor scores showed expected relationships with clinical variables in Cohort 1. SSD diagnosis significantly predicted higher scores in both factors (F1, $B = 0.87$, $p < 0.001$; F2, $B = 0.97$, $p < 0.001$); this relationship remained significant for F1 but became trend-level for F2 when covarying for demographic variables. Accounting for diagnosis group, F1 remained a significant predictor of overall psychosis symptoms ($B = 0.23$, $p = 0.02$), while F2 did not ($B = 0.15$, $p = 0.14$). Both were significant predictors of

negative symptoms (F1, $B = 0.21$, $p = 0.01$; F2, $B = 0.30$, $p < 0.001$), and role functioning (F1, $B = -0.29$, $p = 0.002$; F2, $B = -0.32$, $p = 0.001$). Only F2 predicted interpersonal functioning ($B = -0.27$, $p = 0.001$).

Several notable patterns emerged among clinical factors and speech features in Cohort 2. F1-Associative Dysregulation was negatively correlated with coherence as measured by cosine distance between adjacent sentence embeddings using Word2-Vec ($r = -0.53$), use of pronouns and verbs ($r = -0.61$, $r = -0.50$), while positively correlated with speaking partial words ($r = 0.45$) and negative valence ($r = 0.41$). F2-Impaired Expressivity was negatively correlated with total word count ($r = -0.61$) and the graph metric for number of nodes on the largest strongly connected component ($r = -0.63$), while positively correlated with graph density ($r = 0.76$) and use of interjections ($r = 0.49$).

Conclusions: Speech and language dysfunction in SSD can be described by latent factors representing F1) Associative Dysfunction, principally characterized by derailment, loss of goal, and circumstantiality, and F2) Impaired Expressivity, characterized by poverty of speech, decreased vocal inflections, and increased response latency. Both factors are elevated in SSD, even while covarying for demographic variables, and both predict dimensional clinical and functional status, even when accounting for diagnosis. F1 is more related to overall psychosis symptoms, and can be characterized by speech features reflecting decreased coherence, among others. F2 is more related to functioning, and is reflected by graph metrics for restricted connectivity among words.

Keywords: Schizophrenia (SCZ), Biomarkers, Natural Language Processing (NLP), Machine Learning, Language

Disclosure: North Shore Therapeutics: Stock / Equity (Self)
Winterlight Labs: Grant, Consultant (Self)

P497. Repetitive Transcranial Magnetic Stimulation (rTMS) Treatment Reduces Variability in Brain Function in Schizophrenia: Data From a Double-Blind, Randomized, Sham-Controlled Trial

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Background: Cognitive impairments, particularly deficits in working memory, are a core feature of schizophrenia and predict functional outcome (Green et al. 2004). However, there is a paucity of effective therapeutic interventions targeting these deficits. The dorsolateral prefrontal cortex (DLPFC) plays a critical role in working memory performance. Various studies link working memory deficits to alterations in DLPFC structure and function (Ehrlich et al. 2012; Glahn et al. 2005). Based on our pilot study showing that repetitive transcranial magnetic stimulation (rTMS) to the DLPFC in schizophrenia improves working memory performance (Barr et al. 2013), a four-week rTMS trial was conducted (NCT01880255). As a part of this trial, we used task-based fMRI to examine changes in brain activity during an N-back working memory task following rTMS.

Methods: This double-blinded, sham-controlled trial randomized 81 participants (age 18-59) with schizophrenia or schizoaffective disorder to active or sham rTMS administered bilaterally to the DLPFC five days/week for four weeks (active rTMS at 20 Hz). Participants completed an fMRI letter sequence N-back task (1- and 3-back trials presented as block design) before and after receiving rTMS. Scan data were preprocessed (correction for slice time, motion and susceptibility distortions using fMRIprep; Esteban et al. 2019), transformed onto the cortical surface, and smoothed (Ciftify toolbox; Dickie et al. 2019). N-back fMRI data were available from 42 participants (active/sham: $n = 19/23$) who completed both scans with adequate performance and acceptable motion. Preprocessed data were analyzed using a general linear model (GLM) approach as implemented in SPM12 (http://www.fil.ion.ucl.ac.uk/spm/). Working memory-related activity was identified via the contrast of 3-back vs 1-back. To assess local effects, an 8mm ROI was created bilaterally around the rTMS coordinates, and mean beta weights were extracted for each scan. Between-group (i.e. active or sham) difference in change (local activation post - pre rTMS) was assessed via two-sample t-test. Whole-brain pattern group analysis was performed using 1000 permutations with threshold-free clustering enhancement (FSL PALM; Winkler et al. 2014), comparing the change in activation (post - pre rTMS) between groups. We also calculated the correlational distance between pairs of participants (Hawco et al. 2020). Individual variability was the average distance from each participant to all participants of the same treatment group (i.e. active or sham), with lower distance representing a more 'typical' activity pattern. While paired t-tests were used to assess within-group change (variability post - pre rTMS), between-group (i.e. active or sham) differences in change (variability post - pre rTMS) were assessed via two-sample t-tests.

Results: Our preliminary results show an increase in task-evoked left DLPFC activity in the active (mean change $= 0.20 \pm 0.32$) but not the sham (mean change $= -0.05 \pm 0.38$) group; this group difference was significant ($t = 2.3$, $p = 0.027$, $df = 40$). Although we did not observe differences in the whole brain analysis, the active group showed a reduction in variability in their spatial pattern of task-evoked activation following rTMS (mean change $= -0.074 \pm 0.05$; $t = -6.2$, $p < 0.0001$, $df = 18$), while sham group did not (mean change $= 0.005 \pm 0.06$; $t = 0.3$, $p = 0.78$, $df = 22$); this difference was significant between groups ($t = -4.3$, $p < 0.0001$, $df = 40$).

Conclusions: The current study increases our knowledge about how rTMS treatment may change brain function in schizophrenia. Further understanding of how changes in variability of brain function relates to potential clinical changes will be an important next step.

Keywords: Repetitive Transcranial Magnetic Stimulation (rTMS), Schizophrenia (SCZ), Individual Variability, Variability in Brain Activity Pattern, Working Memory fMRI

Disclosure: Nothing to disclose.

P498. C-Reactive Protein is Associated With Symptoms of Amotivation and Impaired Effort Allocation on the EEFRT Task in Patients With Schizophrenia

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Background: Increasing data implicates inflammation as a driver of negative symptoms in patients with schizophrenia. Negative symptoms are some of the most debilitating aspects of the disorder as they are most predictive of poor functional outcomes and do not respond well to current antipsychotic medications. Moreover, negative symptoms, such as amotivation, have been shown to be related to performance on effort-based decision-making tasks on which patients with schizophrenia show less willingness to exert effort for rewards compared to healthy controls. Indeed, recent evidence from computational modeling approaches have demonstrated that negative symptoms are most strongly related to the perceived effort required in those individuals who take information regarding reward and/or probability of reward into account. Thus, we hypothesized that

inflammation would be associated with impaired effort-based decision making in individuals with schizophrenia.

Methods: 39 patients with schizophrenia (mean age 39.5, standard deviation 12.1; 64.1% male) were recruited from Grady Memorial Hospital in Atlanta, Georgia. Patients were medically screened with history, laboratory testing, and physical exam and patients were excluded if they had evidence of unstable medical conditions, history of inflammatory illness, use of current anti-inflammatory medications, and/or active substance use. Plasma C-Reactive Protein (CRP) (an index of peripheral inflammation) was obtained at study visit and assays were run in the CLIA-certified hospital laboratory. On the same day, subjects underwent clinical interview for negative symptoms and completed the Effort Expenditure for Rewards Task (EEfRT) in which subjects were asked to choose between an easy task (30 button presses in 7s with the dominant index finger) where they would earn $1 or a hard task (100 button presses with the non-dominant pinky in 21s) where they would earn varying amounts of money ($1-$4.30). The probability of obtaining the reward in the hard task varied by probability levels of 12%, 50%, and 88%. We first tested relationships between negative symptoms (using the Brief Negative Symptom Scale; BNSS) and CRP, using two BNSS factors: a motivated behavior factor comprising avolition, asociality, and anhedonia subscales, and an expressivity factor comprising blunted affect and alogia subscales. Computational modeling was applied to the EEfRT data, yielding a measure of model fit for the three different models tested: a full subjective value (SV) model in which subjects incorporate reward and probability information into their decisions, a reward only model (RM) in which subjects incorporate reward but not probability into their decisions, and a bias model in which subjects incorporate neither reward nor probability into their decisions or appear to choose at random. A k parameter was also calculated, reflecting how costly effort is perceived to be. Linear regression models were tested to determine the relationship between CRP and negative symptoms and on EEfRT model parameters, while controlling for negative symptoms.

Results: Increases in CRP were significantly correlated with decreases in the motivated behavior factor ($r = -0.340$, $p = 0.042$) a finding that was primarily driven by the relationship with the avolition subscale ($r = -0.438$, $p = 0.029$). No association was found between CRP and the expressivity factor ($p > 0.8$). Regarding the EEfRT task, higher CRP was associated with poorer model fit for all three models of effort allocation: SV ($\beta = 0.368$, $p = 0.023$), RM ($\beta = 0.379$, $p = 0.016$), and bias ($\beta = 0.346$, $p = 0.032$). The k parameter (perceived effort) was not significantly associated with either CRP or negative symptoms, though there was a trend for higher CRP and increased k parameter in individuals who took both reward and probability into account (SV model; $\beta = 0.276$, $p = 0.091$). When the sample was limited to those subjects who take reward and/or probability into account, greater negative symptoms was associated with a greater perception of effort cost (k parameter for the SV model; $\beta = 0.681$, $p = 0.007$).

Conclusions: This data further supports a relationship between inflammation and negative symptoms, especially for those symptoms related to motivation. It also extends previous work demonstrating "computational phenotyping" of performance on an effort-based decision-making task. In this sample, we demonstrate that CRP was associated with a poorer model fit across the three models tested, implying that increased inflammation was associated with greater impairment in the ability to allocate effort across the three models. Furthermore, effort sensitivity, or the willingness to expend effort, was associated with negative symptoms in individuals who use probability and/or reward information to guide their choices in the task. Thus, inflammation may impair effort allocation for reward in individuals with schizophrenia, whereas negative symptoms may drive individual differences in effort discounting amongst patients who take reward and probability into account. Future work will test the impact of inflammation on relevant reward circuitry in the brain to further investigate how inflammation may impair reward allocation in patients with schizophrenia.

Keywords: Schizophrenia (SCZ), Inflammation, Negative Symptoms, Effort Based Decision Making Task, C-Reactive Protein

Disclosure: Nothing to disclose.

P499. Rare Damaging Variants and Phenotypic Variability in Schizophrenia

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Background: One of the greatest barriers to understanding the core biological processes that underlie schizophrenia (SCZ) and developing better interventions is the clinical and genetic heterogeneity of the disorder. For example, while psychosis onset is most common in late adolescence or early adulthood, some patients experience their first psychotic episode as early as childhood. Similarly, while cognitive functioning is impaired on average among SCZ patients compared to healthy individuals, there is considerable variability in functioning between patients. Determining whether varying phenotypes among patients can be explained by distinct genetic profiles, such as by burden of rare damaging variants (RDV) or RDV affecting specific neurodevelopmental processes, remains a crucial question.

Methods: In a sample of 411 patients with SCZ spectrum disorders (female $n = 118$, male $n = 283$) and clinical and cognitive phenotyping data, we identified protein-truncating variants (PTV) and missense variants from whole exome sequencing data and copy number deletions from genome-wide genotyping data. Rare PTV, damaging missense variants, and copy number deletions were analyzed together as RDV. Total burden of RDV, burden of RDV affecting genes associated with autism spectrum disorder (ASD) or SCZ, and burden of RDV affecting gene-sets involved in distinct neurodevelopmental processes were examined for association with impaired cognitive functioning and age of psychotic symptom onset.

Results: Total burden of RDV and burden of RDV in genes associated with ASD or involved in protein synthesis and catabolism were significantly associated with borderline intellectual functioning among SCZ patients. Restricting analyses to genes that are known to be intolerant to loss of function (i.e., "PLI" genes) revealed that increased burden of RDV in PLI genes involved in regulating gene expression during fetal development was significantly associated with borderline intellectual functioning. Excluding a subsample of 9 patients with known deletions in the well-characterized 22q11.2 locus yielded nominal associations between borderline intellectual functioning and burden of RDV in ASD-associated genes, as well as with burden of RDV in PLI genes involved in regulating gene expression during fetal development or associated with SCZ. There were no significant associations between the RDV burden scores examined in the current study and age of psychotic illness onset.

Conclusions: Results suggest that poor cognitive functioning among SCZ patients may be associated with greater burden of damaging variants in genes that have been previously associated with earlier-onset neurodevelopmental disorders such as ASD, and/or with genes involved in early aspects of brain development. However, future studies in larger samples are needed to confirm these results.

Keywords: Schizophrenia (SCZ), Genetics, Cognitive Impairments

Disclosure: Nothing to disclose.

P500. Evaluation of M4 Muscarinic Receptor Occupancy by CVL-231 Using [11C]MK-6884 Pet in Nonhuman Primates

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Background: CVL-231 is a novel, brain-penetrant, positive allosteric modulator selective for M4 muscarinic acetylcholine receptors in development for the treatment of schizophrenia. Preclinical characterization of CVL-231 in rodents showed favorable brain penetration, direct target engagement, and robust in vivo activity in animal models of psychosis, including reversal of amphetamine-stimulated locomotor activity and prepulse inhibition. Verifying in vivo target engagement in primate brains and quantifying the exposure-occupancy relationship would facilitate clinical dose selection and translation of preclinical data to humans. [11C]MK-6884, an M4 PAM radioligand,1 was used in nonhuman primate positron emission tomography (PET) imaging studies to evaluate M4 receptor occupancy (RO) as a function of CVL-231 dose and plasma concentration.

Methods: Two male adult rhesus macaques underwent a total of 12 PET/CT scans in this study. Both animals had 3 imaging sessions, each consisting of a baseline scan followed by a blocking scan with CVL-231 administration. Prior to each imaging session, animals were sedated with ketamine/xylazine and intubated for maintenance of anesthesia with isoflurane. [11C]MK-6884 was administered intravenously and dynamic PET data were acquired for 90 minutes. Arterial blood sampling was performed to measure radiometabolite-corrected plasma input functions. In blocking scans, CVL-231 was administered intravenously as a loading dose (~48% of the total dose by bolus, 10 minutes before radiotracer) followed by a maintenance dose (~52% of the total dose continuously infused until the end of scan); blood was collected at 1 hour to determine plasma levels of CVL-231. Regional brain PET data were analyzed by pharmacokinetic modeling using blood-based and reference tissue (cerebellum) input functions to quantify radiotracer binding and calculate RO in the blocking scans. Estimates of RO were analyzed in a dose-response fashion against injected CVL-231 dose and plasma concentration.

Results: Among evaluated regions of interest (caudate, cerebellum, cortical gray matter, hippocampus, putamen, central white matter), only caudate and putamen displayed significant blockade of [11C]MK-6884 by CVL-231. Striatal RO was dose-dependent from 18% to 67% over the range of evaluated CVL-231 doses (0.25 to 1.7 mg/kg, total of loading and maintenance components). The respective plasma concentrations of CVL-231 ranged from 126 ng/mL to 1040 ng/mL. The relationship of striatal RO with CVL-231–injected dose and plasma concentration was described by the classical Hill dose-response function, with a half-maximal inhibitory dose (ID50) of 1.1 +/-0.1 mg/kg and a half-maximal inhibitory concentration (IC50) of 581 +/-55 ng/mL.

Conclusions: These data confirm the dose-dependent target binding of CVL-231 to M4 receptors in the striatum of nonhuman primates. Evaluation of M4 RO by CVL-231 in humans using [11C]MK-6884 is being explored.

Keywords: Muscarinic M4, Schizophrenia, PET

Disclosure: Cerevel Therapeutics: Employee (Self)

P502. Effects of Repetitive Transcranial Magnetic Stimulation (rTMS) on Cannabis Use and Cognition in Schizophrenia

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Background: Problematic cannabis use in schizophrenia is high, with ~26% diagnosed with cannabis use disorder (CUD), compared to ~3% in the general population, which negatively impacts psychosocial functioning. These patients have great difficulty quitting cannabis that may reflect putative deficits in the dorsolateral prefrontal cortex (DLPFC), which may constitute a target for treatment development. Neuromodulation interventions such as repetitive transcranial magnetic stimulation (rTMS) warrant consideration in this regard. Our primary objective determined effects of active versus sham high-frequency (20-Hz) rTMS to bilateral DLPFC on cannabis use in outpatients with schizophrenia and CUD. Secondary outcomes included cannabis craving/withdrawal, psychiatric symptoms, and cognition.

Methods: A preliminary double-blind, sham-controlled randomized trial that enrolled $N = 24$ outpatients between October, 2017 and March, 2020. Nineteen participants were randomized to receive either active ($n = 9$) or sham ($n = 10$) high-frequency rTMS five times/week for 4 weeks. Cannabis and tobacco use and symptomatology were monitored weekly. Cognition was assessed pre- and post-treatment.

Results: No significant Treatment Group x Time effects on cannabis use between active and sham rTMS treatments were found. However, linear contrast estimates indicated greater reductions in cannabis use and semi-quantitative urine toxicology for THC-COOH (THC's min metabolite) in the active versus sham groups (Estimates: 4.0-4.6; p's < 0.02; Cohen's d = 0.55-0.72). A trend toward significantly greater reduction in craving over time was found in active vs. sham (Estimate = 3.9, $p = 0.06$). Reductions in PANSS positive (Estimate = 2.4, $p = 0.02$) and total (Estimate = 5.0, $p < 0.01$) symptoms were found in active vs. sham groups. rTMS improved sustained attention (Estimate = 6.6, $p < 0.05$), and suppressed increased tobacco use that was associated with cannabis reductions in these patients (Treatment x Time: $p = 0.01$). rTMS was safe and well-tolerated with high retention (>90%).

Conclusions: Our preliminary findings suggest that rTMS is safe and potentially efficacious for treating CUD in schizophrenia. Further evaluation of neuromodulation methods to address co-occurring psychosis and addiction by targeting DLPFC dysfunction associated with schizophrenia is warranted.

Keywords: Repetitive Transcranial Magnetic Stimulation (rTMS), Dorsolateral Prefrontal Cortex, Randomized-Controlled Trial, Schizophrenia (SCZ), Cannabis Use Disorder

Disclosure: Nothing to disclose.

P503. Disruptions in Agency in Psychosis are Associated With Dysconnectivity Between the Cerebellum and Pons

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Background: Core psychosis symptoms may occur when a person does not recognize themselves as the agent of their self-generated actions. For every action, an efference copy of the motor plan generates a corollary discharge of the expected sensory consequences, which is then compared to the actual sensation; this process is driven by automatic cerebellum side-loops, and motor information is likely sent to the cerebellum via the pons. If mechanisms supporting agency go awry, then sensations that should have been predicted, but were not, may acquire inappropriate salience and lead to delusional schema used

to explain those aberrant experiences. The extent to which expected and actual sensations align can be studied using vocalization paradigms, where neural responses to self-generated sounds are compared to responses to external sources. The N1 component of the EEG-based event-related potential (ERP) is suppressed during vocalizing relative to listening and is indicative of expectancy when self-generating auditory stimuli. N1 suppression is deficient in people with psychosis. Parsing N1 into theta power and intertrial coherence (ITC), we recently showed that theta ITC suppression was more sensitive than N1 suppression to psychosis for individuals early in the illness course. One possibility is that deficient theta ITC suppression in psychosis arises from aberrant cerebellum-pons connectivity. Current study goals are twofold: 1) replicate and extend our work showing theta ITC suppression is greater than theta power suppression in a new sample of psychotic spectrum patients (PSP) and their unaffected 1st-degree relatives (REL) and 2) using resting-state fMRI (rsfMRI) data, test if greater theta ITC suppression corresponds with greater cerebellum-pons connectivity in healthy controls (HC), relative to PSP, their REL, and people at clinical high-risk (CHR) for developing psychosis.

Methods: EEG analyses: 88 PSP, 103 REL, and 43 HC completed the Talk-Listen paradigm at the University of Illinois, Chicago (UIC). In the Talk condition, participants repeated short "ah" vocalizations in a self-paced manner. Speech sounds were transmitted back to the participant through headphones in real time. In the Listen condition, participants simply listened to recorded vocalizations from the Talk condition. N1 peak amplitudes were measured as maximum negative voltage between 60-150 ms (site Cz); theta ITC and power were calculated following a Morlet wavelet decomposition from the same trials. One-sample t-tests in HC determined if the theta ITC suppression effect size was larger than that of N1 and theta power. EEG metrics were age-corrected based on HC data to account for normal brain maturation. A 3x3 mixed-effects model compared age-corrected z-scores for N1, theta ITC, and theta power suppression across groups (3 EEG metrics X 3 Groups). Follow-up contrasts evaluated HC-PSP and HC-REL differences; specifically, whether theta ITC suppression was greater than N1 and/or theta power suppression. rsfMRI connectivity analyses: rsfMRI data from UIC and the University of California San Francisco (UCSF) yielded a sample of 131 PSP, 62 REL, 50 CHR, and 113 HC. A functionally-defined vocalization cerebellum seed was used to generate individual participant whole-brain connectivity maps. We then extracted average connectivity values within the pons and tested if resulting cerebellum-pons average connectivity values correlated with theta ITC suppression in HC, when accounting for N1 and theta power suppression. If significant, we tested if the theta ITC and connectivity relationship in HC was preserved in PSP, REL, and CHR.

Results: EEG results: HC had a larger effect size for theta ITC suppression ($d = 1.86$), compared to N1 ($d = 1.35$) and theta power suppression ($d = 0.70$). The EEG X Group interaction was significant ($F4,460 = 2.47$, $p = .04$), and follow-up contrasts indicated that HC-PSP difference was greater for theta ITC suppression relative to theta power suppression ($t = 2.03$, $p = .04$) but not N1 suppression ($t = -0.70$, $p = .49$). REL did not differ from HC in contrasts between theta ITC suppression versus theta power or N1 suppression. rsfMRI connectivity correlations with EEG: greater theta ITC suppression correlated with greater cerebellum-pons connectivity in HC, when accounting for N1 and theta power suppression (partial $r = .22$, $p = .02$). A significant Connectivity X Group interaction was found for theta ITC ($F3,343 = 3.11$, $p = .03$). The HC positive correlation was significantly greater than the theta ITC-connectivity relationship in REL (z-ratio = -2.71, $p = .007$) and CHR (z-ratio = -2.32, $p = .02$), and greater at a trend-level when compared to PSP (z-ratio = 1.87, $p = .06$).

Conclusions: Extending earlier work, there was greater theta ITC suppression during vocalization relative to N1 and theta power suppression in PSP, but not REL. Findings further point to theta ITC suppression as a marker that is sensitive to fully formed psychosis, but not liability for psychosis. Compared to HC, PSP and those with a liability for psychosis showed less of a relationship between theta ITC suppression and cerebellum-pons connectivity (using a seed associated with agency during vocalization). This suggests that rapid feedback loops between the cerebellum and pons, while relevant for distinguishing self-generated versus external sounds in HC, may be less indicative of sensory processing in those with psychosis. Together, findings suggest that EEG reflections of agency may depend on cerebellum-pons communication.

Keywords: Agency, Corollary Discharge, Cerebellum, Resting-State Functional Connectivity, Theta

Disclosure: Nothing to disclose.

P504. iPSC-Derived Brain Microvascular Endothelial Cells Reveal MMP1 Mediated Blood-Brain Barrier Deficits in a Subgroup of Individuals With Psychotic Disorders

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Background: Blood-brain barrier (BBB) disruption has been implicated in schizophrenia (SZ) and bipolar disorder (BD). Brain microvascular endothelial cells (BMECs) form the BBB, but their role in SZ and BD pathology is unclear. We hypothesized that BMEC function is altered in SZ and BD.

Methods: Induced pluripotent stem cells (iPSCs) from 4 SZ, 4 psychotic BD and 4 healthy control (HC) subjects were differentiated into BMEC-like cells. Gene expression and protein levels of tight junction proteins was assessed. Transendothelial electrical resistance (TEER) and permeability was assayed to evaluate BBB function. Cytokine levels were measured from conditioned medium. Outlier analysis using TEER revealed a BBB-deficit ($n = 3$) and non-deficit ($n = 5$) group.

Results: TEER was reduced in SZ compared to HC, but not for other contrasts. Both CLDN5 expression and protein levels were decreased in SZ ($P < 0.05$). Reduced barrier function and increased permeability were observed in the BBB-deficit group, but not in non-deficit group. In co-culture experiments, TEER was reduced in a HC cell line cultured with BMECs from BBB-deficit patients ($P < 0.01$). MMP1 levels were increased in the BBB-deficit group and MMP1 elevation were correlated with lower CLDN5 levels and poorer BBB function. Inhibition of MMP1 with piroxicam or infliximab improved TEER function in BBB-deficits cell lines.

Conclusions: We identified inherent BBB deficits in a subgroup of individuals with psychosis and found MMP1 to be associated with these deficits, an effect that might be attenuated by MMP1 inhibition. This study identified a targetable mechanism for reversing BBB deficits in psychosis.

Keywords: Brain Microvascular Endothelial Cells, Induced Pluripotent Stem Cells (iPSCs), Blood-Brain-Barrier, Schizophrenia (SCZ), Bipolar Disorder

Disclosure: Nothing to disclose.

P505. Risk of Psychosis With Prescription Amphetamines

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Background: Previous research indicates that prescription stimulant use is associated with an increased risk of psychosis and mania, with a prior cohort study using insurance claims data indicating an increased risk of psychosis with prescription amphetamines compared to methylphenidate.

Methods: A case control study using electronic health records was performed in 4,733 patients age 16 – 35 years admitted to McLean Hospital between January 1, 2005 and December 31, 2019. Cases were admitted for an incident episode of psychosis or mania. Controls were admitted for first psychiatric hospitalization without psychosis or mania. We performed adjusted multivariable logistic regression to estimate the effect of past month prescription amphetamine exposure on the risk of psychosis or mania, adjusting for age, gender, race, immigration, month and year of admission, insurance type; smoking, use of cannabis, alcohol, illicit stimulants, sedative/hypnotic drugs, opioids, or hallucinogens; psychiatric diagnoses prior to admit, medications on admission; and first-degree relative with a history of bipolar or psychosis. Doses of amphetamine were converted to dextroamphetamine equivalents and divided into tertiles to estimate the effect of low, medium and high dose amphetamines on the risk of psychosis/mania. We also assessed the odds of psychosis/mania with prescription methylphenidate use.

Results: Past month use of prescription amphetamines was associated with an increased odds of psychosis or mania: cases with an initial episode of psychosis or mania (12.1%) vs. controls (8.9%): unadjusted odds ratio (OR) 1.41, 95% CI 1.15 - 1.74; adjusted OR 1.96, 95% CI 1.46 - 2.63. Low dose amphetamine (≤ 15 mg dextroamphetamine equivalents) was not associated with a significant difference in the odds of psychosis or mania (adjusted OR 1.44, 95% CI 0.92 - 2.25). Medium (>15 mg to ≤ 30 mg) and high dose (> 30 mg) amphetamines were associated with a significantly increased odds of psychosis or mania (medium dose: adjusted OR 1.95, 95% CI 1.22 - 3.13; high dose OR 2.70, 95% CI 1.57 - 4.63). Past month use of prescription methylphenidate was not associated an increased odds of psychosis or mania (adjusted OR 0.81, 95% CI 0.49 - 1.32).

Conclusions: Prescription amphetamines are associated with an increased risk of psychosis and mania, with higher doses associated with the greatest risk.

Keywords: First Episode Psychosis, Mania, Psychostimulant, Amphetamine, Methylphenidate

Disclosure: Nothing to disclose.

P506. Prefrontal fALFF as a Biomarker of Antipsychotic Efficacy: A Prospective First Episode Study

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Background: While antipsychotic medications have been utilized for decades, many patients experiencing psychosis do not demonstrate a satisfactory positive symptom response, even in the first episode of illness. Resting state functional magnetic resonance imaging (rs-fMRI) has increasingly been studied as a potential biomarker of antipsychotic treatment response, but studies to date remain limited in terms of sample size and brain regions examined. The present study examined fractional amplitude of low frequency fluctuations (fALFF) derived from rs-fMRI to search the whole brain, in a hypothesis-free manner, for regions that might predict response to 12 weeks of treatments with standard antipsychotic medications (risperidone or aripiprazole). To our knowledge, the present study represents the largest first episode psychosis treatment sample studied with rs-fMRI to date ($N = 126$).

Methods: Subjects included 126 patients (33.3% female, mean age = 22.6, SD = 5.7) with first episode psychotic disorders and minimal exposure to APs (median exposure = 5 days; all patients <2 years). All subjects underwent scanning while entering 12 weeks of prospective treatment with second-generation APs (risperidone or aripiprazole). Consistent with our prior studies6,7, stringent treatment response criteria were applied for ratings obtained on weeks 1, 2, 3, 4, 6, 8, 10, and 12: response required 2 consecutive ratings of much or very much improved on the CGI, as well as a rating of ≤3 on psychosis-related items of the BPRS-A. By these criteria, 83 patients were classified as responders; these subjects did not differ from 43 non-responders on age, sex, scanner type (GE or Siemens), or movement during the scan (framewise displacement, FD), as displayed in Table 1. Of the 62 patients who completed a follow-up scan, 41 were responders and 21 were non-responders. In order to examine effects of time and re-scan independent of illness and treatment, we scanned 45 healthy comparison subjects (53.3% female, mean age = 25.6, SD = 4.5). All subjects provided written, informed consent under a protocol approved by the Institutional Review Board of the Feinstein Institutes for Medical Research at Northwell Health.

All fMRI exams were conducted on a 3T scanner (GE Signa HDx, $n = 77$ at baseline, $n = 35$ at follow-up); Siemens PRISMA, $n = 53$ at baseline, $n = 27$ at follow-up). For each patient, the follow-up scan was conducted on the same scanner as the baseline. On the Signa, the resting-state scan lasted 5 minutes, during which 150 EPI volumes were obtained (TR = 2000 ms, TE = 30 ms, matrix = 64*64, FOV = 240 mm; 40 contiguous 3mm oblique axial slices). On the PRISMA, two 7-minute 17-second resting-state runs were obtained, one each with AP and PA phase encoding directions. Resting scans contained 594 whole-brain volumes, each with 72 contiguous axial/oblique slices in the AC-PC orientation (TR = 720ms, TE = 33.1ms, matrix = 104x90, FOV = 208mm, voxel = 2x2x2mm, multi-band acceleration factor = 8). Raw resting state data were preprocessed with despiking, linear trend removal, spatial smoothing (6mm3 kernel FWHM), and grand mean scaling. Utilizing Fourier Transformation at every voxel, we calculated the power of BOLD signal in the low frequency range of 0.01–0.10 Hz and divided it by the power of BOLD signal across the entire frequency range (0–0.25 Hz) to calculate fALFF30. Voxelwise fALFF was compared between responders and non-responders using t-tests implemented in SPM with age, sex, scanner, and movement (FD) as nuisance covariates, and applying a height threshold of $p < 0.005$ and FDR-corrected cluster size $p < .05$.

Results: At baseline, patients who would later meet strict criteria for clinical response demonstrated significantly greater baseline fALFF in bilateral orbitofrontal cortex compared to non-responders. In a subset of 62 patients who were scanned a second time at the end of treatment, responders and non-responders demonstrated significant increases in fALFF in differing brain regions. Specifically, responders demonstrated significant increases in fALFF, from baseline to follow-up, in bilateral dorsal prefrontal cortex; prefrontal changes were not observed in non-responders or in healthy individuals ($n = 45$) scanned at two time points.

Conclusions: Spontaneous activity in orbitofrontal cortex may serve as a prognostic biomarker of antipsychotic treatment, while spontaneous activity in the dorsal prefrontal cortex may serve as a critical target of effective medication. Additionally, we observed increased fALFF in the precuneus/posterior cingulate over the course of treatment in responders; this change was not observed in non-responders. This finding complements two recent studies reporting that increased connectivity of the posterior cingulate was associated with clinical response to antipsychotic treatment in the first episode

of illness. This brain region is considered the hub of the default mode network, and is critical to the ability to shift between self-oriented processing and (external) task-oriented function mediated by dorsal prefrontal cortex. Collectively, our findings are consistent with a recently proposed model of schizophreni that places the inability to shift between internal and external focus at the core of the disorder, and suggests novel pharmacologic and molecular approaches to ameliorating these deficits.

Keywords: Resting-state fMRI, Antipsychotic-Naïve First-Episode Schizophrenia, Antipsychotic Response, Neuroimaging Biomarkers

Disclosure: Nothing to disclose.

P507. PET Clinical Study of Novel Antipsychotic LB-102 Demonstrates Unexpectedly Prolonged Dopamine Receptor Target Engagement

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Background: Amisulpride is an antipsychotic approved in the 1980s in Europe to treat schizophrenia and, in some countries, dysthymia. Despite its successful use in millions of SCZ patients over decades, amisulpride has never been approved in the United States. The poor blood brain barrier permeability of amisulpride led us to develop LB-102, an N-methylated version of amisulpride with a putative higher capacity to cross membranes. Like amisulpride, LB-102 is a strong dopamine D2/3 antagonist (Ki < 1 nM) and moderate 5-HT7 antagonist (Ki ~30 nM). In preclinical animal models, LB-102 recapitulated the pharmacokinetic and behavioral properties of amisulpride. A Phase 1 clinical study examined the pharmacokinetics, safety, and tolerability of LB-102 in healthy volunteers in doses ranging from 10 mg/day to 200 mg/day. In addition, slow CNS exchange kinetics was anticipated to uncouple target occupancy from the plasma concentrations of LB-102. Establishing target engagement of antipsychotics versus dose and schedule is key to the development of new medications to treat schizophrenia (SCZ). We present clinical PET dopamine receptor occupancy (RO) data on LB-102 which confirms and refines our understanding D2/3 occupancy versus dose and schedule.

Methods: In clinical study NCT04588129 we measured LB-102 dopamine RO in four different dose cohorts of $N = 4$ each. LB-102 was examined as a single dose in the first three cohorts. Receptor occupancy in subjects was imaged with [11C]raclopride before dosing and at three timepoints after dosing for up to 48 hours after 50, 75, and 100 mg doses of LB-102 using dynamic PET with PET-CT Vision. Reference tissue modeling was done with the simplified reference tissue method (SRTM) and Logan graphical method to yield percentage occupancy in caudate/putamen, thalamus, and temporal cortex. Blood samples were drawn at 0, 0.5, 1, 2, 3, 4, 8, and 24 h post dose for cohorts 1 and 2, with a draw at 48 h added for Cohort 3 (corresponding with the extended PET scan) and plasma concentration of LB-102 and amisulpride (a minor metabolite of LB-102) were measured.

Results: Dopamine ROs in the caudate for a 50 mg dose of LB-02 averaged (± SEM) 26 % (0.8) for the scan beginning at 2.5 h, 43% (1.7) at 7.5 h, and 51% (1.6) at 23.5 hours; in the putamen the same time points afforded 22% (1.0), 36%, (2.3) and 39% (1.6); in the thalamus, the same values were 36% (2.9), 37% (2.3), and 31% (3.2); in the temporal lobe, 21% (1.6), 27% (3.2), and 26% (3.8), respectively. At 75 mg (note, timepoints for this cohort were extended to 48 h to better capture slow loss of dopamine receptor occupancy with respect to time. Within the caudate D2/3 occupancy averaged (SEM) 36 % (6.0) for the scans starting at 2.5 h, 61% (2.6) at 23.5 h, 41% (4.8) at 47.5 h; in the putamen the same time points afforded 42% (4.2), 53% (0.1), and 38% (0.1); in the thalamus, 29% (5.0), 40% (4.5), and 15% (2.7) ; in the temporal lobe 15% (2.6), 27% (3.1), and 9% (2.1), respectively. At 100 mg dopamine ROs in the caudate averaged (SEM) 43% (3.8) at for scans beginning at 2.5 h, 73% (3.1) at 7.5 h, and 73% (1.0) at 23.5 h; in the putamen the same time points afforded 39% (3.5), 67% (3.2), 65% (1.5); in the thalamus, 34% (1.8), 46% (1.1), and 37% (1.9); in the temporal lobe, 16% (1.1), 20% (1.2), and 18% (1.2), respectively. Values obtained Logan reference method were not materially different from those obtained using the SRTM.

Plasma concentrations of LB-102 were consistent with the prior phase 1 study of LB-102. As predicted, CNS dopamine RO levels were uncoupled from instantaneous plasma concentrations and a global analysis of this study indicates in humans, CNS exchange kinetics are kin = 0.0016 h-1 and kout = 0.0135 h-1, and an apparent D2/3 caudate ED50 of 1.4 ng/g. Notably, under steady state dosing, plasma peak-to-trough ratios of LB-102 are approximately 9-fold but slow CNS exchange kinetics limits CNS peak-to-trough ratios to under 3-fold offering more consistent modulation of D2/3 occupancy over the course of a day.

There were three adverse events in this study, two incidences of mild headache and one incidence of moderate lightheadedness/ dizziness that were possibly drug related: these all resolved without intervention.

Conclusions: Results from this study demonstrated that striatal dopamine RO in the desired range of 60 ~ 75% can be achieved with once daily dosing of 100 mg LB-102, a dose that was well-tolerated in our Phase 1 clinical study. Due to slow CNS exchange kinetics, with a single dose of 100 mg, sustained (>50 %) dopamine receptor occupancy persists for as long as 48 hours post-dose. Notably, our phase 1 clinical study revealed that for a given dose, LB-102 plasma exposure of LB-102 is 2.5 X greater than what is reported for amisulpride. A fourth cohort, including data obtained under steady state dosing conditions, will be presented. Data from this study will be used to inform dosing in a Phase 2 clinical study in SCZ patients currently being planned

Keywords: Antipsychotic, Target Engagement, D2 Dopamine Antagonists, Schizophrenia Novel Treatment, Clinical Psychopharmacology

Disclosure: LB Pharma: Grant (Self)

P509. Effects of Acute Olanzapine Exposure on Central Leptin-Mediated Regulation of Energy Homeostasis

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Background: Effects of illness biology, life-style factors, and metabolic adverse effects of antipsychotic medications contribute to exceedingly high rates of metabolic comorbidity in schizophrenia spectrum disorders. Leptin is a key metabolic hormone secreted from adipose tissue, which acts through central and peripheral mechanisms to reduce food intake, increase energy expenditure and lipolysis and maintain glucose homeostasis. Leptin dysregulation and hyperleptinemia have been observed in schizophrenia and other severe mental illnesses. However, it is unknown if antipsychotic drugs cause leptin resistance, and whether this occurs through direct impairments of leptin action in the brain, or as a secondary effect of antipsychotic-induced weight gain and increased adipose mass. In the present study, we set out to explore whether the antipsychotic drug olanzapine, prior to increased adiposity, could interfere with intracerebroventricular (ICV) leptin-mediated regulation of food intake and energy homeostasis.

Methods: Male Sprague Dawley rats were assigned to 4 treatment groups (ICV-peripheral): Vehicle (VEH)-VEH ($n = 6$), Leptin (LEP)-VEH (n

= 6), LEP-olanzapine (OLZ) ($n = 7$), VEH-OLZ ($n = 6$). Following acclimatization to metabolic cages, rats received acute injections of LEP (3ug) or VEH into the 3rd ventricle, and OLA (2mg/kg) or VEH subcutaneously at the beginning of the light (8AM, $t = 0$) and dark (8PM, $t = 12$h) cycles. Indirect calorimetry was used to calculate respiratory exchange ratio (RER), and heat production. RER is indicative of substrate utilization, with higher values representing greater carbohydrate utilization, and heat production is indicative of energy expenditure. Cumulative food intake was measured at 12-hour intervals ($t = 12$h and 24 h). Intraperitoneal glucose tolerance tests (IPGTTs) were performed in overnight fasted rats. Repeated measures ANOVA was employed for the analysis of longitudinal RER and heat production values. Cumulative food intake and blood glucose levels acquired during IPGTTs were analyzed using one-way ANOVAs. Alpha value was set at $p < 0.05$ and Tukey's post-hoc test was employed for analysis of between-group differences when appropriate.

Results: Treatment with OLZ (VEH-OLZ) in the light cycle resulted in a rapid decrease in RER ($p < 0.05$, vs. VEH-VEH), recovering to levels of VEH-treated rats, approximately 3 hours post-treatment. During the dark cycle when rats are feeding and should be shifting to carbohydrate utilization, retreatment with OLZ resulted in a rapid and sustained reduction in RER in the absence of changes in food intake, suggesting an inappropriate shift to fat utilization ($p < 0.05$, vs VEH-VEH). Treatment with ICV-leptin resulted in a gradual reduction in RER in the light cycle which became significant ~6 hours post-treatment (LEP-VEH, vs VEH-VEH, $p < 0.05$), and was sustained throughout the dark cycle. Hence, as expected, central LEP caused a shift to fat oxidation. This was also accompanied by reductions in food intake. Similar to LEP-VEH animals, co-treatment with LEP and OLZ resulted in sustained reductions in RER as compared to VEH-VEH ($p < 0.05$) in the light and dark cycle, and were also accompanied by reductions in food intake. Leptin (LEP-VEH) and LEP-OLZ treated animals demonstrated reduced cumulative food intake in the dark cycle relative to VEH-VEH and VEH-OLZ groups ($p < 0.01$). Heat production was increased by OLZ in the light phase, though this effect was not consistently maintained. During IPGTTs, acute OLZ exposure resulted in significantly higher blood glucose levels in both VEH-OLZ and LEP-OLZ groups compared to VEH-VEH and LEP-VEH groups ($p < 0.01$).

Conclusions: Both leptin (LEP) and olanzapine (OLZ) reduce RER during the light and dark cycles, suggesting a shift in fuel preference from carbohydrates to fats. In the case of OLZ, this occurred independently of changes in food intake, and has previously been shown to represent an inappropriate response given simultaneous impairment of lipolysis and reduced availability of fat as a substrate. Intriguingly, we demonstrate that central LEP is able to reduce food intake in the presence of OLZ, suggesting feeding regulation mediated by LEP signalling in the brain is not acutely impaired by antipsychotics. Conversely, LEP is unable to mitigate OLZ-induced dysglycemia, suggesting involvement of disparate pathways regulating feeding and glucose homeostasis, and possible induction of acute LEP resistance by OLZ.

Keywords: Antipsychotics, Leptin Resistance, Schizophrenia Spectrum Disorders, Central Nervous System, Feeding

Disclosure: Alkermes: Advisory Board (Self)

P510. Xanomeline Augments the Therapeutic Actions of Antipsychotic Medications in Animal Models of Psychosis via Agonism of Central Muscarinic Receptors

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Background: All currently approved antipsychotic medications for schizophrenia have direct dopamine (DA) D2 receptor antagonist activity and continued reliance on DA D2 antagonism is likely to be a limitation in advancing therapeutic potential of new treatments. There is now rather compelling data that muscarinic cholinergic agonists have antipsychotic properties. KarXT (xanomeline-trospium), a central muscarinic receptor agonist devoid of direct DA D2 antagonist activity, reduced positive and negative symptoms of schizophrenia in a phase 2 trial and is currently in phase 3 trials in this indication. Xanomeline, the centrally active component of KarXT, preferentially binds to and stimulates M1 and M4 receptors and exhibits robust activity across numerous preclinical behavioral models predictive of antipsychotic activity, suggesting these muscarinic receptor subtypes are likely to regulate neurotransmitter systems and neural circuits implicated in schizophrenia. Using two well-validated behavioral models of psychosis, we verified that xanomeline's antipsychotic activity is mediated by activation of central muscarinic receptors and tested the potential for xanomeline to augment the antipsychotic activity of agents that directly antagonize DA D2 receptors.

Methods: Conditioned Avoidance Responding (CAR): Mice were first trained to avoid a foot-shock to a performance criterion of >85% avoidance responses. Additional experiments evaluated augmentation by combining subthreshold doses of xanomeline with various antipsychotics that directly antagonize DA D2 receptors. The CAR assay was set up in a repeated-measures and counter balanced design.

Locomotor Activity (LMA): In mice, varying doses of xanomeline and antipsychotic agents were co-formulated and dosed 30 minutes before pharmacological challenge with a psychostimulant. The LMA assay was set up using a between-subjects design.

Pharmacokinetics (PK): Plasma and brain tissue were collected from mice to determine drug exposure and potential for drug-drug interactions.

Results: Results obtained using centrally-active (scopolamine) and peripherally-restricted (N-methylscopolamine) muscarinic antagonists confirmed that xanomeline's antipsychotic activity is mediated through activation of central muscarinic receptors. In CAR, significant augmentation of response inhibition was observed following administration of subthreshold doses of antipsychotic agents with xanomeline, and the magnitude of effect was greater than administration of either agent alone. No dose combination produced escape failures, indicative of lack of motor side-effects. Similarly, co-administration of xanomeline with antipsychotics that directly antagonize DA D2 receptors resulted in a larger reversal effect on psychostimulant-induced hyperlocomotor activity than did either agent alone. This augmented behavioral activity was not attributable to PK interactions affecting drug exposure.

Conclusions: Xanomeline's antipsychotic activity in CAR, a predictive animal model, is mediated by central, and not peripheral, muscarinic receptors. Combined low doses of xanomeline with antipsychotics significantly augmented CAR and LMA effects over those observed for each agent alone. No motor side-effects were evident in these studies. Finally, the augmented activity observed in LMA was not an artifact of increased compound exposures. These data support further research evaluating xanomeline's ability to augment the effects of antipsychotics.

Keywords: Antipsychotic Drugs, Schizophrenia, Muscarinic Agonist, Adjunctive Treatment

Disclosure: Karuna Therapeutics: Employee (Self)

P511. Retest Reliability: An Untapped Source for Strengthening the Predictive Power of Functional Connectivity When Predicting Psychosis

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Background: Neurometric properties, such as retest reliability, are now receiving attention from imaging scientists. However, there are few examples to our knowledge that use neurometrically-informed analyses to improve predictive power. To demonstrate this principle, we examined resting state fMRI (rsfMRI) predictors of psychotic-like experiences (PLEs) across two samples (the Human Connectome Project or HCP, and the Psychosis Human Connectome Project or P-HCP).

Methods: In Step 1, resting-state functional connectivity (rsFC) between 25-350 independent components from the HCP rsfMRI data was tested for optimal retest reliability, which occurred around 100 components (Ma and MacDonald [in press], Brain Connectivity). In Step 2, an elastic net model for PLEs using reliable rsFC features (intraclass correlations > 0.4) from the HCP ($n = 855$ healthy young adults) was compared to a model using all features regardless of reliability. Models controlled for age, sex, and known confounds of rsFC including handedness, relative movement, brain volume, and multiband reconstruction algorithm. We used leave-one-family-out cross-validation to ensure independence between the training and test sets. For Step 3, we derived an rsFC composite score based on the previous model to examine psychotic traits and symptoms from the P-HCP (118 patients with psychosis, 71 non-psychotic first-degree relatives, and 45 healthy controls) that used similar acquisition parameters.

Results: The cross-validated model from the HCP using all available features only predicted 1.7% of variance, whereas the model excluding low reliability features nearly doubled that, predicting 3.3% of variance in PLEs ($p = .002$). Predictive connections largely included the default, frontoparietal, cingulo-opercular, and dorsal attention networks. The model built from reliable features generalized to younger P-HCP participants, explaining two additional measures of positive/disorganized psychotic traits (the Structured Interview for Schizotypy: $b = 0.25$, p(1-tail) $= 0.027$, the Schizotypy Personality Questionnaire positive factor: $b = 0.14$, p(1-tail) $= 0.041$).

Conclusions: CONCLUSIONS: Prescreening based on a very liberal threshold for retest reliability, an established procedure widely available to imagers, resulted in nearly doubled variance in PLE's predicted by rsFC metrics despite using fewer features. This resulted in a significantly predictive model that was found to generalize across the psychosis continuum. Because many regions of the brain are acquired with unreliable signals, eliminating these sources may improve the prediction of external variables.

Keywords: Neurometrics, Retest Reliability, Psychotic-Like Experiences, Cross Validation, Resting State Functional Connectivity

Disclosure: Nothing to disclose.

P512. Prevalence of Rate of Deleterious Copy Number Variants Similar in Early Onset Psychosis and Autism Spectrum Disorders: Implications for Clinical Practice

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Background: Copy number variants (CNVs) have some of the highest estimated relative risks for psychiatric disorders. Specifically, rare recurrent CNVs are strongly associated with schizophrenia, with 22q11.2 deletion syndrome widely considered as the single biggest genetic risk factor for the disorder. A nearly 3-fold increase of CNV carriers in childhood-onset schizophrenia (COS) relative to adult-onset schizophrenia suggests an even greater genetic component to the childhood form of the disorder. However, COS is rare, representing <4% of all schizophrenia cases, making studies of rare CNVs in this disorder doubly challenging. Moreover, many children with psychosis do not yet meet the strict diagnostic criteria for schizophrenia. The CNV burden in these more common forms of early onset psychosis (EOP) remains unclear, despite evidence that this information may improve treatment outcomes.

Here, we model the biological consequences of CNV burden in EOP and compare this burden to individuals with autism spectrum disorders (ASD) and population controls. Specifically, we performed clinical chromosomal microarrays on 138 EOP children and adolescents. CNVs were called using identical pipelines as in our prior work, such that CNV burden in EOP participants could be directly compared to CNV burden in 5,756 ASD participants, as well as to 16,516 participants from epidemiologically ascertained samples (CT). We hypothesized that (1) the prevalence of recurrent CNVs in the EOP sample will be greater than in ASD and CT, and that (2) the pathogenicity of CNVs in EOP would be similar to ASD and higher than in unselected CT samples.

Methods: CNVs were called, annotated, cleaned and LOEUF scores for deletions and duplications were calculated using pipelines described previously (Huguet et al., JAMA Psychiatry 2018). Briefly, each coding gene fully encompassed in a CNV was annotated using the inversed LOEUF (1/LOEUF) score, which is available for ~20K genes and ranges from 0.5 (gene tolerant to haploinsufficiency) to 33.3 (gene intolerant to haploinsufficiency). To measure the pathogenicity of CNVs, we compared 1/LOEUF and sums of genes scores in EOP, ASD, and unselected populations using logistic regression models.

Statistical analyses were performed in R. Demographic differences between groups were examined with Standard t-tests and chi-squares. To test for group differences in the incidence of recurrent CNVs was performed with a Fisher's exact test using the fisher.test() function. To measure pathogenicity of deletions and duplications CNVs for clinical phenotypes, we compared 1/LOEUF and sums of genes scores in EOP, ASD, and unselected populations using logistic regression models. These models included the sum of genetic scores for deletions and duplications as two independent main effects, adjusting for sex:

$\ln(o \cdot s(Yi = \text{diagnosis}|xi)) \sim \alpha X + \beta 0 + \beta_1$ SumScoreDELi $+ \beta_2$ SumScoreDUPi + sexi

Where SumScoreDEL/DUP are CNV scores (1/LOEUF or sums of genes) for deletions (DEL) and duplications (DUP) as explanatory variable; and α, β_1, and β2 are the vectors of coefficients for fixed effect.

Results: The prevalence of recurrent CNVs was higher in EOP patients (10%, 14 carriers) compared to both ASD (5%, 261 carriers, $X2 = 9.54$, $p = 0.002$) and CT populations (3%, 537 carriers, $X2 = 20.33$, $p < 0.00001$). The number of deleted genes (Odds ratio 1.12, $p = 8$x10-4) and the pathogenicity of those genes (1.28, $p =$ 6x10-5) was increased in the EOP vs. CT cohorts. In contrast, neither the number of genes (1.02, $p = 0.50$) not their pathogenicity (1.06, $p = 0.30$) differed between groups for duplications. EOP and ASD cohorts were similar for deletions (number 1.01, $p = 0.80$; pathogenicity 1.02, $p = 0.60$) and duplications (number 0.97, $p =$ 0.43; pathogenicity 0.95, $p = 0.48$).

Conclusions: Our unpublished results indicate that EOP has a higher prevalence of recurrent CNVs than ASD or unselected samples. The number and pathogenicity of deleted genes was greater in EOP compared to unselected samples, without an effect of duplications. In contrast, the number nor pathogenicity of altered genes differed between EOP and ASD. Our results suggest

that assessing CNVs should be part of standard clinical care for EOP youth, as is recommended in ASD.

Keywords: Copy Number Variants, Early Onset Psychosis, Schizophrenia (SCZ), Autism Spectrum Disorder and Related Syndromes

Disclosure: Nothing to disclose.

P514. Luvadaxistat, a D-Amino Acid Oxidase Inhibitor, Improves Mismatch Negativity in Patients With Schizophrenia

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Background: D-amino acid oxidase (DAAO) is the main enzyme that degrades D-serine, which is highly expressed in glia and neurons. D-serine is a co-agonist with glutamate of N-methyl-D-aspartate (NMDA) receptors, which are hypoactive in schizophrenia according to the dominant current hypothesis. Luvadaxistat (also known as TAK-831, NBI-1065844) is an investigational highly selective and potent inhibitor of DAAO that is being developed for the treatment of cognitive impairment associated with schizophrenia (CIAS). The objective of this study was to assess pharmacodynamic (PD) biomarkers of luvadaxistat on measures related to cerebellar circuitry function and NMDA pharmacology that are known to be affected in schizophrenia.

Methods: This was a randomized, double-blind, placebo-controlled cross-over Phase 1b clinical trial (NCT03359785) in schizophrenia patients at a single site to evaluate PD effects, safety, tolerability, and pharmacokinetics of multiple oral doses of luvadaxistat. Participants received either 50 or 500 mg of luvadaxistat and placebo for 8 consecutive days in two periods separated by a 2- or 3-week washout. Eye-blink conditioning (EBC) was used to assess the impact of luvadaxistat on cerebellar circuitry function. Evoked potentials in the electroencephalogram were also tested to assess impact on NMDA-sensitive physiological responses that are affected in schizophrenia. Mismatch negativity (MMN), auditory steady state response (ASSR) and P300 were measured at baseline and following 8 days of treatment in both periods. All endpoints were analyzed as change from baseline compared to placebo within the same subjects. Levels of D- and L-serine were also analyzed for all groups. Statistical comparisons were conducted with an ANOVA model, with treatment sequence, period, and treatment as fixed effects, and subject as a random effect.

Results: COVID-19 impacted the study resulting in fewer completers than initially planned, affecting the power of the study. Therefore, the data are considered exploratory in nature. This study enrolled 31 patients, aged 18 to 60 years. Participants were symptomatically stabilized patients with schizophrenia, receiving stable antipsychotic medication over 2 months. 12 patients completed both active and placebo periods for each dose. Patients were primarily male (26/31) and African-American (26/31).

The primary endpoint was change from baseline in EBC, which was slightly but not significantly favorable for 50 mg compared to placebo ($p = 0.240$). Estimated logit transformed percent conditioned responses LS means (90% CI) were 0.597 (-0.210 to 1.405) for 50 mg and 0.319 (-0.448 to 1.086) for placebo. For the 500 mg dose, there was no significant difference between active and placebo in change from baseline ($p = 0.905$). The 500 mg dose responses were 0.578 (-0.192 to 1.348) and placebo responses were 1.344 (0.602 to 2.087).

On secondary endpoints, the MMN amplitude improved (became more negative than baseline) with 50 mg, but not with 500 mg, compared to placebo. The difference between 50 mg and placebo in LS means change from baseline was statistically significant (50 mg: -0.239 (-0.863 to 0.386); placebo: 0.669 (0.012 to 1.326); $p = 0.0497$; effect size: -0.691). The 500 mg dose did not show a significant difference from placebo in change from baseline (500 mg: 0.594 (-0.245 to 1.432); placebo: -0.154 (-1.008 to 0.700); $p = 0.8517$; effect size: 0.389).

The ASSR gamma band power showed numerical increase from baseline with 50 mg but not 500 mg compared to placebo. The 50 mg dose showed a greater change from baseline than placebo in ASSR gamma power, but it showed only a trend towards significance (50 mg: 2.135 (-13.127 to 17.396); placebo: -16.282 (-32.353 to -0.211); $p = 0.056$; effect size: 0.575). The 500 mg dose change from baseline was similar to placebo (500 mg: 9.411 (-26.018 to 44.480); placebo: 9.203 (-26.981 to 45.387); $p = 0.495$; effect size: 0.003).

Luvadaxistat was generally well tolerated. The only adverse events (vomiting, salivation and drowsiness) observed in one patient were mild and not related to study drug. There were no significant findings in safety, laboratory or ECG assessments.

Conclusions: Luvadaxistat demonstrated a statistically significant improvement in MMN amplitude and a numerical improvement in ASSR gamma band power in stable patients with schizophrenia. These effects were observed only at the lower dose of 50 mg and not with 500 mg. These results are consistent with the findings of improvement in cognitive performance with 50 mg but not with 500 mg reported in the Phase 2 INTERACT study (see accompanying poster). The observed higher impact of the lower dose (potential inverted U-shaped dose response) is consistent with preclinical data with luvadaxistat and with NMDA receptor pharmacology in general. Although the effects on the primary EBC endpoint were not significant, this may be due to the sample size being underpowered for this assay. Overall, the data suggest low doses of luvadaxistat improve the outcome of neural circuitry activity that has been associated with NMDA receptor pharmacology and correlated with cognitive performance in schizophrenia.

Keywords: D-serine, Mismatch Negativity, Pharmacodynamics, Schizophrenia (SCZ)

Disclosures: Takeda: Employee (Self)
Takeda: Stock / Equity (Self)

P515. Glutamate in Key Reward-Related Brain Regions is Associated With Social Anhedonia in First-Episode Psychosis: A Preliminary Finding

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Background: Social anhedonia—a reduced tendency to enjoy interpersonal relationships and/or reduced interest in social interaction—is one of the most pervasive and debilitating features of schizophrenia. A substantial body of work showed that social anhedonia is prevalent and influences social functioning in schizophrenia. However, despite the overwhelming evidence of demonstrating social anhedonia as an integral feature of schizophrenia, the relationship between social anhedonia and the underlying pathophysiological processes of the disorder is poorly understood. Emerging work in behavioral neuroscience suggests that reduced social motivation is related to aberrant glutamatergic system. Building upon this preclinical work, this study examined the relationship between glutamate levels in key reward-related brain regions and social anhedonia in first-episode psychosis using 1H magnetic resonance spectroscopy (1H MRS).

Methods: Sixteen patients (6 females) who are within two years of their first psychotic episode and 25 community controls (11 females) participated in this study. Social anhedonia was measured with a Revised Social Anhedonia Scale-Brief (SAS-B). 1H MRS data were acquired on a 3T Siemens Prisma scanner using a single-voxel PRESS sequence (TE = 30ms, TR = 2000ms, spectral width = 2000 Hz) with and without water suppression. The high-resolution 1T-weighted scan was re-sliced to position voxels in the right ventral striatum (VS) and ventromedial prefrontal cortex (vmPFC). For the vmPFC, a voxel (25 x 30 x 25 mm) was placed anterior to the genu of the corpus callosum centered around the interhemispheric fissure. For the VS, a voxel (20 x 20 x 20 mm) was centered on the right VS including as much gray matter as possible. The LCModel (version 6.3) was used to analyze 1H MRS data. Any spectra with Cramer Rao lower bounds (CRLB) equal to or greater than 20%, a mean linewidth greater than 0.1 ppm, or signal-to-noise (SNR) ratio less than 10 were excluded. The relationship between creatine-normalized glutamate levels and social anhedonia was examined with Spearman rank correlations.

Results: Patients with first-episode psychosis and controls did not differ for age (21.1 (SD = 3.3) years and 22.5 (SD = 2.7) years, respectively; $F(1,37) = 2.46$, $p = .12$) and sex (Pearson chi-square = .27, $p = .60$). One spectrum of one control in the vmPFC was excluded due to a mean linewidth greater than 0.1 ppm (a mean linewidth of 0.128 ppm). All remaining spectra showed adequate quality. In the right VS, patients and controls did not differ in glutamate levels (1.25 (SD = .17) and 1.28 (SD = .11), respectively; $F(1,37) = .28$, $p = .59$, d = .15). Similarly, patients and controls showed comparable levels of glutamate in the vmPFC (1.22 (SD = .14) and 1.31 (SD = .29), respectively; $F(1,37) = 1.24$, $p = .27$, d = 0.26). Patients showed higher scores on SAB-B than controls (6.5 (SD = 2.4) and 3.1 (SD = 2.9), respectively; $F(1,37) = 13.54$, $p < .001$, d = 1.26), indicating more severe social anhedonia in patients. In the patient group, glutamate levels were negatively associated with a SAS-B total score both in the right VS (rs = -.55, $p < .05$) and the vmPFC (rs = -.46, $p = .09$). Controls did not show any significant relationship between glutamate levels and a SAS-B score in either region (VS, rs = -.02, NS; vmPFC, rs = 00, NS).

Conclusions: In this study, we examined the association between aberrant glutamatergic system and social anhedonia in first-episode psychosis. Patients and controls showed similar levels of glutamate in the VS and vmPFC. This finding is consistent with previous 1H MRS studies that showed no abnormal levels of glutamate in medicated patients with psychosis. Notably, lower levels of glutamate in the VS and vmPFC were correlated with greater levels of social anhedonia in first-episode psychosis, suggesting that reduced levels of glutamate in key reward-related regions may play an important role in social anhedonia in psychosis. Considering the potential therapeutic relevance of this relationship between glutamate and social anhedonia, it will be important to replicate this finding with a larger sample.

Keywords: Glutamate, Social Anhedonia, First Episode Psychosis

Disclosure: Nothing to disclose.

P516. COMPASS-10: A Reduced Item Scale for Assessment of Symptoms in Individuals With First Episode Psychosis

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Background: Use of symptom assessment instruments are routine in research but rare in mental health community clinical practice despite the demonstrated advantages of measurement-based care. The lack of routine symptom assessments in clinical care impedes research initiatives to understand outcomes from large clinical databases. NIMH recently funded the EPINET project that collects data on a large number of patients with early phase psychosis being treated in coordinated specialty care clinics across the country. EPINET includes academic and community sites so a symptom assessment tool that could be readily administered by clinicians was needed.

One barrier to clinician use of symptom assessments has been the large number of items in typical research scales. Reduced item versions of psychosis scales have been developed, notably for the Positive and Negative Symptom Scale. These have been developed based on data from patients with multi-episode psychosis and ratings done in a research context. The RAISE-ETP study of first episode psychosis included COMPASS, a decision support system for first episode psychosis treatment. COMPASS included a clinician-rated symptom assessment that was the basis for our effort to develop a reduced item severity measure for first episode psychosis.

Methods: COMPASS symptom assessment were completed at 17 sites located across the US. Inclusion criteria for RAISE-ETP included age 15-40 years old, DSM-IV diagnoses of schizophrenia, schizoaffective disorder, schizophreniform disorder, brief psychotic disorder, or psychotic disorder not otherwise specified, had experienced only one psychotic episode and had taken antipsychotic medication for < six months. COMPASS symptom assessments were done by clinicians (physicians or nurse practitioners) at every outpatient participant visit; these occurred at least monthly for a minimum of 2 years. Each item is rated on a 7-point scale. Item reduction was a two-stage process. The first stage was a statistical analysis of the scale using a non-parametric item response theory (NIRT) framework for the analyses. The relevant metric is Mokken's scalability coefficient H, with a range between 0 and 1. H can be calculated for a scale item or for the scale as a whole. H is recommended to be > 0.3. In the second stage, COMPASS versions with acceptable scalability were presented to clinicians for their feedback on the importance of the domains assessed.

Results: Data for the scalability analyses included 3600 COMPASS ratings. Two reduced item versions had acceptable H values, a 10-item version with H = 0.359 (SE = 0.007) and a 5-item version with H = 0.396 (SE = 0.009). The 5-item version includes positive symptom items for unusual thought content, hallucinations and conceptual disorganization and negative symptom items for apathy and asociality. The 10-item version has these items plus items for depressed mood, anxious mood, suicidal thoughts, hostility and suspiciousness.

H (SE) values for items of the 5-item version are: unusual thought content 0.357 (0.012), hallucinations 0.355 (0.012), conceptual disorganization 0.294 (0.014), apathy 0.469 (0.010) and asociality 0.461 (0.010). H (SE) values for items of the 10 -item version are: depressed mood 0.371 (0.011), anxious mood 0.357 (0.011), suicidal thoughts 0.338 (0.016), hostility 0.328 (0.011), suspiciousness 0.424 (0.009), unusual thought content 0.375 (0.010), hallucinations 0.363 (0.010), conceptual disorganization 0.227 (0.012), apathy 0.368 (0.010) and asociality 0.359 (0.010).

Clinicians for the focus sessions about the two reduced item COMPASS versions included clinicians who had used the full COMPASS scale in the RAISE-ETP study and clinicians who are current EPINET prescribers who had no experience with the tool. The consensus across both groups was that the additional items in the 10 item version were important domains for clinical decision making and could be rated within the time frame available in routine clinical practice.

Conclusions: The 10 item version of the COMPASS scale (named COMPASS-10) is a potentially useful scale for clinicians to assess symptoms with individuals with first episode psychosis and for use in analysis of large clinical databases. The scale has been

adopted as one of the options for symptom assessment in the NIMH EPINET program.

Keywords: First-Episode Psychosis, Clinical Assessment, Rating Scales, Non-Parametric Item Response Theory

Disclosure: Teva: Consultant (Self)

P517. Modeling Reaction Time Distributions Increases the Statistical Power of Cognition Testing

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Background: Drug development clinical trials often include tests of cognition to assess participants' cognitive performance during individual testing sessions. The testing sessions, designed to measure cognitive domains (such as processing speed, attention, visual learning and working memory) collect subjects' response data as reaction time (RT) and accuracy, and report subject-level metrics (eg, mean log-RT) that are used to quantify cognition. We examined whether current approaches of using mean log-RT to reduce an individual subject's performance data results in information loss relative to other approaches that more fully model individual subject's RT distributions, and thereby reduce the power to test specific hypotheses of cognition.

Methods: Reaction times were extracted from subject-level performance data during computerized cognitive tests (Cogstate tests of Detection, Identification, One Card Learning, One Back). Data from 7 drug development clinical trials (schizophrenia, bipolar disorder, $N = 1{,}890$ subjects) were compared to normative data obtained from healthy subjects ($N = 7{,}108$). Parameters describing subject-level RT distributions were obtained by Bayesian estimation of population models using either ex-Gaussian or Wiener diffusion model residual likelihoods. Information loss was examined by comparing the ability of single parameter mean log RT to reject null hypotheses of cognition versus the parameters of RT distribution models (ex-Gaussian, Wiener Diffusion Model). Here we evaluated the sensitivity and specificity of the discrimination (AUC) between subjects with schizophrenia or bipolar disorder versus healthy subjects.

Results: An individual subject participating in a cognitive test session performed approximately 170-180 responses across the 4 tests. Subject-level RT distributions were well-described by ex-Gaussian and Wiener diffusion models, resulting in parameter estimates for each subject. The sensitivity/specificity of cognitive performance data alone to classify adults with schizophrenia or bipolar depression from healthy subjects was improved for each task. For example, correctly categorizing disease status was improved for the diffusion model versus mean log-RT, with AUC values of 79% vs. 73% for Identification, 75% vs. 65% for One Card Learning, and 74% vs. 61% for Detection.

Conclusions: Analyzing subject-level responses during cognitive testing recovers information lost by mean log-RT, the latter being most typically used in analyses of cognitive performance data. The ability separate individuals with schizophrenia or bipolar disorder from healthy controls using cognitive domains was improved by 10-13 percentage points across cognition tasks. In conclusion, modeling subject-level RT distributions is superior to the typical use of single performance metrics and improved analysis methods may increase the statistical power to test specific hypotheses of cognition in clinical trials.

Keywords: TAAR1, Schizophrenia (SCZ), Cognitive Functioning

Disclosure: Sunovion: Employee (Self)

P518. Population Pharmacokinetic Modeling and Stochastic Simulations to Support Pediatric Dose Selection of Pimavanserin

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Background: Pimavanserin is a selective serotonin modulator with inverse agonist/antagonist activity at the 5HT2A receptor and to a lesser extent at the 5HT2C receptor. It has no appreciable affinity for dopamine receptors, including dopamine D2 receptors. The safety and efficacy of pimavanserin 34 mg has been characterized in both placebo-controlled trials and open-label extension studies in adult patients with Parkinson's disease psychosis, as well as in development programs in adult patients with schizophrenia, major depressive disorder, and dementia-related psychosis. The goal of this report was to perform model based simulations of pimavanserin steady-state exposures to identify a dose in pediatric patients that results in exposure comparable to the target exposure achieved with the oral 34-mg dose that has demonstrated safety and efficacy in different patient populations.

Methods: A population pharmacokinetic (PK) model was generated using pooled plasma drug concentration data from 13 phase 1/2b/3 clinical studies of pimavanserin, including a phase 1 study of adolescent patients (13–17 years) to assess patient factors that may contribute to interpatient variability in PK in this population. The final PK model was used to perform model-based stochastic simulations to explore the expected range of pimavanserin exposures in virtual pediatric subjects, 5–17 years of age, based on age and body weight distributions obtained from the Centers for Disease Control and Prevention Growth Chart. Steady state pimavanserin exposures, including the area under the plasma concentration-time curve (AUC) and maximum drug concentration levels (Cmax), for 10, 20, and 34-mg doses were simulated for virtual pediatric and virtual adolescent populations. Simulations were also performed using the adolescent and adult patients (<50 years of age) from the actual population PK analysis population, who served as the reference groups for steady state drug exposures associated with the 34-mg daily dose regimen.

Results: Graphical and tabular comparisons of simulated pimavanserin drug exposures illustrated that steady-state AUC levels following 34-mg dosing were generally not different across the range of age groups or body weight groups. There was a trend for higher Cmax with decreasing age and body weight in pediatric groups. In the adult population (18–49 years), mean AUC ranged from 47.41 to 54.73 ng x d/mL and mean Cmax ranged from 41.13 to 50.07 ng/mL. In the young pediatric population (5–9 years), simulations predicted that a 20-mg dose would produce a distribution of Cmax (mean [SD]: 45.30 [21.31] ng/mL) that would be similar to Cmax associated with the 34-mg dose in the adult population. In the older pediatric population (aged 10–17 years), a 34-mg dose was predicted to yield a Cmax distribution (mean [SD], 56.54 [24.58] ng/ML) similar to steady state exposures associated with a 34-mg dose in the adult population.

Conclusions: Model-based stochastic simulations exploring 4 dose levels of pimavanserin indicate that pimavanserin 20 mg may be most appropriate in a young pediatric population (5–9 years) and pimavanserin 34 mg may be most appropriate in an older pediatric population (10–17 years) to yield steady-state pimavanserin exposures similar to a daily pimavanserin 34-mg oral dose in adults (18–49 years). These results could inform dose selection in pediatric patients.

Keywords: Schizophrenia, Pimavanserin, Pharmacokinetics

Disclosure: Acadia Pharmaceuticals Inc.: Employee (Self)

P520. Medial Frontal Cortex GABA in Psychosis and Depression: A Meta-Analysis of Magnetic Resonance Spectroscopy Studies

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Background: Substantial evidence suggests that abnormalities of GABAergic systems may play an important role in psychosis spectrum disorders and mood disorders. Post-mortem studies have consistently reported deficits in the expression of multiple GABA-related proteins in these patients, and medications that potentiate GABAergic transmission are frequently used to treat patients in the psychosis spectrum for anxiety or as mood stabilisers. Proton magnetic resonance spectroscopy (1H-MRS) allows non-invasive in vivo quantification of the metabolites of the human brain. However, 1H-MRS studies of frontal cortex GABA concentrations in the psychosis spectrum have thus far yielded inconsistent findings. There are several possible reasons for such inconsistencies. First, findings may be complicated by different voxel placements that have been grouped together in meta-analytic studies. The medial frontal cortex is a functionally heterogeneous region and lumping together disparate voxels may obscure true GABA differences. Second, a number of factors such as stage of illness, medication status and age all may confound meta-analyses.

Methods: We systematically searched the PubMed database for 1H-MRS studies of medial frontal GABA in male and female participants with schizophrenia, bipolar disorder, depression, and individuals meeting ultra-high risk (UHR) for psychosis criteria. For each study, voxel placements were reviewed, and classified as rostral anterior cingulate cortex (rACC), dorsomedial prefrontal cortex (dmPFC), or posterior prefrontal cortex (pMFC). Studies of schizophrenia were classified as acute (within 5 years of illness) or chronic (more than 5 years following illness onset). Random-effects meta-analyses were conducted separately for each clinical group, for each of the MFC sub-regions. Sub-group analyses exploring the influence of illness stage (acute vs chronic) in the schizophrenia sample were conducted where more than 8 studies were found for that sub-region. Effect sizes were estimated using Hedges' g. Heterogeneity of effect sizes and publication bias were assessed for each meta-analysis performed.

Results: Of 326 screened articles, we included 21 studies of Schizophrenia (Sz; 699 patients, 795 controls), 5 studies of bipolar disorder (BPD; 111 patients, 79 controls), 18 studies of depression (MDD: 370 patients, 403 controls), and 6 studies of individuals at an ultra-high risk of developing psychosis (UHR; 222 patients, 164 controls) in the meta-analysis. Meta-analysis revealed significantly lower pMFC GABA concentrations in schizophrenia (g = -0.31, 95% confidence interval [CI]: -0.56 to -0.06, $p < .05$, I2 = 19%) and increased rACC GABA concentrations in bipolar disorder (g = .76, 95% CI = .25 to 1.26, $p < .05$, I2 = 0%). No other significant differences in GABA concentrations were found. Of note, we did not find any evidence for GABA concentration changes in depression, in any of the subregions (rACC: g = -0.29, 95% CI = -0.67 to 0.08, $p = .10$, I2 = 40%; dmPFC: g = -.35; 95% CI = -0.82 to 0.11, $p = .11$, I2 = 24%; pMFC: insufficient studies). We did not find evidence for a confounding effect of illness duration, however there were insufficient studies to explore this at each MFC sub-region to fully explore this question. We found no evidence of publication bias.

Conclusions: The current results substantiate the relevance of GABA in the ethology of psychosis spectrum disorders and underline the importance of careful consideration of voxel placement. Additional studies are required to fully understand GABA concentrations in bipolar disorder and UHR, and to elucidate the relationship between frontal GABA concentrations and illness duration.

Keywords: Magnetic Resonance Spectroscopy, GABA, Meta-Analysis, Psychosis

Disclosure: Nothing to disclose.

P521. An Investigation of Variable Number Tandem Repeat (VNTR) Variation in Schizophrenia Post-Mortem Brain

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Background: Tandem repeats (TRs) are prevalent throughout the genome, with more than one million TR loci constituting at least 3% of the genome, including short tandem repeats (STRs, 1-6bp in motif length) and variable number tandem repeats (>7bp in motif length). A pathogenic role of STR variation has been established for some neuropsychiatric disorders (e.g. trinucleotide repeat expansion disorders, Huntington's Disease), whereas VNTRs are less well studied, overall. Interestingly, previous one-off studies of select SCZ risk loci indicate that VNTRs may influence SCZ genetic risk warranting a more thorough, systematic analysis. The current project therefore aimed to begin to elucidate the potential contribution of VNTRs to schizophrenia genetic risk, by genotyping VNTRs within known SCZ genome-wide association studies (GWAS)-positive loci in DNA derived from post-mortem brain, in schizophrenia and control samples, and querying for an effect of VNTR copy number on cis-gene expression.

Methods: The next-generation sequencing based tool, adVNTR, genotyped VNTRs in whole genome sequenced (WGS) DNA extracted from post-mortem cerebellum and dura matter ($n = 54$ SCZ, $n = 54$ controls (CONT). The adVNTR algorithm used hidden Markov models to model each VNTR, count repeat units, and detect sequence variation. Then, for VNTR loci with high variance, gene expression was regressed against VNTR copy number, covarying for age, sex and RIN.

Results: The adVNTR algorithm genotyped $n = 3{,}189$ VNTR loci, each within 100kb of $n = 270$ index single nucleotide polymorphisms (SNPs), derived from the most recent SCZ GWAS meta-analysis. VNTR loci with the highest variance and within genes of known functional neurobiological import included a BDNF intronic VNTR (CCGCCCC)n, annotated to be located in an alternative 5′ untranslated region, located 73kb from a SCZ GWAS index SNP, for which adVNTR genotyped copy numbers of 7 and 21. (BDNF VNTR Copy Number Distribution, SCZ $n = 12$ (7/7); $n = 25$ (7/21); $n = 11$ (21/21); CONT $n = 7$ (7/7); $n = 35$ (7/21); $n = 4$ (21/21) Multi-allelic variance was noted for a VNTR within the promoter region of SLC45A1 (ACCCATCCACATATCC)n, a gene encoding a glucose transporter in the brain, located 14 kb from a SCZ GWAS index variant, for which adVNTR genotyped copy numbers of 3, 4, and 10. (SLC45A1 VNTR Copy Number distribution: SCZ $n = 22$ (3/10), $n = 9$ (4/10), $n = 18$ (10/10); CONT $n = 25$ (3/10), $n = 8$ (4/10), and $n = 17$ (10/10) An exonic VNTR within RERE (CTCCCGCTCTCGGAT) n, overlapped a SCZ GWAS index variant, for which adVNTR genotyped a copy number of 4, with no copy number variability identified across the $n = 108$ WGS samples. Of $n = 387$ VNTR loci with high variability in copy number across the $n = 108$ WGS samples, there was no detected association of cis-gene expression with VNTR copy number in DLPFC or hippocampus ($n =$ 55 samples), after multiple testing correction.

Conclusions: VNTR variation in SCZ/CONT WGS post-mortem brain was identified in some regions proximal and distal to schizophrenia GWAS-positive loci, but the current assessment of SCZ-association with VNTRs was limited by sample size. Future analyses will expand to include additional VNTR calling methods and additional post-mortem brain samples, as well as additional SCZ samples derived from a hospital-based biobank. The linkage disequilibrium of VNTR loci with SCZ GWAS index variants will also be investigated.

Keywords: Tandem Repeat Variation, Schizophrenia, Whole Genome Sequence, Post-Mortem Brain

Disclosure: Nothing to disclose.

P522. Thalamocortical Abnormalities During Sleep in Schizophrenia

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Background: Mounting evidence suggests thalamocortical deficits in patients with schizophrenia (SCZ) manifested as impaired sleep spindles in NREM2 sleep. Here, we used a large clinical dataset to conduct an extensive investigation of spindle density and morphology in SCZ. In addition, we aim to characterize spindle deficit with respect to other less studied sleep EEG measures including slow oscillations (SO), coupling between SO and spindles and global connectivity patterns. Deficits of auditory Event related Potentials (ERP) have been widely reported in SCZ, suggesting abnormal auditory processing during wake. Leveraging the unique combination of sleep and wake data collected on the same individuals, we investigate whether the sleep EEG abnormalities and auditory ERP alterations in SCZ reflect similar thalamocortical circuit dysfunction.

Methods: As part of a larger ongoing study, whole night high-density EEG and wake auditory ERP data were collected for 72 patients with SCZ (25 females, mean age of 34.8 ± 6.96 years) and 57 healthy volunteers (22 females, mean age of 31.7 ± 6.29 years). Clinical, medication, and demographic information was collected for each subject. Using our open-source package Luna, we automatically detected slow, fast sleep spindles (target frequencies of 11 and 15 Hz, respectively) and SOs in NREM2 EEG segments. Multiple parameters characterizing spindles, SOs and their coupling were estimated. We also computed spectral power and phase-slope index (PSI) quantifying connectivity across wide frequency ranges. In addition, ERP analysis was performed using BrainVision Analyzer, yielding measures of auditory steady state response (ASSR) at 40Hz, mismatch negativity and sensory gating. Statistical significance of group differences was tested using a general linear model, with age and sex as covariates. All results were corrected for multiple comparisons using permutation rounds to determine the probability of significant *p*-values due to chance.

Results: Our results indicated that sleep macrostructure was comparable between groups except from reduced sleep efficiency ($p = 0.0012$) in SCZ. Spectral composition of EEG signals was altered in SCZ with profound decrease in delta/theta power (2-5 Hz) and sigma frequency range (11-14 Hz) with the latter suggesting alterations in spindles. We observed a marked and highly significant decrease in density and amplitude of slow and fast spindles in SCZ, that was not explained by medication. The largest effects were observed for the fast spindle density with significant differences (padj < 0.05) in 53 out of 57 channels with effect size ranging from -0.55 to -1.27 SD. Decreased density and amplitude of slow spindles correlated with higher PANSS scores. Patients with SCZ also displayed significand reductions in fast spindle duration and chirp, as well as integrated slow spindle activity across multiple channels. They also have increased SO duration (10 channels with padj<0.05, effect sizes ranging from 0.68 - 0.91 SD), reduced negative peak amplitude and slope between negative peak and positive zero-crossing in frontal channels. The abnormalities in the last two metrics, however, vanished when we accounted for adjunctive medication. Both control and SCZ groups displayed comparable strength of coupling between spindles and SO, but the proportion of slow spindles overlapping with SO was significantly reduced in SCZ in 13 frontal channels (effect sizes from -0.75 to -1.25 SD) and fast spindles tended to occur earlier during SO in SCZ (236.8°±25.81) compared to CTR group (249.2°±29.1). Phase slope index averaged for each channel revealed hyperconnectivity at 5-8 Hz and 11-14 Hz in the SCZ group, while the general patter of information flow was unchanged. Sleep spindles abnormalities, power reduction and hyperconnectivity were replicated in an independent demographically distinct sample of SCZ patients. Logistic regression model built based on the principal components derived from spindle parameters, spectral power and connectivity was able to correctly classify SCZ subjects with area under ROC curve (AUC) equal to 0.93, and with AUC = 0.73 in the replication sample. In wake auditory ERP analysis, we confirmed the diminished 40 Hz ASSR power and phase synchrony, reduced MMN amplitude and altered sensory gating in SCZ patients. Wake alterations, however, were largely independent from sleep EEG abnormalities and with respect to each other, suggesting that sleep and wake EEG abnormalities index unique SCZ pathophysiology.

Conclusions: Sleep spindle deficit is a highly reproducible trait of SCZ pathophysiology. Despite similar reduction in density and amplitude, slow and fast spindle displayed distinct alterations in their morphology. In addition, our analyses revealed the SO/spindle coupling and global connectivity patterns were altered in patients of SCZ, while spindle deficits are the most robust and spread across many channels. Similarly, spindle abnormalities in SCZ appeared to be stronger than the auditory alterations during wake. Interestingly, our findings suggest that sleep and wake EEG alterations are statistically independent, potentially indexing different facets of thalamic and cortical dysfunction in SCZ and conveying unique information that could expand our knowledge of neurobiological underpinnings of SCZ.

Keywords: Sleep Spindles, Slow Oscillation, EEG, Thalamus - Reticular Nucleus, EEG Connectivity

Disclosure: Nothing to disclose.

P524. Clinical Consequences of Lack of Scalability of the Positive and Negative Syndrome Scale (PANSS)

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Background: The Positive and Negative Syndrome Scale (PANSS) is considered the 'gold standard' when evaluating the efficacy of experimental drug interventions in patients with schizophrenia. The scale was developed several decades ago in response to a two-dimensional model of schizophrenia to cover a positive and a negative symptom domain as reflected in the PANSS subscales. However, it is mainly the PANSS total score that has gained importance as a clinically relevant composite score of schizophrenia symptom severity. The total score is in widespread use in

research but less so in clinical care due to considerable requirements of time and training to administer the scale. The PANSS has previously been psychometrically evaluated in terms of assessment of the validity of the 30-item total score. However, clinical interpretation of a poor fit to applied statistical models has not been examined.

The aim of this study was to examine the scalability of the total 30-item scale (PANSS-30) - and of a shortened version (PANSS-6) that has recently been advocated for use - in a large sample of first-episode patients with schizophrenia. Scalability was assessed using Rasch analyses. Subsequently, we assessed the clinical consequences of a possible non-fit to the Rasch model.

Methods: We analyzed a composite data set consisting of 1,073 subjects from three European first-episode schizophrenia studies (the OPTiMiSE, EUFEST and PECANS studies). Study participants were clinically assessed at baseline and again after 4-6 weeks of treatment with antipsychotic monotherapy (a different compound in each study). We evaluated the scalability of the PANSS-30 and the PANSS-6 using Rash modelling. This statistical analysis investigates whether a total score across a set of items is a meaningful measure of total symptom severity as expressed by the individual items. In order to assess the clinical consequences of a non-fit, we applied Rasch structure analysis for the PANSS-30 and the person separation index for the PANSS-6. For the Rasch structure analysis, we dichotomized the response categories (present/not present) to demask the most basic properties of the PANSS scale. This was a necessary methodological step since the results showed that the administration of the 7-point Likert scale was inconsistent across items.

Results: Neither the PANSS-30 nor the PANSS-6 fulfilled the Rasch model and as such were not meaningful measures of overall symptom severity as expressed by the two sets of items in this patient sample. The non-fit resulted in a marked systematic error which together with the random error summed up to a clinically significant level where misclassifications into response versus non-response (based on PANSS-30 total score) might be the consequence. For the PANSS-6, the problems were so severe that individual patients were not necessarily separable by using this scale in the current sample. Discrepancies to previous Rasch analyses with a positive outcome for the PANSS-6 were observed and will be discussed.

Conclusions: Academia and industry need to recognize the manifest psychometric limitations when using the total PANSS-30 score. Reducing the number of items as in the PANSS-6 does not seem to solve the problem. Until future analyses confirm the current findings, the results are limited to patients with first-episode psychosis. Important methodological technical details separate this and previous Rasch analyses of the PANSS-30.

Keywords: Validity, Panss, First-Episode Schizophrenia

Disclosure: Nothing to disclose.

P525. D-Serine Augmentation of Neuroplasticity-Based Auditory Learning in Schizophrenia

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Background: Schizophrenia (Sz) is a major public health problem associated with core cognitive deficits that are amongst the strongest predictors of impaired functional outcome. In addition, Sz patients show reduced cortical neuroplasticity, defined as reduced learning during training on exercises that place implicit, increasing demands on early auditory information processing. As improved auditory processing can facilitate gains in those cognitive processes that are more proximal to daily functioning (e.g., verbal memory, executive functioning), enhancing neuroplasticity for better auditory processing represents an unmet clinical need and a rate-limiting first step prior to remediating cognition and overall function.

Over recent years, NMDAR glycine site agonists have increasingly been shown to facilitate neuroplasticity in both Sz and healthy volunteers. Using the R61 mechanism, we conducted a study to assess target engagement, pharmacodynamics, functional relationships and the optimal dose (80 vs.100 vs. 120 mg/kg) of the NMDAR modulator D-serine combined with 3 sessions of a neuroplasticity-based auditory remediation program (AudRem) in Sz.

Methods: The study was conducted in three separate 15 subject, double-blind, randomized dose-cohorts (12 subjects on D-serine 80, 100 or 120 mg/kg and 3 on placebo). Subjects were randomized to double-blind D-serine/placebo, 30 minutes before three AudRem sessions administered 1x per week to allow for training during peak levels. EEG was recorded pre/post and during sessions, to assess early auditory processing measures mismatch negativity (MMN) and theta.

In AudRem, participants were presented with paired tones (e.g., Stimulus 1 ("reference") and Stimulus 2 ("test"): S1 and S2) and indicated which tone is higher in pitch (frequency). In the first pair, the ratio is 50% (e.g. 1000 ± 500 Hz). A two-down/one-up staircase procedure was used to adjust the ratio to maintain a steady (~70% correct) level of performance across the 80 repeats. Ratios in each pair are averaged across 10-trial pairs (e.g., pairs 1-10, 11-20, etc.).

Milestones were designed to confirm target engagement, functional relationships and safety, and were assessed with three preplanned outcomes: plasticity, theta and mismatch negativity (MMN). As previously, plasticity was defined as improved pitch thresholds between successive auditory stimuli after AudRem, operationalized as (1) End of session plasticity: an improved (smaller) threshold (%frequency, S2/S1) at the end of the titration period (mean of trials 70-80) and (2) Within session plasticity: increase from initial plateau (trials 20-30) to end of titration period (trials 70-80). As previously, MMN was defined as change from baseline after treatment and theta as change during AudRem sessions. Milestones required at least a moderate effect size difference ($d = 0.5$) between D-serine and placebo groups.

Results: 45 Sz subjects were randomized, meeting our preplanned "n." Consistent with the study eligibility criteria, subjects were on a stable antipsychotic dose for at least 4 weeks, had normal kidney function with an estimated Glomerular Filtration Rate (GFR) greater or equal to 60 and had moderate or lower cognitive disorganization (PANSS P2 less than or equal to 4).

As assessed by the Verbal Memory and overall composite domains of the MATRICS and the Tone Matching Task (TMT), subjects had impairments in both overall (27.6 ± 10.9) and auditory cognition (35.9 ± 8).

Target engagement was demonstrated by significant improvement in plasticity within the D-serine treatment arms in all three cohorts (all $p < 0.001$). Furthermore, moderate to large effect sizes vs. placebo for within session plasticity were seen for the 80 mg/kg cohort ($d = 1.25$) and 120 mg/kg cohort ($d = 0.70$). The 100 mg/kg cohort showed moderate effect size differences for both end of session ($d = 0.70$) and within session ($d = 0.52$) plasticity. D-serine treated subjects showed continued 2 to 3% improvement from their initial plateau, while placebo treated subjects worsened.

Target engagement was also demonstrated by moderate to large effect sizes vs. placebo for post baseline MMN change for all three cohorts (all $d > 0.7$) and for theta-ITC for Cohort 2 ($d = 0.74$) and theta power for both S1 ($d = 0.53$) and S2 ($d = 0.60$) for cohort 3. As previously, MMN tended to show worsening with placebo and theta showed increases relative to placebo.

A moderate effect size correlation between plasticity and auditory cognition and both end of session (TMT $r = 0.41$) and

within session (MATRICS verbal learning r = 0.4) plasticity across cohorts. Correlations remained within the "medium-large" (r = 0.3-0.5) range within the D-serine group. Moderate effect size correlations were also seen for overall cognition, speed of processing and reading, suggesting that changes on the plasticity task are predictive of sustained functional improvement.

Safety of D-serine was demonstrated by a complete absence of renal toxicity or clinically significant adverse events in any subject.

Conclusions: All prespecified Go/No Go milestones were met. Findings strongly support engagement of the NMDAR system by D-serine. We did not find a clear dose response, with improvements across all three cohorts. This was potentially due to the narrow range of doses tested under our IND. D-serine was well tolerated across all three doses. Furthermore, while we focused on auditory plasticity and Sz in the present project, results are relevant across learning disorders across conditions.

Keywords: NMDA Receptor, D-serine, Neuroplasticity, CNS Clinical Trials, NMDA Receptors, MMN, ERP, Auditory, Training

Disclosure: Nothing to disclose.

P526. Dopamine Synthesis Capacity, Glutamate, and GABA Concentrations as Diagnostic Markers in Antipsychotic-Naïve Schizophrenia Spectrum Patients

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Background: Dysregulation of striatal dopamine synthesis capacity (DSC), as well as cortical concentrations of glutamate and glutamine (Glx), and gamma-aminobutyric acid (GABA) are considered key factors in the development of psychosis, however, the interaction between these neurotransmitters needs further investigation.

Combined measures of two neurotransmitters in relevant regions of interest (ROI) in first episode psychotic patients suggest that DSC and glutamate concentrations in anterior cingulate cortex (ACC) are inversely related in patients, but not in controls, and that concentrations of glutamate in thalamus is higher and GABA concentrations in ACC lower in patients as compared to controls. The latter finding in an overlapping sample.

In this study, we applied novel, conjoint analyses of all three neurotransmitters; DSC, concentrations of Glx in thalamus, and GABA in ACC to separate a group of antipsychotic naïve schizophrenia spectrum patients from a group of healthy controls.

Methods: Twenty-five patients (median age 22, nine males) and 21 controls (median age 21, nine males) matched on age, gender and parental educational level were included.

All participants underwent two different neuroimaging sessions within one week: dynamic positron emission tomography (PET) with fluorine-18 labeled fluoro-DOPA (18F-DOPA) yielding measures of striatal DSC, and 3 Tesla magnetic resonance spectroscopy (MRS) yielding spectra of Glx concentrations in thalamus and GABA concentrations in ACC.

The DSC variable Ki4p, expressing net influx (mL* g-1 * min-1) of dopamine to striatum, was obtained using an arterial input function and PET acquisition till 150 minutes post injection. Variables were fitted using a Four-parameter model. Based on previous findings of an association between DSC (Ki4p), and additionally the decarboxylation rate of 18F-DOPA to 18F-dopamine, k3, in nucleus accumbens (NAcc) and psychotic symptoms, implicating a role of dopaminergic activity specifically in this striatal subregion in psychopathology (Sigvard et al. under review), NAcc was selected as the ROI for the DSC data.

The Glx spectra from the MRS data were obtained using PRESS acquisition with voxel placement in left thalamus and analyzed with LCModel. The GABA spectra were obtained from a bilateral voxel in ACC using the MEGAPRESS sequence and analyzed with Gannet.

As statistical model, we used binominal logistic regression implemented in SPSS to separate patients from controls. DSC, Glx, and GABA were used as predictors. Since the interaction term DSC*GABA was significant, this term was included as a fourth predictor.

Results: The overall model was significant ($\chi2(4) = 18.788$, $p = 0.001$), and explained 44.8% of the variance. The model accuracy was 82.6%, sensitivity was 84.0%, specificity was 81.0%. The positive predictive value was 84.0%, and the negative predictive value was 81.0%. Of the predictor variables three were statistically significant: GABA ($p = 0.012$), DSC ($p = 0.016$) and the interaction-term GABA*DSC (0.017).

Conclusions: The significance of the overall model supports the notion that all three neurotransmitters, DSC in NAcc, Glx in thalamus and GABA in ACC, contribute to the discrimination between patient and controls. The finding that DSC adds important information when attempting to identify patient is in line with previous work and existing hypotheses. According to the present model, the GABA concentration in ACC is, however, at least as strong a predictor as DSC in NAcc. Information on the interaction between DSC and GABA improves chances of identifying patients further hereby suggesting that the interrelation between DSC and GABA is disturbed in patients. Our findings support the growing body of evidence (yet predominantly in preclinical studies) that GABAergic disturbances play a key role in the development of psychosis.

Keywords: Striatal Dopamine Signaling, Glutamate GABA, Antipsychotic-Naive Schizophrenia, Positron Emission Tomography (PET), 1H MRS

Disclosure: Nothing to disclose.

P527. The Road Not Taken: Disconnection of a Human-Unique Cortical Pathway in Schizophrenia and Its Effects on Naturalistic Social Cognition

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Background: Efficient processing of complex and dynamic social scenes relies on intact connectivity of many underlying cortical areas and networks, but how connectivity deficits affect this functioning in social cognition remains unknown. Here we measure these relationships using functional localization of social cognition areas, resting-state functional connectivity, and movie-watching data.

Methods: In 42 schizophrenia participants (SzP) and 41 healthy controls (HC), we measured the functional connectivity of areas localized by face-emotion processing, theory-of-mind, and attention tasks. We quantified the weighted shortest path length between visual and medial prefrontal theory-of-mind areas in both populations to assess the impact of functional connectivity deficits on network structure. We then correlated connectivity along the shortest path in each group with movie-evoked activity in a key node of the theory-of-mind network (TPJp).

Results: SzP had pronounced connectivity deficits in temporoparietal junction/posterior superior temporal sulcus (TPJ-pSTS) areas involved in face-emotion processing ($t(81) = 4.4$, $p = 0.00002$). In HC the shortest path connecting visual and medial

prefrontal theory-of-mind areas passed through TPJ-pSTS, whereas in SzP the shortest path passed through prefrontal cortex (PFC). While movie-evoked TPJp activity was correlated with connectivity along the TPJ-pSTS pathway in both groups ($r = 0.43$, $p = 0.002$), it was additionally correlated with connectivity along the PFC pathway only in SzP ($rSzP = 0.56$, $p = 0.003$).

Conclusions: Connectivity along the human-unique TPJ-pSTS pathway affects both the network architecture and functioning of areas involved in processing complex dynamic social scenes. These results demonstrate how focal deficits can have widespread impacts across cortex and potential compensatory mechanisms.

Keywords: Resting State Functional Connectivity, Right Temporo-Parietal Junction, Theory of Mind

Disclosure: Pfizer Inc.: Employee (Spouse)

P528. Experience-Dependent Observational Fear in Hippocampal-Amygdala Memory Engram Networks

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Background: The empathic ability to vicariously experience the other's fearful situation, a process called observational fear (OF), is critical to survive in nature and function in any social context. Considerable progress in our understanding of OF has been made by OF models in primates and rodents. In particular, the neural processes in the anterior cingulate cortex (ACC) and basolateral amygdala (BLA) networks are currently considered to be the center for OF. Remarkably, OF can be facilitated by prior experiences, including both prior similar fear experience in the observer and social familiarity with the demonstrator, which allow individuals to understand other's fearful situation even if the demonstrator has ambiguous reaction. However, the neural circuit mechanisms of experience-dependent OF (Exp OF) have so far been unknown, because they are largely based on the Naive OF model, which delivers strong aversive stimuli to the demonstrator eliciting a robust reaction and thus induces OF without prior experiences. Furthermore, virtually nothing is known about the roles of dorsoventral hippocampal (HPC) function on Exp OF, despite the importance of hippocampal function for memory process about prior similar fear experience in dorsal HPC and social familiarity with the demonstrator in ventral HPC.

Methods: We established two models of OF in mice: Naive OF, which does not require prior experience to induce OF, and Exp OF, which requires prior similar fear experience with contextual fear conditioning (CFC) and social familiarity (SF) with the demonstrator to induce OF. For both models, the OF apparatus consists of two chambers separated by a transparent plexiglass partition. The observer chamber has a plexiglass floor, and the demonstrator chamber has an exposed stainless-steel rod floor to deliver electrical footshocks. In Naive OF, after a habituation period (HP), the strong OF protocol (1.0mA, every 10 sec) was applied to the demonstrator during the shock period (SP), which produced a robust demonstrator's fear reaction and then induced the freezing response in the observer. In Exp OF, on Day 1, observers received contextual fear conditioning experience (CFC) in a different chamber as prior similar fear experience. On Day 2, after a habituation period, the weak OF protocol (0.5mA, every 15 sec) was applied to the demonstrator, which produced an ambiguous demonstrator's reaction and induced observer freezing. Exp OF required both CFC experience in the observer and social familiarity with the demonstrator.

Results: We first established Exp OF and Naive OF models in mice and investigated how ACC, BLA, dorsal HPC and ventral HPC activity and the relationship across these brain regions contributes to both Exp OF and Naive OF. We discovered that dorsoventral HPC to BLA pathways, without involving ACC function, facilitate Exp OF ($P < 0.05$ by unpaired t-test, $N = 9$-11 mice / group), while the ACC to BLA pathway mediates Naive OF ($P < 0.05$ by unpaired t-test, $N = 9$-11 mice / group). These two distinct neural pathways for Exp OF and Naive OF are evoked by different sensory perception modalities.

Second, to understand the neural mechanisms about how the HPC-BLA networks mediate Exp OF, we addressed the role of memory engram cells in HPC and BLA that encode prior similar fear experience in Exp OF. We found that fear memory engram cells in BLA encoding prior similar own fear experience are significantly reactivated during Exp OF compared with non-engram cells ($P < 0.01$ by paired t-test, $N = 5$ mice / group)) and are necessary for Exp OF ($P < 0.01$, by unpaired t-test, $N = 11$, 13 mice / group). Dorsal HPC neurons generate fear memory engram cells in BLA during prior own fear experience ($P < 0.05$, One-way ANOVA, Post-hoc test: Tukey-Kramer test, $N = 5$-6 per group), while ventral HPC neurons reactivate the fear engram cells in BLA to elicit Exp OF ($P < 0.05$, One-way ANOVA, Post-hoc test: Tukey-Kramer test, $N = 5$-6 per group) by sending the information about vicarious fear experience in the familiar demonstrator. in vivo calcium imaging further revealed that the fear memory engram cells in BLA represent emotional mirror neuron activity during both own and the familiar demonstrator's aversive moment. Therefore, we propose that the memory engram circuits in the HPC-BLA networks govern the integration of perception-action coupling as a principle of Exp OF.

Conclusions: In this study, we found that HPC to BLA pathways, without ACC, facilitate Exp OF, while the ACC to BLA pathway mediates Naive OF. dHPC neurons is necessary for the generation of fear memory engram cells in BLA encoding CFC memory on Day 1. The fear memory engram cells in BLA are reactivated during Exp OF and the reactivation is necessary for Exp OF on Day 2. On the other hand, vHPC neurons respond to the familiar demonstrator's fearful situation that reactivate the fear memory engram cells in BLA to elicit Exp OF on Day 2. BLA fear engram cells are activated during both own shock and the familiar demonstrator's shock moment. The two distinct neural pathways for Exp OF and Naive OF facilitate OF in a parallel manner.

Keywords: Emotional Empathy, Mirror Neuron, Memory Engram Cell, Hippocampus, Anterior Cingulate Cortex

Disclosure: Nothing to disclose.

P529. Neural Circuit Recruitment and Adult Neurogenesis Abnormalities in the Dentate Gyrus of the Df(h22q11)/+ Mouse Model of 22q11.2 Deletion Syndrome

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Background: 22q11.2 deletion syndrome (22qDS) confers the highest known genetic risk for schizophrenia and is also associated with several other neurodevelopmental conditions such as autism. Several mouse models with genetic deletions orthologous to those in human 22q11.2 deletion syndrome have been instrumental in revealing cellular and circuit deficits in many brain regions, especially the hippocampal cornu ammonis and prefrontal cortex. However, far less is known about whether networks within the dentate gyrus (DG) are abnormal in 22qDS mice and which cell types are involved, which might enable a greater mechanistic understanding of DG-dependent cognitive

deficits in neurodevelopmental conditions. We investigated this question in the Df(h22q11)/+ mouse model of 22qDS.

Methods: Male and female Df(h22q11)/+ (originally purchased from Taconic Biosciences) and wildtype littermates were used. To evaluate neuronal activation in vivo in response to novel contexts, adult mice were exposed to a novel cage containing several novel small toys for 10 mins, then transcardially perfused 80 mins later. Control mice were perfused directly from their home cage. For electrophysiological experiments, 300-micron horizontal sections were prepared from juvenile mice and whole-cell patch-clamp recordings from DG granule cells performed while electrically stimulating the inner molecular layer to activate mossy cell inputs. The object location memory task was performed in adult mice by habituating mice to an arena containing spatial cues for 6 days, followed by a training day where mice are exposed to the arena containing 2 identical novel objects for 10 mins. 24 hrs later, mice are returned to the arena which contains the same objects, one in a familiar location and the other in a novel location, and time spent investigating each object is quantified for a 5 min test session. Statistical analyses were performed using t-test, ANOVA, or Kolmogorov-Smirnov test, as appropriate.

Results: Consistent with previous studies, Df(h22q11)/+ mice were unable to recall the moved object in the object location memory test whereas WT performance differed significantly from chance ($n = 7$-8/genotype). No significant differences were found in the number of dorsal or ventral DG mossy cells as revealed by staining for the mossy cell marker GluR2/3, the number of parvalbumin (PV)-positive interneurons, or the number of GABA-positive neurons ($n = 4$/genotype). However, PV-staining intensity was reduced in Df(h22q11)/+ mice ($n = 4$/genotype, 171 total cells, $p = 0.051$). Consistent with a functional inhibitory deficit, inner molecular layer electrical stimulation to activate mossy cell inputs resulted in greater excitability of granule cells in Df(h22q11)/+ mice (action potentials elicited in 3 of 3 cells from 2 Df(h22q11)/+ mice vs. 1 of 5 cells from 2 WT mice). As expected, exposure to a novel context increased granule cell cFos expression (main effect of context: $p < 0.0001$), but context x genotype interaction was not significant ($p = 0.26$, $n = 5$-7/genotype). Interestingly, activation of hilar cells as revealed by cFos staining in response to novel context was impaired in Df(h22q11)/+ mice (context x genotype interaction: $p < 0.05$; Sidak post-hoc home cage vs. novel cage: WT: $p < 0.0001$, Df(h22q11)/+: $p = 0.14$). Finally, the number of immature granule cells as revealed by Dcx-staining was modestly but significantly reduced in Df(h22q11)/+ mice (~14% reduction, $p < 0.05$).

Conclusions: The results of this study suggest several abnormalities in excitation-inhibition balance in the DG of Df(h22q11)/+ mice, including impaired recruitment of PV-interneurons and neurons in the hilus, but not granule cells. Future studies will determine the identify of this aberrantly underactivated hilar cell population, whether this deficit is cell autonomous, and whether restoration of activity in these neurons can improve performance in tasks involving context encoding.

Keywords: Dentate Gyrus, 22q11 Deletion Syndrome, Hippocampus, Neurogenesis, Spatial Memory

Disclosure: Nothing to disclose.

P530. Hippocampal Blood-Brain Barrier Damage and Peripheral Vascular Compromise in Schizophrenia Share Endothelial Dysfunction

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Background: Schizophrenia spectrum disorder (SSD) is one of the most severe forms of mental illness, yet mechanisms remain unclear. A widely established brain finding in SSD is hippocampal atrophy, and a coherent explanation similarly is lacking. Epidemiological evidence suggests increased cerebrovascular and cardiovascular complications in SSD independent of lifestyle and medication, pointing to disease-specific pathology. Endothelial cell contributions to blood-brain barrier (BBB) compromise may influence neurovascular unit and peripheral vascular function, and we hypothesize that downstream functional and structural abnormalities may be explained by endothelial deficits.

Methods: Postmortem human hippocampus sections ($n = 27$ controls, $n = 25$ SSD) were obtained from the NIH NeuroBioBank and Maryland Brain Collection, that were age, gender, race, and postmortem interval (PMI) frequency-matched to interrogate BBB integrity. Leakage phenomena was observed using a secondary IgG-only (Vector Laboratories, 1:250) staining technique, to demonstrate endogenous IgG extravasation. IgG leak was quantified using unbiased stereology (MBF Bioscience, VT) to measure the areas of IgG immunoreactivity under blinded analysis. A ratio was then calculated of fraction of leak in the tissue compared to overall tissue area. To translate findings to in vivo endothelial testing methods, post-occlusive reactive hyperemia was used with simultaneously administered brachial artery reactivity testing (Philips iE33 Ultrasound, Germany) and laser speckle contrast imaging (Perimed Inc., Sweden) of the macrovascular- and microvascular endothelial cell response, respectively, in $n = 26$ community controls and $n = 34$ SSD participants. Flow-mediated dilation was captured in the brachial artery by calculating the percent dilation at one-minute post-occlusion compared to baseline, while perfusion measures captured using the Laser Contrast Imager with area under the curve (AUC) and time relative to baseline perfusion measures. The primary measure of BBB leak between diagnostic groups was compared using raw means as well as covarying for age, sex, and PMI with reported estimated marginal means. Multiple comparisons were corrected using false discovery rate with q-value set at <0.05.

Results: Postmortem samples demonstrated 11% BBB leakage in SSD compared to 5% in controls ($t50 = -2.3$, $p = 0.02$). Linear regression was performed to determine age, sex, and PMI in addition to diagnosis as predictors of BBB leak, and the model remained significant ($F4,47 = 3.0$, $p = 0.03$) with a significant diagnosis ($t = 3.3$, $p = 0.002$) but no significant age ($p = 0.5$), sex ($p = 0.2$), or PMI ($p = 0.5$) effects. We further explored the impact of age on BBB permeability by splitting our sample population in two age-groups, 30-50 or 50-70 years-old, based on mean and medians. BBB leak fraction was also significantly increased in older aged schizophrenics vs controls ($t39 = 2.5$, $p = 0.02$). We found no significant sex difference in BBB permeability in the combined sample ($p = 0.99$), however, there was a significant increase in BBB permeability only in male schizophrenia subjects compared to male control subjects ($t51 = 2.7$, $p = 0.009$) on post-hoc analysis, while females did not ($p = 0.9$), with a diagnosis x sex interaction ($p = 0.03$). Post-occlusive reactive hyperemia experiments were performed in the peripheral vascular compartments to translate the findings of central endothelial cell dysfunction in the context of BBB permeability. Group differences were significantly present amongst the peripheral endothelial vascular measures shown using flow-mediated dilation of the brachial artery, a gold-standard cardiovascular measurement. Flow-mediated dilation was significantly reduced in the SSD group compared to controls, indicated endothelial damage (12% vs 8%, $p = 0.02$), after covarying age, sex, and body-mass index. Microvascular endothelial perfusion of the time to reach maximal flow was significantly shorter in SSD compared to controls (16 seconds vs 24 seconds, $p = 0.006$) as well.

Conclusions: The contributions that vascular abnormalities may have, even after accounting for psychopharmacologic and lifestyle regimens, may shed light onto mechanistic underpinnings that can be harnessed in both detection and treatment approaches for schizophrenia. Psychoneuropathology within SSD may be mediated by endothelial function within the various vascular compartments, including that of the brain. These results provide evidence for robust explorations of how endothelial dysfunction underserves impairments in neural correlates of SSD, with supporting preliminary data.

Keywords: Aging, Blood-Brain Barrier, Neuroinflamation, Brain Microvascular Endothelial Cells, Cardiovascular Physiology, Neuropsychiatric Disorders [Schizophrenia, Parkinson's Disease, Major Depressive Disorder], Hippocampus

Disclosure: Nothing to disclose.

P531. Load-Dependent Functional Connectivity Deficits During Visual Working Memory in First-Episode Psychosis

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Background: Aberrant network connectivity is increasingly viewed as a core dysfunction in psychosis and may underlie many of its associated cognitive and functional deficits. Working memory, the ability to retain sensory information in a readily accessible form, relies upon coordinated activity across distributed brain regions and has consistently exhibited impairments in schizophrenia. Recent studies in first-episode schizophrenia spectrum (FE) populations suggests a preservation of working memory network function during low-load conditions with disruptions becoming apparent as task complexity increases. The present study assessed visual network connectivity and its contribution to load-dependent working memory impairments in FE.

Methods: Magnetoencephalography was recorded from 35 FE and 27 matched healthy controls (HC) during a lateralized change detection visual working memory task. Structural MRI was acquired separately to facilitate localization of cortical activity. Impaired task-related alpha-band desynchronization was previously identified among FE within bilateral dorsal occipital (Occ) regions during high, but not low-load conditions. Whole-brain functional connectivity was assessed using phase-locking value (PLV) with bilateral Occ identified as connectivity seeds. Connections exhibiting significant modulation of PLV by memory load identified across all participants using paired t-tests (FDR-corrected) were compared between groups and across conditions. Correlations between functional connectivity, performance, and symptomatology were performed (FDR-corrected across comparisons).

Results: The effect of task condition on performance differed by group ($p = .004$) with greater impairment in memory capacity among FE during high ($p = .002$) compared to low ($p = .02$) load conditions. Across groups, significant load-dependent modulation of functional connectivity was observed between 7 region pairs. Again, the effect of task condition on connectivity differed by group ($p = .042$), though this interaction differed across region ($p = .03$). While HC exhibited significantly larger PLVs during the high-load condition across all 7 region pairs (p's < .05), FE failed to exhibit this enhancement between the right Occ and left inferior frontal gyrus (IFG), lateral occipito-temporal sulcus, and anterior intermediate parietal sulcus (AIPS) (p's > .1). Smaller PLVs between right Occ and both left IFG ($r = -.51$, $p = .002$) and AIPS ($r = -.48$, $p = .004$) during high-load condition were associated with increased SAPS Reality Distortion scores in patients.

Conclusions: As previously observed, FE exhibited impaired working memory capacity during high, but not low load conditions compared to HC. Examination of functional connectivity across the visual working memory network revealed a similar load dependent deficit in FE who were unable to enhance communication between perceptual and executive networks in response to increasing cognitive demands. Furthermore, the degree of impairment in the connectivity between occipital and fronto-parietal regions was associated with increased positive symptoms in these patients. These findings highlight the contribution of network connectivity to cognitive control deficits and symptoms in early psychosis and provide potential targets for future interventions. Future studies examining effective connectivity would also enhance our knowledge of information flow through this executive network by describing the directional nature of communication between regions.

Keywords: First Episode Psychosis, Visuospatial Working Memory, Magnetoencephalography

Disclosure: Nothing to disclose.

P532. Diurnal Alterations in Gene Expression Across the Human Dorsal and Ventral Striatum in Psychosis

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Background: Schizophrenia is a debilitating psychiatric disorder associated with major disruptions in sleep and circadian rhythms. A recent study from our group used a time-of-death analysis of RNA-seq data and found that subjects with schizophrenia show altered gene expression rhythms in the human postmortem dorsolateral prefrontal cortex (dlPFC), a region associated with the cognitive symptoms of schizophrenia. However, the role of molecular rhythms in other regions associated with core symptoms of schizophrenia remains unknown. Positive symptoms (i.e., psychosis) are associated with altered dopamine functioning in the striatum, particularly the dorsal striatum. In a recent study, we measured gene expression rhythms in the NAc, caudate, and putamen of a non-psychiatric disease cohort to determine how gene expression is normally fluctuating across the ventral and dorsal striatum. Here, we investigate how these gene expression rhythms are altered across the striatum in subjects with psychosis.

Methods: In the current study, we performed both differential expression and rhythmicity analyses to determine diurnal alterations in gene expression across the nucleus accumbens (NAc), caudate, and putamen in subjects with psychosis relative to unaffected comparison subjects. Nucleus accumbens, caudate, and putamen tissue samples were collected from male and female schizophrenia, bipolar disorder, and unaffected comparison subjects. We generated a psychosis cohort ($n = 34$) consisting of subjects with schizophrenia or bipolar disorder with psychosis and a matched comparison cohort ($n = 34$). Total RNA-seq was performed on the striatal tissue samples. Differential expression (DE) analysis was first performed between psychosis and comparison subjects in each region regardless of time of death (TOD). Given our previous findings in the dlPFC, we then split the cohorts into subjects that died either during the day or during the night and performed DE analysis. Transcripts were considered DE if corrected $p < 0.01$ and log2 fold change (FC) ≤ -0.26 or ≥0.26. Circadian patterns of gene expression were detected using nonlinear regression based on individual subject's TOD. Sinusoidal curves were fitted to the expressed data using the nonlinear least-squares method and coefficient of determination (R^2) was used as a measure of goodness-of-fit. Transcripts with either a gain or loss of rhythmicity between psychosis and matched comparison subjects were determined using the difference in R^2 between the cohorts.

Results: We detected many DE transcripts in each striatal region, with more in the caudate and putamen compared to the NAc. When we split our cohorts into subjects that died during the day or during the night, more DE transcripts were observed during the day in the caudate and putamen and during the night in the NAc. Using threshold-free comparison approaches, we determined that differential expression between psychosis and comparison subjects was largely similar across striatal regions. However, at cutoffs of corrected $p < 0.01$ and log2FC ≤ -0.26 or ≥0.26, we observed some region-specific differences in pathway enrichment. Notably, in the NAc we observed a downregulation in mitochondrial-related transcripts in subjects that died at night. Our rhythmicity analyses revealed many rhythmic transcripts across the striatum in comparison subjects. However, there were fewer rhythmic transcripts in psychosis subjects and these transcripts were distinct from comparison subjects. Overall, there was a substantial loss of rhythmicity in core circadian clock genes across the striatum in psychosis subjects. Some region-specific changes in rhythmicity were observed in the NAc, with a loss of rhythmicity in non-coding small nucleolar RNAs and a gain of rhythmicity in glutamatergic signaling. In comparing overlap of rhythmic transcripts across striatal regions, we found a striking degree of overlap between the caudate and putamen in psychosis subjects, suggesting a synchronization of rhythms across the dorsal striatum in psychosis.

Conclusions: Overall, we have identified multiple gene expression differences in the striatum in subjects with psychosis, as well as changes in rhythmic gene expression that could underlie disease pathology or response to treatment. Some of these changes are specific to particular striatal regions, such as the downregulation in mitochondrial-related transcripts at night in the NAc, while others are common to all three regions, including a substantial loss of rhythmicity in core circadian clock genes. Future studies in animal and cell culture models will be necessary to elucidate the mechanisms by which these rhythmic changes impact cellular function and behavior.

Keywords: Postmortem, Circadian Rhythms, Striatum, Schizophrenia, Psychosis

Disclosure: Nothing to disclose.

P533. Repetitive Transcranial Magnetic Stimulation (rTMS) for Catatonia– A Case Report

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Background: Catatonia is one of the most common severe motor syndromes, with an estimated prevalence among psychiatric inpatients of about 15 %. Benzodiazepines and electroconvulsive therapy (ECT) are the most widely studied treatment methods and are recommended as first-line therapy. However, no uniform treatment has yet been brought forward. Recent studies of the last two years show a successful utilization of rTMS to treat catatonic symptoms by an inhibitory stimulation of the supplementary motor area (SMA).

We present the case of a 55-year-old female patient with paranoid schizophrenia and severe

life-threatening catatonia who remitted under a short series of rTMS.

Methods: The point of resting motor threshold (RMT) for the musculus rectus femoris was determined for the left hemisphere. A straight line 3 cm anterior and parasagittal from that point defined the SMA. A total of three sessions, each with 1000 pulses at intensity 66 % of the RMT, were performed within 24 and 120 hours apart. Stimulation protocol was set to 1Hz in the area of the left SMA with 25 series of 40 pulses, pulse width 25 ms, angle of attack 45°.

Hardware: MagVenture, 8-coil "cool-B65 butterfly-shaped coil from Medtronic.

Results: Within 24 hours after the first session, a marked improvement of catatonic symptoms like independent locomotion and verbal communication were recognized. One week after the whole rTMS treatment, a food intake without a gastric tube was possible.

Conclusions: The present case demonstrates that pronounced catatonia may be successfully treated with inhibitory rTMS. Even though the validation and reproducibility of rTMS in this single case are somewhat limited, we are convinced that our results underline the importance of non-invasive brain stimulation as a valuable addition to the existing treatment spectrum for catatonia in certain patients. Future research is empowered to path the way for a significant expansion of treatment, advanced and possibly highly effective therapy.

Keywords: Repetitive Transcranial Magnetic Stimulation (rTMS), Supplementary Motor Cortex, Catatonia, Schizophrenia (SCZ)

Disclosure: Nothing to disclose.

P534. Developmental Inhibition of Striatal Indirect Pathway Decreases Adult Motivation and Alters Dopamine Release in Nucleus Accumbens

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Background: Despite being a major source of burden to patients, motivational deficits of schizophrenia (SCZ) are not effectively treated by available medications. Studies have shown enhanced dopamine (DA) release and increased occupancy of striatal DA D2 receptors (D2Rs) in SCZ patients. Striatal indirect pathway neurons predominantly express D2Rs (the main target of antipsychotics) and project to midbrain DA neurons. D2Rs are Gi-coupled and have an inhibitory effect on neuronal activity upon DA binding. Although a growing body of evidence supports a neurodevelopmental basis for SCZ, the mechanisms are not well understood. Studies in mice have shown that selective upregulation of D2Rs in the striatum from early development on lead to deficits in cognition and motivation, thereby linking alterations in the DA system to cognitive and negative symptoms, which are largely resistant to antipsychotics. One possibility is that early D2R upregulation induces long lasting changes in striatal circuitry that cannot be reversed by D2R antagonism. Thus, my hypothesis is that increased D2R signaling during neonatal development causes persistent circuitry changes that lead to behavioral abnormalities in the adult animal. To start addressing this hypothesis, I have used hM4DGi, which like D2R, is Gi-coupled and systemic injection of its synthetic ligand allows for activation during defined developmental periods. Using this approach, Kozorovitzkiy et al (Nature, 2012) developmentally inhibited the indirect pathway in mice from postnatal day P8 to P15. The developmental inhibition persistently altered the balance of cortical input into the striatum that lasted beyond the period of CNO treatment by 10 days. However, the long-term effects of this manipulation into adulthood are currently unknown.

Methods: Viral hM4D expression and CNO injections: AAV5-hSyn-DIO-hM4D-mCherry or -GFP (control) viruses were stereotaxically injected in P1 Adora2a-Cre (A2a) mouse neonates (both sexes included). Injections targeted the bilateral nucleus accumbens (NAc) and dorsal striatum (dSTR). From P8 to P15, pups received twice daily intraperitoneal (i.p.) injections of CNO (1 mg/kg) or saline. No other treatment was given after P15.

Behavior: Open Field activity was assessed at P21, P40, and P90 for 60 minutes each. Following OF, appetitive motivation was assessed by Progressive Ratio (PR). After being trained to press the operant lever at a rate > 200 presses per 30-min session, mice were subjected to a PR session, where the ratio of presses per reward doubled after each time.

Fiberphotometry: the virally encoded DA sensor, dLight1.2 was stereotaxically delivered to the right NAc of 3 A2a/hM4DCNO and 2 A2a/hM4DSAL adult mice (P120). After 4 weeks, mice were tested in a Fixed Ratio 5 (FR5) operant task while dLight1.2 fluorescent signal was being simultaneously recorded. The dLight1.2 signal, expressed as ΔF/F, was aligned to task-relevant epochs.

Results: Neuroanatomically restricted expression of viral hM4D-mCherry (and GFP control) in the indirect pathway was detected by immunostaining as early as postnatal day P7. Open Field (OF) locomotion analysis revealed a significant decrease in activity only in adult (P90) mice and not earlier ($N = 15$, linear regression, $p < 0.0001$). Analysis of A2a/GFP mice (CNO or SAL) showed no effect of CNO alone at P90 in OF activity ($N = 8$-9, linear regression, $p = 0.6838$). Analysis of PR experiments showed that, compared to saline controls, A2a/hM4DCNO mice displayed lower press ratio breakpoint ($N = 10$-18, unpaired t-test, $p = 0.0378$), fewer total presses ($N = 10$-18, unpaired t-test, $p = 0.0162$), and shorter session durations ($n = 10$-18, Mantel-Cox Log-rank, $p = 0.0277$). Analysis of A2a/GFP mice ($N = 10$-11) showed no statistically significant differences between the two groups in total presses (two-tailed unpaired t-test, $p = 0.8989$); breakpoint (two-tailed unpaired t-test, $p = 0.9003$); and session duration (Mantel-Cox Log-rank, $p = 0.6681$). In the preliminary analysis of fiberphotometry experiments measuring DA release in the NAc core, adult A2a/hM4DCNO mice (developmentally inhibited) displayed lower DA response (expressed as AUC) (2.951 +/-0.0729, $N = 3$) than A2a/hM4DSAL controls (3.904 +/-0.0572, $N = 2$) in response to the lever presentation (two-tailed unpaired t-test, $p = 0.0027$).

Conclusions: These preliminary results suggest that the developmental chemogenetic inhibition of the indirect pathway by hM4D led to alterations in basal ganglia-related behaviors that only manifest in adulthood, despite the early onset of the manipulation. This would be in line with growing evidence of a prolonged postnatal development of dopamine projections and basal ganglia circuits. The lower performance on progressive ratio by developmentally inhibited mice also hints at long-term alterations in cost-benefit decision making that could be, at least in part, attributable to circuit-level mechanisms involving DA release in the NAc in response to reward-predicting stimuli, to the perceived cost of work requirement, or reward value. Preliminary results from dLight1.2 fiberphotometry recordings during an operant fixed ratio task suggest that developmentally inhibiting the indirect pathway in mice could lead to prolonged alterations in accumbal DA release. Additional experiments will be required to elucidate the nature of DA release alterations in A2a/hM4Ddev mice and how they relate to motivational deficits.

Keywords: D2 Dopamine Receptor, Motivation, Fiberphotometry, Developmental Trajectory, Indirect Pathway

Disclosure: Nothing to disclose.

P535. Striatal Dopamine Regulates Auditory Discrimination

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Background: Perceptual decision-making requires a complex integration of sensory inputs, such as auditory signals to produce an executive order to the motor system. While dopamine (DA) has been shown to be involved in decision-making from lines of pathological and physiological studies, it remains largely unclear how DA dynamically regulates neural circuits to impact the process of perceptual decision-making. In this study, we aim to address the role of DA in regulating auditory decision-making, focusing on the key neural circuit locus, the auditory striatum.

Methods: We employed viral tracing, optogenetic, chemogenetic, and microendoscopic optical imaging approaches to study mice performing a two forced choice auditory frequency (low vs. high tone cloud) discrimination task. We used implanted optic fibers and a dual viral strategy (CAV-Cre and AAV-flex-ArchT) to label and inhibit DA input into the auditory striatum of mice performing the task. To directly study DA dynamics, we recorded DA sensor fluorescence (AAV-DA2M) in well-trained mice. Lastly to study the neuronal source of DA, we used a dual viral strategy (CAV-Cre and AAV-flex-GCaMP6f) to label and record DA neurons projecting to the auditory striatum. To investigate the role of DA in modulating striatal activity, we recorded GCaMP6f-based neuronal calcium activity in the auditory striatum using microendscopic cameras and employed chemogenetics to silence DA inputs.

Results: Injection of CAV-Cre into the auditory striatum primarily labeled DA (Tyrosine Hydroxylase-expressing) neurons. The majority of these neurons populated the lateral portion of the substantia nigra pars compacta (SN). Cue-locked optogenetic inhibition of this population impaired auditory choice performance on a trial-by-trial basis. To determine the neural circuit mechanism governing this regulation, we set out to examine the auditory striatal dynamics corresponding to task stimuli during decision-making. Using microendoscopic DA sensor measurements in the auditory striatum, we observed robust responses towards both low and high frequency tone clouds during the decision making. We next investigated the dynamics when the mice were presented with tone clouds of intermediate evidence strengths and tone mixtures. We found that auditory striatal DA levels scaled inversely with evidence strength such that DA responses were higher when mice were presented with more mixed ambiguous stimuli (lower evidence strength). We then retrogradely labeled auditory striatal-projecting SN neurons with GCaMP6f and monitored task activity. A subset of SN neurons similarly responded to tone cue stimuli with an inverse scaling towards evidence strength. To test the impact of SN activity on auditory striatal neuron task responses, we recorded GCaMP6f neuronal signals in the auditory striatum while chemogenetically silencing the SN in mice performing the auditory task. We observed a decrease in auditory striatal cue responses during the decision task. Lastly, we investigated which DA-recipient population within the auditory striatum may mediate auditory choice. Using immunohistochemical methods, we and others have noted that the auditory striatum is D1R-biased, with a region devoid of D2R medium spiny neurons (MSN). Imaging of the D1R-expressing population demonstrated that a subset of neurons expressed tonal cue responses during task performance. Furthermore, local infusion of D1R antagonist into the auditory striatum impaired choice performance, suggesting that the DA signaling at D1R MSNs participates in auditory discrimination. Altogether, our findings revealed that DA regulates choice cue responses in the auditory striatum, at least largely via D1R-expressing MSNs, providing a novel insight into the understanding of how striatal DA regulates auditory choice.

Conclusions: We find that auditory striatal DA consistently rises towards cue stimuli and is enhanced by stimulus difficulty. Auditory striatal DA regulates auditory discrimination and striatal cue responses. Combined, our study reveals a mechanism through which the nigrostriatal system regulates auditory discrimination behavior, by modulating ongoing striatal perceptual performance.

Keywords: Auditory Perception, Dopamine, Auditory Striatum, Perceptual Decision-Making, D1 Dopamine Receptors

Disclosure: Nothing to disclose.

P536. Schizophrenia-Associated MAP2 Phosphorylation Affects Interactions With Microtubules and Actin

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Background: Microtubule-associated protein 2 (MAP2) is the predominant dendritic cytoskeletal regulator, influencing dendritic arbor and spine morphology, which are altered in schizophrenia (SZ). MAP2 is regulated by multiple mechanisms, most prominently phosphorylation (MAP2-P). We have previously demonstrated that MAP2-P state is altered in SZ, with multiple phosphopeptides being upregulated in disorder. Moreover, a subset of identified phosphopeptides (10 sites) were negatively correlated with dendritic spine density in primary auditory cortex, suggesting that SZ-associated MAP2-P has consequences for protein function, in turn compromising neuronal morphology/function. Here, we set out to characterize the consequences of SZ-associated MAP2-P events on protein function by assessing the 1) tubulin polymerization, 2) microtubule-binding, and 3) actin-binding abilities of purified phosphomimetic MAP2c constructs.

Methods: Phosphomimetic MAP2c mutants were expressed in Bl21(DE3) bacteria and purified by boiling. Ultracentrifugation-based microtubule- and actin-binding assays as well as a turbidometric tubulin polymerization assay were performed by manufacturer's instructions (Cytoskeleton Inc) ($N = 3$-6 independent experiments).

Results: Phosphomimicry at multiple residues in the proline-rich and C-terminal domains- including T293, T300, S426, S439 and S443- was sufficient to delay microtubule nucleation. S426 additionally slowed polymerization rate. T293, T300, S426 and S439 inhibited actin-binding, while only S426 and S439 inhibited microtubule-binding. Other tested sites, including T249, S252, Y253, S297 and S446, showed no effects.

Conclusions: These data indicate that SZ is characterized by both regulatory MAP2-P events and events which are irrelevant to its interactions with cytoskeletal filaments. We put forward MAP2-P-mediated changes to microtubule nucleation kinetics and microtubule/actin crosslinking as potential mechanisms for dendritic dysmorphogenesis in SZ. Future studies will examine effects of MAP2 phosphomimicry on microtubule dynamics and dendritic architecture in neuronal culture.

Keywords: Cytoskeleton, Schizophrenia (SCZ), Dendritic Morphogenesis

Disclosure: Nothing to disclose.

P537. Adolescent Thalamic Inhibition Leads to Long-Lasting Impairments in Prefrontal Cortex Function

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Background: The prefrontal cortex is a cognitive structure that is implicated in several psychiatric disorders, including schizophrenia. It receives reciprocal inputs from the thalamus, and this thalamo-cortical circuitry supports cognition. In patients with schizophrenia, who have impaired cognitive functioning, thalamo-prefrontal connectivity is disrupted. This finding is also seen in adolescents at high risk for the disorder, even before diagnosis.

While impaired cortical maturation has been postulated as a mechanism in the etiology of schizophrenia, the postnatal development of thalamo-prefrontal circuitry is still poorly understood. In sensory cortex, activity relayed by the thalamus during a postnatal sensitive period is essential for proper cortical maturation. However, whether thalamic activity also shapes maturation of the prefrontal cortex is unknown.

We hypothesize that adolescence represents a sensitive period, during which the prefrontal cortex is susceptible to transient perturbations in thalamic input activity, resulting in persistent changes in circuitry.

Methods: We developed an approach in mice whereby we can transiently reduce thalamic activity. We used both male and female mice, injecting an inhibitory pharmacogenetic receptor into the medial thalamus and administering its ligand during specific time windows. We chose adolescence, a vulnerable period in the progression of schizophrenia. As a comparison, we also manipulated thalamic activity during adulthood. Forty days following the manipulation, we tested for persistent effects on prefrontal cortical circuitry and behavior. Sample sizes ranged from 10-30 animals per group. Statistical analyses include t-tests, ANOVA, as well as a linear decoder algorithm custom-made in Python for this experiment.

Results: We show that inhibiting the medial thalamus during adolescence leads to a long-lasting decrease in thalamo-prefrontal projection density and prefrontal cortical excitation. Adolescent thalamic inhibition also causes impairments in adulthood in two prefrontal-dependent cognitive behavioral tasks: a non-match to sample working memory task and an attentional set-shifting task. These cognitive deficits are associated with disrupted prefrontal cross-correlations and task outcome encoding. In contrast, thalamic inhibition during adulthood has no long-lasting consequences on behavior or outcome encoding. Strikingly, exciting the thalamus in adulthood during the attentional set-shifting task rescues prefrontal cross-correlations, task outcome encoding, and the cognitive deficit.

Conclusions: These data point to adolescence as a sensitive window of thalamo-prefrontal circuit maturation as adolescent thalamic inhibition has long-lasting consequences on prefrontal circuitry, while adult thalamic inhibition has no persistent effects. Moreover, these results highlight the role of the thalamus as a non-specific facilitator of prefrontal activity, expanding our understanding of this thalamic function to cognitive contexts outside of delay-response tasks. Finally, by supporting prefrontal network activity, boosting thalamic activity provides a potential therapeutic strategy for rescuing cognitive deficits in neurodevelopmental disorders.

Keywords: Medial Prefrontal Cortex, Thalamo-cortical Interactions, Adolescence- Critical Period, Cognitive Functioning, Schizophrenia (SCZ)

Disclosure: Nothing to disclose.

P538. Dorsolateral Prefrontal Cortex GABA/Glutamate Abnormalities in Clinical High Risk and First-Episode Psychosis: A 7-T Magnetic Resonance Spectroscopic Imaging Study

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Background: Gamma aminobutyric acid (GABA) and, to a lesser extent, Glutamate (Glu) neurotransmission abnormalities in the dorsolateral prefrontal cortex (DLPFC) have been reported in patients with schizophrenia (SCZ) by post-mortem studies. However, in vivo GABA-ergic and Glutamatergic DLPFC alterations

in these patients, including in early course and in individuals at-risk of psychosis remain largely unknown, mainly due to methodological challenges. The availability of 7 Tesla (7 T) magnetic resonance imaging (MRI) scanners, which provide higher signal-to-noise ratio and magnetic resonance spectroscopy imaging (MRSI) that enables measuring GABA and Glu simultaneously in more than one brain region, have helped mitigate these challenges. However, no prior studies have investigated the relationship between GABA and Glu (, which are served as the primary inhibitory and excitatory neurotransmitter, respectively), measured as GABA/Glu, in the DLPFC across the early stages of psychotic illness.

Methods: 7 T MRSI was utilized to investigate GABA/Glu abnormalities in right and left DLPFC in 16 individuals with first episode of schizophrenia (FES), 17 clinical high risk for (CHR) individuals, and 25 healthy control (HC) participants. Freesurfer was used to segment the anatomical brain image (MPRAGE) into different cortical regions of interest. LCModel was utilized to quantify the spectral data (in the range of 1.8 to 4.0 ppm), which consist of default macromolecule components and 14 basis metabolite functions (e.g., creatine, γ-aminobutyric acid, glucose, glutamate, and glutamine). The Cramer-Rao lower bound (CRLB) values for each ratio were utilized to filter out data of poor spectral quality. Spectra were excluded if CRLB > 10 for the major singlet resonances tNAA, Cre, or tCho, or CRLB > 20 for GABA and Glu. A two-way analysis of covariance (ANCOVA) with hemisphere and group as independent variables, GABA/Glu as the dependent variable, as well as age and sex as covariates was conducted. Post-hoc independent samples t-tests were performed to investigate GABA/Glu concentration differences between groups for both left and right DLPFC. In addition to measuring the GABA/Glu in DLPFC, we compared right and left DLPFC GABA/Glu values to assess for between hemisphere asymmetry (e.g., left DLPFC GABA/Glu higher than right DLPFC GABA/Glu) using paired t-test among each group of subjects. Furthermore, in FEP-SCZ participants, we performed Spearman correlation analyses between DLPFC GABA/Glu parameters and working memory performance, which was assessed with the Spatial Span (Wechsler Memory Scale [WMS-SS]) and the Letter–Number Span test (LNS) from the Measurement and Treatment Research to Improve Cognition in Schizophrenia Consensus Cognitive Battery (MCCB).

Results: ANCOVA analysis for DLPFC GABA/Glu revealed significant differences between groups ($p < 0.001$), sex ($p = 0.016$), and group × hemisphere ($p = 0.005$). No significant effects of age ($p = 0.364$) and hemisphere ($p = 0.724$) were found. Post-hoc paired t-test analysis revealed higher GABA/Glu in the right DLPFC of both FES patients and CHR participants compared with HC subjects, $p < 0.001$ and $p = 0.019$, respectively. No significant differences were observed in the left DLPFC GABA/Glu among the three groups. We also established that HC individuals had higher GABA/Glu in the left DLPFC compared to the right DLPFC (L > R, $p = 0.008$), whereas the opposite relationship (R > L, $p = 0.042$) was observed in the DLPFC GABA/Glu of FES patients. Furthermore, in FES patients, working memory performance (i.e., LNS) was inversely correlated with right DLPFC GABA/Glu ($p = 0.022$, r = -0.626) and GABA/Glu asymmetry ($p = 0.004$, r = − 0.878).

Conclusions: By employing 7 T MRSI in individuals with CHR, FES, and HC subjects, we demonstrated that GABA/Glu DLPFC alterations: a) were present before illness onset; b) worsened in first-episode patients; and c) were associated with their cognitive deficits, thus representing a putative target engagement biomarker for early interventions in patients with SCZ and related psychotic disorders.

Keywords: MR Spectroscopy, Early Psychosis, Cognitive Performance

Disclosure: Nothing to disclose.

P539. Electrophysiological Measures From Human iPSC-Derived Neurons are Associated With Schizophrenia Clinical Status and Predict Individual Cognitive Performance

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Background: Schizophrenia (SCZ) is a complex, polygenic disorder with marked clinical heterogeneity and no clear pathological mechanism or cellular pathology. The advent of human induced pluripotent stem cell (hiPSC) technology has enabled our ability to capture the polygenic nature of SCZ in vitro. Several previous studies using hiPSC to model SCZ have identified interesting phenotypes, but their relevance to the clinical illness in adults remains unclear. Here, we describe results of our investigation into the relationships between the electrophysiological properties of hiPSC-derived cortical neurons and the individual's clinical status, severity of symptoms, and cognitive performance. Importantly, we look beyond case-control differences, and identify within-case patterns of association that could serve to illuminate dimensions of illness and subgroups of patients that may be suitable for targeted treatments.

Methods: We have established a systematic and quantitative iPSC platform to model common risk variation associated with SCZ. We studied iPSCs from 13 SCZ patients with high polygenic risk scores (PRS) and 15 neurotypical individuals with low PRS. hiPSCs underwent four independent rounds of directed differentiation into cortical neurons. To identify phenotypes differing between high PRS SCZ cases and low PRS controls, a battery of assays were performed on cortical neurons in a double-blind manner. Lastly, we correlated electrophysiology measures to the individual's rating on the Positive and Negative Syndrome Scale (PANSS), six cognitive domains (Verbal Memory, Nback, Visual Memory, Processing Speed, Card Sorting, and Digit Span), estimated IQ, and general cognitive ability composite ("g").

Results: Five electrophysiological measures related to Na+ channel function were associated with diagnosis. Lines derived from SCZ donors showed an increase in membrane resistance (SCZ effect = 29.41, $p = 0.025$, $N = 1074$ cells from 28 genomes), an increased number of Na+ current peaks in response to a voltage ramp protocol (SCZ effect = 0.093, $p = 0.038$, $N = 1074$ from 28 genomes), a decrease in the activation threshold of the second Na+ peak (SCZ effect = -0.74, $p = 0.009$, $N = 1074$ from 28 genomes), a significantly hyperpolarized shift in Na+ channel activation and V1/2 activation voltage (CON V1/2 = -39.2 ± 0.6 mV; SCZ V1/2 = -41.1 ± 0.7 mV, Delta = 1.9 mV, $p = 0.039$, $N = 173$ from 13 genomes), and a significant hyperpolarized shift in the voltage dependence of inactivation and V1/2 inactivation voltage (CON V1/2 = -58.1 ± 0.6 mV; SCZ V1/2 = -61.8 ± 0.7 mV, Delta = 3.7 mV, $p < 0.0001$, $N = 162$ from 13 genomes). In SCZ, the number of Na+ peaks showed directionally-consistent, positive associations with some positive symptom ratings (SCZ $N = 12$, R = 0.77, $p = 0.0033$, coeff.=0.77), whereas there were no correlations with negative symptom ratings or cognitive measures. In striking contrast, sEPSC amplitude showed no correlation with positive symptoms, but showed a negative correlation (SCZ $N = 11$, R = -0.93, $p = 2.94E-05$, coeff.=-0.93) with performance on the Wisconsin Card Sort Test (WCST).

Conclusions: Our results demonstrate for the first time that common genetic variants associated with SCZ converge on electrophysiological mechanisms that relate to clinically relevant features of SCZ, and therefore underscore the potential of this approach for biomarker identification and perhaps downstream drug development.

Keywords: Schizophrenia (SCZ), Induced Pluripotent Stem Cells (iPSCs), Cognition, Electrophysiology, Ion Channels

Disclosure: Nothing to disclose.

P540. Utility of Polygenic Embryo Screening for Disease Depends on the Selection Strategy

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Background: Preimplantation genetic diagnosis (PGD) has been utilized for years to avoid implantation of embryos harboring rare monogenic disease-causing alleles. However, recent progress in complex trait genetics, coupled with the technical ability to generate accurate genome-wide genotypes from single-cell input, has made it possible to genetically screen embryos for common polygenic traits and disease risk. As of 2019, polygenic risk scores have been utilized to screen in vitro fertilization embryos for genetic liability to adult diseases, despite a lack of comprehensive modeling of expected outcomes. While popular media has raised the specter of "designer babies," little empirical work has been done to assess the utility of polygenic embryo screening (PES), examine its ethical implications, and inform stakeholders.

Recently, we have published a study demonstrating the statistical limitations of applying polygenic scores for the purpose of selecting embryos on the basis of quantitative traits (Karavani et al., Cell, 2019). Given current technology, we found that the average gain due to screening would be modest, with extremely wide confidence intervals, resulting in considerable uncertainty for any couple seeking PES. Here, we will present new (unpublished) data suggesting a very different pattern of results for PES in the context of disease risk reduction.

Methods: Our initial analysis consisted of mathematical modeling of polygenic scores and their relation to disease risk. For obvious ethical and practical reasons, we could not validate our modeling predictions with actual experiments. Nevertheless, we could perform realistic simulations based on genomes from case-control studies, similarly to our previous work (Karavani et al., 2019). For schizophrenia, we used GWAS data ≈900 cases and ≈1600 controls of Ashkenazi Jewish ancestry, all unrelated (Lencz et al., 2013), to generate 5,000 "virtual couples" by randomly mating pairs of individuals. For each such "couple", we simulate the genomes of *n* hypothetical embryos, based on the laws of Mendelian inheritance and by randomly placing cross-overs according to genetic map distances. In parallel, we used the same genomes to learn a logistic regression model that predicts the risk of disease given a PRS computed from the most recently available summary statistics (excluding the samples in our test cohorts). We then computed the PRS of each simulated embryo, and predict the risk of disease of that embryo. We finally compared the risk of disease between one randomly selected embryo per couple vs one embryo selected based on PRS. To develop the polygenic risk score for schizophrenia, we used summary statistics from the most recent schizophrenia GWAS of the Psychiatric Genomics Consortium (PGC) (Schizophrenia Working Group of the Psychiatric Genomics et al., 2020). Note that we specifically used summary statistics that excluded our Ashkenazi cohort. The *P*-value threshold was chosen based on results from the PGC study. After clumping, the final score included 23,036 SNPs. To construct the score, we used the effect sizes reported in the GWAS summary statistics, without additional processing.

Results: Results demonstrate that a strong determinant of the potential utility of such screening is the selection strategy employed, a factor that has not been previously studied. Minimal risk reduction is expected if only extremely high-scoring embryos are excluded, whereas risk reductions are substantially greater if the lowest-scoring embryo (for a given disease) is selected.

We also systematically examined the relative contributions of the variance explained by the score, the number of embryos, the disease prevalence, and parental scores and disease status on the utility of screening. For both highly prevalent disorders (such as major depression), and less common disorders (such as schizophrenia), PES may provide meaningful reductions in relative risk. However, absolute risk reduction would necessarily be low for disorders with low base rates.

Conclusions: The use of PRS in adults has focused on those at highest risk, for whom there may be maximal clinical benefit of screening and intervention. However, as PRSs have relatively low sensitivity, such a strategy is relatively ineffective in reducing the overall population disease burden. Similarly, in the context of PES, exclusion of high-risk embryos will result in relatively modest risk reductions. By contrast, selecting the embryo with the lowest PRS may result in large reductions in relative risk. This is fundamentally a property of a threshold character with an underlying normally distributed continuous liability. For such traits, most of the individuals in the extreme of the liability distribution (i.e., the ones affected) are concentrated very near the threshold. Thus, even slightly reducing their liability can move a large proportion of affected individuals below the disease threshold. However, it should be noted that conventional thresholds for defining presence of disease may contain some degree of arbitrariness if the underlying distribution of pathophysiology is truly continuous. Consequently, the effects on ultimate morbidity may depend on the validity of the threshold itself.

Keywords: Polygenic Risk Score, In Vitro Fertilization, Embryo Screening, Eugenics

Disclosure: Nothing to disclose.

P541. Atypical Antipsychotic Agent Use From Diagnosis to Long-Acting Injectable Antipsychotic Agent Implementation Among Patients With Schizophrenia in the United States: An Analysis of a Commercial Claims Database

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Background: Schizophrenia is a chronic, disabling psychiatric disorder that affects approximately 1% of adults in the United States (US). The early initiation of an appropriate long-term treatment with antipsychotic agents after diagnosis is necessary to control burdensome symptoms and decrease the risk of relapse. Long-acting injectable antipsychotic agents (LAIs) demonstrated improved clinical effectiveness and adherence versus oral antipsychotic agents (OAs); however, there are limited data about the utilization of these formulations, especially in early-stage disease. In order to understand the gap between recommended and actual practice, we characterized utilization of atypical antipsychotic agents in general, and atypical LAIs in particular, in a US-based, real-world dataset of mainly commercially insured patients with newly diagnosed schizophrenia. Commercially insured populations were of particular interest as previous analyses typically did not utilize these populations, which are

more likely to include patients in the early phases of schizophrenia who are still covered by their own or their family's insurance. The objective of this study was to evaluate treatment and utilization patterns among patients with schizophrenia from a commercial claims database who were treated with atypical LAIs to understand potential obstacles faced by patients and clinicians prior to the successful implementation of a continuous LAI regimen.

Methods: Patients with newly diagnosed schizophrenia in the US were identified using claims data from IBM® MarketScan® Commercial and Medicare Supplemental databases between January 1, 2013, and September 30, 2019. Key inclusion criteria were first diagnosis of schizophrenia in study period (index date) without prior schizophrenia diagnosis or OA/LAI treatment in the 12-month pre–index period; ages 18–40 years; successful LAI implementation (treatment ≥ 90 consecutive days with ≤7-day gaps); continuous enrollment in pre–index period and from index date to successful LAI implementation; and ≥1 OA prior to LAI initiation. Key outcomes were (1) time between the index date and first LAI administration; (2) time between first LAI administration and successful implementation (defined as uninterrupted treatment for ≥90 days); (3) number of distinct LAIs between first LAI administration and successful implementation; (4) duration of LAI treatment following successful implementation; (5) number of distinct OAs between the index date and first LAI administration; and (6) proportion of patients for whom the initial LAI was followed by successful implementation. All outcomes were examined descriptively.

Results: Of the 41,391 patients with newly diagnosed schizophrenia in the database during the study period, 1836 patients received treatment with an LAI, and 202 patients met the inclusion criteria for this analysis (mean age: 23.8 years; male: 83.2%). Median time between the index date and the first LAI administration was 289.5 days (range: 0–2171 days), and median time between the first LAI administration and successful LAI implementation was 90 days (range: 90–1061 days). Overall, LAIs were successfully implemented from the first LAI for 86.1% of patients. The median duration of LAI treatment following successful LAI implementation was 166.5 days (range: 91–799 days). Prior to the first LAI administration, 58.4% of patients had received ≥2 OAs (28.2% received 2 OAs, 15.3% received 3 OAs, 11.4% received 4 OAs). The most frequently prescribed first-line OA was risperidone (34.7%). For second line OAs, aripiprazole (22.9%) and paliperidone (22.0%) were the most prescribed. Overall, the most frequently prescribed LAI was paliperidone (first-line: 57.9%; second-line: 50.0%), followed by aripiprazole (27.7%, 28.6%) and risperidone (12.9%, 21.4%).

Conclusions: In this population of commercially insured patients with newly diagnosed schizophrenia in the US using antipsychotic agents, the utilization rate of atypical LAIs was very low. This is in sharp contrast with growing evidence in support of the greater effectiveness of LAIs. While more than half (58.4%) of patients with schizophrenia received ≥2 OAs before initiating an LAI, indicating that LAIs are not often used as first-line treatment, most (86.1%) patients received continuous LAI treatment following the first administration, reflecting a positive uptake of LAIs in this population. Given growing evidence that overall favors LAIs over OAs in early-phase schizophrenia, these data suggest a gap between evidence-based recommendations and actual practice.

Keywords: Long-Acting Injectable Antipsychotics, Schizophrenia, Claims Database, Commercial Insurance

Disclosure: Alkermes, Intracellular Therapies, Merck, Neurocrine, Roche, Saladex, Teva, Lyndra: Consultant (Self)

Dainippon Sumitomo, H Lundbeck, Janssen, Otsuka, Indivior: Honoraria (Self)

Acadia: Advisory Board (Self),

LB Pharma, Vanguard Research Group, Health Rhythms: Stock / Equity (Self)

P542. Shedding Patterns of Glycocalyx in Plasma Discriminates First-Episode, Antipsychotic-Naive Schizophrenia Spectrum Patients From Healthy Controls: A Novel Blood Marker for Psychosis?

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Background: Glycocalyx (GLX) is a layer of biomolecules, proteoglycans and glycosaminoglycans on the luminal surface of endothelial cells in blood vessels. As the first interaction between the blood and the brain, the GLX plays a vital role in regulating blood flow, immune cell trafficking, and blood brain barrier (BBB) function.

A loss of GLX, denoted 'shedding', can lead to inflammation, oedema and disruption of homeostasis in the brain. Shedding of GLX is increased in response to brain insults such as stroke as well as in neuroinflammatory/neurodegenerative disorders such as multiple sclerosis.

The etiology of schizophrenia is largely unknown, but prenatal infection, infection or autoimmune disease later in life are well-established risk factors for development of mental illness, such as schizophrenia spectrum disorders. Peripheral inflammation, neuroinflammation, and BBB dysfunction in schizophrenia could be linked through a loss of GLX components in the neuroendothelia.

As a novel approach, we investigated whether a panel of 15 biomolecules in the GLX complex measured from plasma can be used to discriminate antipsychotic-naïve, first episode schizophrenia spectrum patients from healthy controls (HC). Levels of the 15 individual GLX markers were described using conventional univariate statistics.

In the main analyses, we applied seven machine learning (ML) algorithms to test if the GLX shedding patterns, denoted 'the composite glycocalyx signal' could be used to classify groups. Within the patient group only, we next applied the same ML framework to explore whether the composite GLX signal could classify high vs. low symptom severity.

Methods: Patients ($n = 49$) fulfilled ICD-10 criteria for schizophrenia spectrum disorder (schizophrenia, schizoaffective disorder, or non-organic psychosis), and all were lifetime naïve to antipsychotic exposure. HC ($n = 51$) were matched to patients on gender, age, and parental educational status.

Blood samples were collected in the morning and in a fasting state. Plasma was immediately isolated and stored at -80C until further analysis. In the GLX analyses, plasma was spun at 4C, and the hydrophilic layer was extracted and 2ul was dotted in duplicate on a negatively charged membrane. Fifteen GLX markers were detected with immunoblotting using optimized protocols. Signals were imaged, background corrected, and analyzed for optical density.

Patients' psychopathology was assessed by trained raters using the Positive and Negative Syndrome Scale (PANSS).

Seven ML models were run, each passing through a double validation loop using nested cross-validation to minimize overfitting. Models yielded estimates of clasification accuracy, as well as estimates of the contribution of each marker for the composite GLX signal to the prediction using SHapley Additive exPlanations (SHAP) values.

Within the patient group, we explored associations between the composite glycocalyx signal and psychopathology. We applied a

mean split on PANSStotal, PANSSpositive, PANSSnegative, PANSSgeneral, splitting each domain by the mean score in order to classify either 'high' or 'low' impact of psychopathology. Gender, CRP values, BMI and smoking status were included as covariates.

ML accuracy was tested with a Wilcoxon nonparametric test against a null model. Differences were deemed significant when $p < 0.05$ after Holm-Bonferroni correction.

Results: In the univariate analyses, five of the 15 GLX markers were significantly increased in patients compared to HC ($p < 0.0001$), passing Holm-Bonferroni correction.

In the ML models of the composite GLX signal, the mean classification accuracy for patients versus controls was 82% (range 89-72%; $p = <0.0001$ to 0.009). All models remained statistically significant after Holm-Bonferroni correction.

Regarding psychopathology, the composite GLX signal classified patients with high vs low PANSSpositive, and PANSSnegative with varying accuracy (p-value range: 0.02 to >0.05) subscores, however, these results did not survive Holm-Bonferroni correction.

Conclusions: To our knowledge this is the first study to investigate the impact of glycocalyx shedding patterns in patients with schizophrenia spectrum disorder. We find that composite GLX signal from plasma differentiates antipsychotic-naive patients from HC with very high accuracy. Although not significant after correction for multiplicity, our findings suggest that the GLX signal may partially map onto core symptom domains, i.e. positive and negative symptoms in schizophrenia spectrum patients.

Biologically, the collective pattern of increase in glycocalyx-related markers in plasma indicates a thinning of the glycocalyx layer, which may in turn be associated with BBB disruption and neuroinflammation.

We are currently engaged in validating the diagnostic accuracy of the glycocalyx signature in independent cohorts of patients with schizophrenia spectrum disorder. Moreover, we will investigate associations of glycocalyx with cognition, brain structure- and function, and treatment response.

In conclusion, these encouraging findings suggest that soluble glycocalyx shedding patterns may be a potential peripheral biomarker for patients in the schizophrenia spectrum, opening the prospect of identifying high-risk individuals, monitoring treatment effects, and testing new therapeutics from a blood sample.

Keywords: Antipsychotic-Naïve First-Episode Schizophrenia, Glycocalyx, Machine Learning Classification, Peripheral Blood Marker, Psychopathology

Disclosure: Boehringer Ingelheim: Contracted Research, Honoraria (Self)

Lundbeck Pharma A/S: Honoraria (Self)

P543. Prospective, Longitudinal Multi-Modal Imaging of Altered Brain Development and Behavior in a Non-Human Primate Model of Maternal Immune Activation

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Background: Evidence has been accumulating for an immune-based component of psychiatric disorder etiology, particularly schizophrenia, including genetic links to the major histocompatibility complex, maternal infections linked to the maternal immune response, pro-inflammatory cytokine elevations in cerebrospinal fluid and plasma, an altered gene expression in post-mortem tissue. The COVID pandemic has heightened the significance of these findings.

Human studies may be limited by the heterogeneity of the sample and inability to make strong inferences about causality. Consequently, we have developed a non-human primate (NHP) model of maternal immune activation (MIA) using a modified form of the viral mimic polyIC (polyICLC) to test the hypothesis that maternal immune response contributes to changes in the developing brain and behavior of NHP offspring.

Aim. To use multiple neuroimaging methods (structural volume, diffusion free water, and striatal dopamine PET) to identify immune-related neurodevelopmental changes in the brains of NHP offspring from infancy to adolescence.

Methods: Fourteen pregnant rhesus monkeys (Macaca mulatta) received polyICLC and 10 pregnant monkeys received saline injections at the end of the first trimester. An additional four offspring were added to reach target enrollment of 14 control animals. Offspring from both groups underwent high resolutions structural and multi-shell diffusion weighted MRI scans on a 3T Siemens Skyra scanner at the UC Davis Center for Neuroscience. Data was collected at 6, 12, 24, 36 and 45 months. Additionally, the animals underwent detailed assessments of their physical, social and cognitive development beginning at birth and continuing till the end of the study.

Results: MIA offspring were grossly typically developing during the first year of life. Subtle and selective changes in social behavior (response to an unfamiliar conspecific at 24 moths, performance on CANTAB reversal learning task, false alarms on the CPT task, and increased errors on ID/ED task (all $p < .05$), while episodic memory performance and progressive ratio task performance were intact.

Selective reductions in bilateral frontal and prefrontal cortical volumes were observed beginning at age 6 months and continuing through the course of the study. In additional prefrontal white matter showed a selective reduction in volume from ages 36-45 months ($p < .05$). Multi-shell diffusion imaging results showed an increase in extracellular free water (FW) in the cingulate cortex, beginning at age 6 months and continuing throughout the study ($p < .01$). The magnitude of the FW increase in the cingulate gyrus significantly positively correlated with the increase in IL6 measured in the dams in response to MIA ($p < .01$).

Conclusions: MIA offspring showed evidence of altered neurodevelopment including reduced prefrontal and frontal lobe volumes and increased FW, a putative marker of neuroimmune dysfunction in the cingulate present at 6 months of age and persisting through adolescence. These changes in neural systems were accompanied by subtle changes in social behavior and cognitive control that were largely evident only using laboratory procedures including testing with the CANTAB system.

These findings confirm the clinical relevance of the non-human primate MIA model, which has heightened relevance due to the COVID pandemic. Additional findings regarding dopamine dysregulation and results from histopathological and transcriptomic analyses of brain tissue from the MIA offspring will be discussed.

Keywords: Prenatal Immune Exposures, Prefrontal Cortex, Schizophrenia (SCZ), Cortical Circuit Development, Neuro-Inflammation

Disclosure: Nothing to disclose.

P544. Infection, Mortality and Vaccinations for COVID-19 in Patients With Schizophrenia and Bipolar Disorder: Data From an Entire Population

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Background: Previous studies have shown that patients with schizophrenia and bipolar disorder have higher rates of infection, hospitalization and mortality due to COVID-19, there are very little

data on rates of vaccination in these patients. We studied these outcomes on all patients with schizophrenia and bipolar disorder in an entire country.

Methods: The study linked national population-wide databases in Israel, including data on infection with COVID-19, history of psychiatric hospitalization, hospitalization in general hospitals, mortality and vaccinations. Cases were patients diagnosed with schizophrenia or bipolar disorder, currently or previously hospitalized in psychiatric hospitals at any point in their life. As patients with schizophrenia and bipolar disorder have higher rates of mortality compared to general population regardless of COVID-19, we compared rates of hospitalization and mortality in the general population, patients with schizophrenia and bipolar disorder who were not infected with COVID-19, and patients with schizophrenia and bipolar disorder who were infected with COVID-19. Analyses were stratified according to age and sex.

Results: Controlling for age and gender, patients with schizophrenia or bipolar disorder were less likely to be infected with COVID-19. However, if infected, patients with schizophrenia or bipolar disorder had higher rates (17.2%) of hospitalization in medical wards compared with in the general population (6.2%). Mortality rates were also higher in patients with schizophrenia or bipolar disorder (3.7% vs. 1.1% in the general population). Persons with severe psychiatric disorders had fewer vaccinations at all ages. Total age-adjusted vaccination rate was 83% for the total population compared to 62% for those with schizophrenia/psychotic disorders and 72% for those with affective disorders. All differences were statistically significant.

Conclusions: Compared to the general population, patients with schizophrenia or bipolar disorder were less likely to be infected with COVID-19, but once ill are more likely to be hospitalized in medical wards and are more likely to die. Patients are at increased risk not to be vaccinated. Medical authorities should reach out to patients with schizophrenia or bipolar disorder, encourage them to be vaccinated, and monitor them closely if infected.

Keywords: COVID-19, Schizophrenia and Bipolar Disorders, Epidemiology

Disclosure: Nothing to disclose.

P545. Effect of Inflammatory Markers on Acoustic Startle in the North American Prodrome Longitudinal Study (NAPLS-2) Cohort

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Background: Schizophrenia (SCZ) typically emerges with prodromal symptoms in late adolescence. A greater understanding of brain changes in the prodrome could lead to improved early treatment and improved course. Acoustic startle is used extensively to probe brain function in SCZ. Latency of the startle response, a putative index of speed of neural transmission, is slower in SCZ than in healthy controls (HC). Slowing of latency predicted conversion to psychosis in subjects at clinical high risk for psychosis (CHR) in the North American Prodromal Longitudinal Study (NAPLS-2) cohort, and this was sensitive to the effects of cannabis use and sex. Markers of inflammation, including cytokines, are also associated with conversion to psychosis in this cohort. Further, we have found associations of inflammatory cytokines with slowing of latency in a cohort of adult subjects with SCZ and HCs. We now analyze whether inflammatory markers predict slowing of latency in the NAPLS cohort, and whether these associations differed between HC and CHR subjects.

Methods: Those subjects in the NAPLS-2 cohort with startle data and inflammatory markers at the baseline visit were included: 47 CHR subjects (31.9% female; age (mean ± SD) = 19.3 ± 4.3 years) who met criteria for the psychosis risk syndrome, and a demographically similar group of 27 HC (25.9% female; age = 19.7 ± 4.9 years). Based on 24 months' follow-up, CHR individuals were further classified as converters to full psychosis (CHR-C; $n = 18$) and non-converters (CHR-NC; $n = 29$). Startle magnitude, latency, and prepulse inhibition were assessed with a standard acoustic startle paradigm. Startling stimuli were 115dB[A] 40 msec white noise bursts delivered through headphones in pulse-alone trials. Prepulse trials were nonstartling 85dB[A] 20 msec white noise bursts presented 30, 60, or 120 msec prior to the startling stimuli. The eyeblink component of the startle response was recorded with surface electrodes placed below the eye to capture the electromyographic signal of the startle eyeblink. After preprocessing and data cleaning, acoustic startle latency for each trial type and amplitude to pulse-alone trials were log transformed to improve normality. Inflammatory markers were analyzed with the Human Discovery Map assay, a Luminex bead-based multiplex immunoassay (Perkins et al, Schizophr Bull 2015). Assay results were transformed to z-scores, reflecting deviations in standard units from the HC subjects in this subset of the full NAPLS-2 cohort, and log transformed. We included markers that had associated with startle variables or cognition in prior studies: C-reactive Protein (CRP), Interleukin (IL)-1 receptor antagonist (IL-1ra), IL-6 receptor (IL-6r), IL-8, IL-10, IL-12 subunit p40 (IL-12p40), monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein-1 alpha (MIP-1 alpha), tumor necrosis factor alpha (TNF-alpha), tumor necrosis factor receptor 2 (TNFR2), and malondialdehyde-modified low-density lipoprotein (MDA-LDL). Linear regressions were used to examine the prediction of latency by inflammatory markers, with models including age, race, sex, cannabis use, subject group, and pulse-alone trial magnitude. These regressions were repeated separately for HC, CHR, CHR-NC, and CHR-C subgroups (but omitting the subject group term). Only those results surviving the Benjamini-Hochberg correction for multiple comparisons are reported herein.

Results: For the cohort analyzed as a single group, two inflammatory markers predicted startle latency for the 30 msec prepulse trials. Lower IL-10 ($B = -0.334$; $p = 0.001$) and lower TNFR2 ($B = -0.290$; $p = 0.004$) predicted slower (i.e. longer) latency. Male sex was a predictor of slower latency in models on pulse-alone trials ($B = -0.249$, $p = 0.024$) although no inflammatory markers were significant in those models. Regressions on latency in subgroups of subjects revealed somewhat different patterns. In HC subjects, lower IL-10 predicted slower latency in the 30msec trials ($B = -0.487$, $p = 0.004$), but TNFR2 and other markers were not significant predictors. For the CHR subjects, lower values for IL-10 ($B = -0.304$, $p = 0.006$), TNFR2 ($B = -0.317$, $p = 0.009$), and CRP ($B = -0.296$, $p = 0.012$) predicted slower latency in the 30 msec trials. For the CHR-NC and CHR-C subgroups, no regressions of inflammatory markers on startle latency were significant. However, given the smaller sample size of these latter two subgroups, we cannot exclude the presence of false negatives.

Conclusions: This analysis of the NAPLS-2 cohort confirm that some inflammatory markers predict slowing of startle. Prior work indicates that latency is slower in SCZ than controls, and predicts conversion to psychosis in the NAPLS-2 cohort. Inflammatory cytokines are associated with slowing of startle latency and slowing of psychomotor performance. IL-10 is generally considered to be anti-inflammatory. Thus, it is consonant with the above findings that lower IL-10 concentrations associate with slower

latency. TNFR2 was reduced in the cerebrospinal fluid of medication naïve SCZ subjects and those at risk for SCZ. We report lower TNFR2 predicting slowing of latency in CHR but not HC subjects. Lower CRP predicted slower latency only in our CHR subjects. This was unexpected, particularly given the finding that genetically lower CRP may exert a protective effect on the risk of SCZ. Understanding the interplay of inflammation and slowing of latency may enhance our understanding of brain changes during the prodrome.

Keywords: Prodromal Schizophrenia, Latency of Acoustic Startle, Inflammatory Markers

Disclosure: Nothing to disclose.

P546. A Phase 3 Trial of Roluperidone, a Drug for the Treatment of Negative Symptoms in Schizophrenia

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Background: There currently exist no approved drugs specifically for the treatment of primary negative symptoms. Roluperidone (MIN-101) is a compound with high affinities for 5-HT2A, sigma2 and α1A-adrenergic receptors but no affinity for DA, cholinergic, and histaminergic receptors.

Methods: Following a positive multi-national trial, the trial reported here is a confirmatory trial, in which 513 schizophrenia patients with moderate to severe negative symptoms were administered roluperidone 32 mg/day, 64 mg/day or placebo in a 1:1:1 ratio for 12 weeks followed by 40 weeks open-label roluperidone administration. The primary endpoint was the PANSS-derived Negative Symptoms Factor Score (NSFS) and the key secondary endpoint was Personal and Social Performance (PSP) total score.

Results: NSFS scores were lower (better) on roluperidone 64 mg compared to placebo for the modified Intent-to-Treat population ($p \leq 0.044$) but not for the Intent-to-Treat population ($p \leq 0.064$). PSP total score was statistically significantly superior on roluperidone 64 mg than on placebo ($p \leq 0.021$). During the 40 week of open-label drug administration negative symptoms, daily functioning, and total PANSS scores continued to improve while positive symptoms remained stable, and < 12% of the patients experienced symptom relapses during the whole 52-week study duration. Roluperidone was well tolerated without major or unexpected adverse events.

Conclusions: Taken together, results of this and of a previous trial indicate that roluperidone administered as monotherapy has the potential to improve negative symptoms and maintain stability or even improve the rest of the schizophrenia symptoms.

Keywords: Negative Symptoms, Sigma2, 5 HT2, Alpha Receptors, Schizophrenia

Disclosure: Minerva Neurosciences: Employee (Self)

P547. Optimization of ML321: A D2 Dopamine Receptor-Selective Antagonist for the Treatment of Neuropsychiatric Disorders

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Background: Schizophrenia is a devastating illness that affects approximately 1.1% of the adult human population. It is characterized by a combination of positive symptoms (hallucinations, etc.), negative symptoms (flat affect) and cognitive impairment. Current treatment consists of antipsychotic drugs that are primarily effective in treating the positive symptoms of the disorder. These consist of typical (first generation) and atypical (second generation) antipsychotics, the latter of which are defined by fewer extrapyramidal side-effects (EPS). Despite much research, the mechanism of "atypicality" observed with second generation antipsychotics is unknown. There is a consensus, however, that antagonism of the D2 dopamine receptor (D2R) is primarily responsible for the efficacy of these drugs in treating the positive symptoms of schizophrenia. Unfortunately, all antipsychotics, both typical and atypical, produce numerous side effects (sedation, weight gain, diabetes, etc.), primarily due to their interaction with various receptors, transporters and other signaling proteins. A potent and highly selective D2R antagonist, which has not been previously available in the pharmacological armamentarium, might thus be particularly effective for treating schizophrenia while leading to fewer side effects than the antipsychotics in current use.

Methods: The global pharmacology of ML321 was evaluated using the DiscoverX gpcrMAX screening panel (168 known GPCRs) and the NIH Psychoactive Drug Screening Program (48 GPCRs, transporters and ion channels). Additional experiments were performed as will be described in the figure legends. All animal experimentation was approved by an Institutional Animal Care and Use Committee and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Results: Using high throughput screening to identify novel D2R antagonist scaffolds, followed by chemical optimization, we developed a lead antipsychotic drug candidate, ML321. This compound demonstrates global selectivity for antagonizing the D2R, and to a lesser extent the D3R, with little activity at other GPCR targets. ML321 has the desired D2R binding kinetics for an antipsychotic with slow association and fast dissociation rates from the receptor – properties that may limit commonly observed extrapyramidal side effects (EPS). In fact, ML321 does not produce catalepsy in rodents, a behavior that models the EPS observed with many antipsychotics. Moreover, behavioral assays demonstrate that ML321 is effective in animal models that are predictive of antipsychotic efficacy in humans (e.g., attenuation of both amphetamine and PCP-induced locomotor activity and prepulse inhibition). Importantly, no other known D2R antagonist exhibits this pharmacological and behavioral profile, supporting its development into an advanced clinical lead. No concerns were noted during safety/toxicity studies including hERG channel activation, cytotoxicity, the AMES test, and a CYP inhibition study. While promising as a therapeutic, it has a short metabolic half-life, impeding its clinical development. To this end, we have determined the metabolic ID of ML321 and designed, synthesized, and tested a series of ML321 analogs with modifications of the site of primary metabolism (the alkyl-thiophene moiety) to metabolically stabilize the scaffold while maintaining its favorable pharmacological properties. Over 100 ML321 analogs have been synthesized and pharmacologically characterized using radioligand binding and functional beta-arrestin recruitment assays. Simultaneously, in vitro ADME properties including metabolic stability, permeability, and solubility have been determined for each analog. These studies have led to the optimization of the compound into a collection of lead clinical candidates that show similar pharmacology to ML321 but with marked increases in metabolic stability and ADME properties.

Conclusions: These findings refine our understanding of ML321 structure-activity relationships, particularly around the alkyl-thiophene moiety. Based on this information, additionally optimized analogs have been designed and synthesized supporting the notion that ML321 can be developed into an advanced drug lead with the potential to be a superior therapeutic for the treatment of schizophrenia and other psychotic disorders.

Keywords: Dopamine, Antipsychotic, D2 Receptor, Drug Development

Disclosure: Nothing to disclose.

P549. Layer Specific Proteome Alterations in the Primary Auditory Cortex of Schizophrenia

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Background: Reduced cortical layer 3 dendritic spine density has been reproducibly observed in multiple brain areas in schizophrenia (Sz) and is believed to underlie deficits in cortical processing. Dendritic spine plasticity is regulated by synaptic protein networks and a significant number of Sz genetic risk loci are associated with genes that code for postsynaptic proteins. However, altered expression of postsynaptic proteins in cortical tissue from patients has not been consistently observed. For example, in a recent study we observed decreased levels of mitochondrial proteins, but not canonical postsynaptic proteins, in primary auditory cortex homogenates from Sz and matched control subjects. Each cortical layer is composed of distinct cell populations with differing connectivity. Here we sought to answer two questions 1: Are mitochondrial protein alterations ubiquitous across cortical layers, and 2: Are alterations in the expression of canonical postsynaptic proteins in Sz "hiding" in the layers in which dendritic spines are decreased (e.g. layer 3). To answer these questions, we utilized laser capture microdissection – quantitative mass spectrometry (LCM-MS) to quantify 2,762 proteins in primary auditory cortex layers 3 and 5 from 40 pairs of Sz and matched control subjects.

Methods: Frozen auditory cortex tissue from 40 pairs of Sz and matched control subjects was obtained from the University of Pittsburgh NeuroBioBank. 12 μM thick tissue sections were cut and 4.5E6 μM2 of layer 3 and layer 5 collected by LCM. Tissue from pairs was kept together throughout all processing steps, utilizing a randomized block design. Protein was extracted from each layer, digested with trypsin, and TMT labeled. Pooled TMT labeled peptide digests were quantified on an Eclipse Tribrid Mass Spectrometer utilizing Realtime search and synchronous precursor selection. Database search and TMT reporter ion quantification was conducted with Proteome Discover (FDR < 0.05). Normalization across TMT batches and protein level calculations were done in MSStats and case control comparisons in Limma-Voom.

Results: 2762 proteins were quantified with > 60% present call across both layers in all subjects. Of these, 250 differed significantly between Sz and control in layer 3 and 107 differed in layer 5 ($q > 0.05$). Proteins differing in layer 3 were enriched for the GO terms Mitochondrion ($p = 8.9E\text{-}35$), Gene Expression ($p = 3.7E\text{-}7$), Protein Folding ($p = 1.2E\text{-}5$), and Adherens Junction ($p = 4.90E\text{-}4$); those differing in layer 5 were enriched for Adherens Junction ($p = 1.6E\text{-}5$), Gene Expression ($p = 9.9E\text{-}6$), and Protein Folding ($p = 1.1E\text{-}4$). Levels of canonical postsynaptic proteins did not differ between Sz and control in either layer.

Conclusions: Decreased mitochondrial protein levels are localized to layer 3 while differences in Gene Expression, Protein Folding, and Adherens Junction proteins were found in layers 3 and 5. This suggests that mitochondrial impairments are not cell autonomous in Sz and maybe downstream of decreased local activity in layer 3. Furthermore, that the expression of canonical postsynaptic proteins was not decreased suggests that these proteins are relocated, not lost, as dendritic spine density decreases in Sz.

Keywords: Schizophrenia, Postmortem, Proteomics

Disclosure: Nothing to disclose.

P550. Examination of Differential Cancer Rates in Schizophrenia Using Epidemiology, Bioinformatics, and Protein-Kinase Activity Based In Vitro Drug Screening: Identification of Novel Off Target Effects of Typical Antipsychotics

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Background: Schizophrenia is a devastating psychiatric disorder that has a global prevalence of about 1%. Interestingly, overall cancer rates in schizophrenia are lower than the general population. Previous epidemiological studies of cancer rates in schizophrenia did not fully account for confounding variables such as smoking and substance abuse. We sought to confirm lower cancer rates in schizophrenia and explore mechanisms responsible for this unexpected observation.

Cell culture studies have shown dysregulation of kinase networks driven by antipsychotic administration with a focus on Dopamine 2 Receptor antagonism. Gene expression analysis of cancer cells treated with antipsychotic drugs has shown decreased expression of kinases involved in cell survival, proliferation, and drug resistance including AKT and PIM kinases. We hypothesize that typical antipsychotics are competitive inhibitors of AKT and PIM kinases. We postulate that inhibition of these and other kinases by antipsychotics accounts for reduced cancer incidence in patients with schizophrenia. We applied a three-pronged approach to answer this question: 1) epidemiological confirmation of reduced cancer incidence rates in patients with schizophrenia, 2) bioinformatics analyses of perturbagen structural moieties and gene expression data, and 3) in vitro kinase activity drug screening.

Methods: The Healthcare Cost and Utilization Project (HCUP) dataset was used to identify health outcomes for patients with schizophrenia. Schizophrenia patients were matched to controls by age and sex ($n = 140476$/group). TriNetX, an international administrative healthcare dataset was used as a confirmation dataset. A stratified analysis was conducted to determine cancer risk among patients with schizophrenia ($N = 36{,}695$) as compared to controls ($N = 237, 488$).

Structure-Activity relationship analyses were conducted to assess antipsychotics antineoplastic properties. 53 antipsychotics were virtually screening for similarity in gene expression across a library of 41,000 LINCS small molecules. Chemical similarity was determined across the same library to identify shared moieties between antipsychotics and LINCS compounds.

Protein kinase activity arrays and biochemical protein kinase assays were used to assess the effects of study drugs on recombinant PIM1 protein kinase activity.

Results: Cancer incidence risk for patients with schizophrenia were decreased across all cancer subtypes in the HCUP dataset despite patients with schizophrenia having higher smoking rates (Imputed lung cancer RR = 0.15). The TriNetX dataset confirmed decreased cancer incidence among patients with schizophrenia with an especially pronounced decrease in bladder and lung cancer when accounting for smoking (RR = 0.24, 0.22).

In our bioinformatics analyses, a known PIM and AKT inhibitor, 10-DEBC, was identified as structurally and transcriptionally similar to several phenothiazine antipsychotics. Thioridazine tanimoto structural similarity coefficient as compared to 10-DEBC was 0.87 and the transcriptional similarity in A549 NSCLC cells was 0.475. This indicates phenothiazine typical antipsychotics as likely contributing to the reduced cancer rates in this patient population.

PIM1 activity was inhibited on the protein kinase activity array in a concentration dependent manner by typical antipsychotics. Additional studies are underway to confirm in inhibition of PIM1 activity at clinically relevant concentrations. Data for AKT1 will also be presented.

Conclusions: Epidemiological data confirmed reduced risk of cancer incidence in patients with schizophrenia when accounting for age and smoking rates using the Healthcare Cost and Utilization Project (HCUP). Bioinformatics analyses of perturbagen structure and transcriptional profiles suggest a medication effect, as opposed to genetic factors (such as single nucleotide polymorphisms risk alleles for schizophrenia), as a main contributor to the observed decrease in cancer rates. In vitro kinase activity studies using a microarray platform indicate an inhibitory effect of our study drugs on PIM1 kinase in vitro. In summary, we have identified AKT and PIM kinases as possible direct targets of typical phenothiazine antipsychotics. Taken together, our data suggest that phenothiazine antipsychotics act directly on protein kinases to lower lifetime cancer risk.

Keywords: Schizophrenia, Epidemiology, Bioinformatics, Protein Kinase Activity, Kinome Array

Disclosure: Nothing to disclose.