Neuropsychopharmacology (2016) 41, S116–S288

www.neuropsychopharmacology.org

Poster Session I
December 5, 2016

Sponsorship Statement: Publication of this supplement is sponsored by the ACNP.
Presenter disclosures are found within the abstracts. Disclosure Format: Company Name (Financial Contributor), Type of Financial Disclosure, Funding Received for Self or Spouse.
Asterisks in the author lists indicate presenter of the abstract at the annual meeting.

M1. Dopamine Receptors and Presynaptic Synthesis Capacity Predict Behavioral Flexibility in Foraging Decision-Making in Humans

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Background: Foraging is experimentally defined as a reward-guided behavior that involves deciding whether to engage with the current environment or leave and search elsewhere. This type of decision is crucial for survival and depends on a widespread circuit in the brain (including the midbrain, striatum, medial prefrontal cortex, and anterior cingulate cortex) that is important for encoding and comparing values of different options in order to appropriately adjust behavior (6,7,8). Furthermore, neuromodulators such as dopamine and norepinephrine have been implicated in foraging behavior due to their role in value coding, through integration of reward prediction errors, as well as by controlling the tradeoff between exploitation of a known reward environment and exploration of other unknown options (1,3,4,11). Abnormalities in this type of decision-making have been found in addiction, Parkinson's disease, and schizophrenia, all associated with dysfunction of the dopamine system. Although previous studies have identified an important role of the anterior cingulate cortex (ACC) and of neuromodulators in foraging behavior, it is unclear how regional variation in dopamine synthesis and receptors impacts behavior in humans. Here we directly measured dopamine presynaptic synthesis capacity and D1 and D2 receptor binding potential with PET imaging. In these same individuals, we measured foraging behavior using a computer-based task and tested for relationships between dopamine measures and adaptive foraging behaviors.
Methods: Fifty-one healthy adults (mean age 33.9 ± 1.3 years; 25 females) were recruited from the local community and screened by a physician to rule out psychiatric, neurological, or major medical illness. Participants were also excluded if they were taking medications that could affect neural function. Foraging behavior was assessed with a computer-based task in which subjects collect apples (later converted to money and added to their compensation) from trees in four different apple orchards (hereafter referred to as reward environments) that differ in how quickly the trees run out of apples with each harvest (depletion rate) and how long it takes to travel from tree to tree, both of which affect the average reward rate (2). With each presented decision, subjects can either harvest apples from the tree they are currently at or leave and move on to a new tree. The behavioral measure of interest is the threshold for leaving a tree (patch-leaving threshold) and how the threshold changes between reward environments that differ in average reward rates. On separate days, subjects completed three PET scans to directly measure presynaptic dopamine synthesis capacity ([18F]-FDOPA, 45 participants) and D1 ([11C]NNC112, 39 participants) and D2-3 ([18F]Fallypride, 39 participants) receptor binding potential (BPnd), while resting with their eyes open. Subjects also completed a T1-weighted MRI scan used for registration and brain segmentation (with Freesurfer and manual adjustments) to generate native-space regions of interest (ROIs) in the basal ganglia (putamen, caudate nucleus, ventral striatum, and dopaminergic midbrain). The FDOPA uptake rate (Ki) was calculated with the Gjedde-Patlak method (5,10) and dopamine D1 and D2 receptor BPnd was calculated with the SRTM method (9) using a cerebellar reference region. We tested for correlations between PET measures and adaptive foraging behavior, measured as the change in patch leaving threshold between the two reward environments with maximally different average reward rates, using statistical thresholds of $p<0.05$ (hypothesis-driven basal ganglia ROIs) and $p<0.005$ (whole-brain voxelwise data), uncorrected.
Results: Adaptive foraging behavior was positively correlated with FDOPA Ki in extrastriatal regions including the ACC ($r=0.50$, $p=0.00073$) and posterior midbrain ($r=0.47$, $p=0.0015$), with D1 BPnd in the ventral striatum ($r=0.43$, $p=0.0071$), and with D2-3 BPnd in the dopaminergic midbrain ($r=0.41$, $p=0.033$).
Conclusions: We found PET correlates of decision-making in a patch foraging task that supports previous work in animals and patients with Parkinson's disease suggesting a role of neuromodulators in tracking the average reward rate of the environment and adjusting the threshold for deciding to leave a particular potentially rewarding environment (3,6). Specifically, the change in patch exit threshold between environments with maximally different average reward rates was correlated with dopamine synthesis capacity in the anterior cingulate cortex and posterior midbrain as well as D1 receptor BPnd in the ventral striatum and D2 receptor BPnd in the midbrain. These data provide direct insights into the roles of dopamine synthesis capacity and receptor availability in frontostriatal and midbrain in modulating adaptive behavior in humans.
Keywords: Dopamine, Reward-Based Decision-Making, Patch Foraging.
Disclosure: Nothing to disclose.

M2. Acute Elevation of Corticosterone Enhances Working Memory and Associates With Preserved Cognitive Ability in Aging

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Background: Working memory, or the ability to transiently hold information "in mind" to support goal-directed behavior, is a foundational cognitive process that requires optimal neural activity in the prefrontal cortex (PFC). The PFC is enriched in glucocorticoid (GR) and mineralocorticoid (MR) receptors that bind the stress hormone corticosterone (CORT) and this brain region is highly susceptible to the effects of advancing age. Indeed, impaired working memory is a hallmark of stress-related neuropsychiatric disorders and age-related executive dysfunction. In addition to its role in higher order cognition, the PFC innervates limbic and hindbrain nuclei that modulate the hypothalamic-pituitary-adrenal (HPA) axis to dynamically regulate circulating CORT levels. As such, the PFC is well positioned to couple affective and cognitive processes. While long-standing theory posits that the cumulative effects of stress precipitate deleterious cognitive and neuroendocrine changes that emerge later in life, little is known about the relationship between stress, HPA axis function, and age-related impairment of working memory. The current study integrated behavioral, pharmacological, and physiological approaches in rats to determine the extent to which CORT signaling influences PFC-dependent working memory and HPA output and also whether HPA integrity is a physiological correlate of working memory in aging.
Methods: All experiments used male, F344 rats at 4-6 mo (young adult) or 22-24 mo (aged). Experiment 1 used a PFC-dependent, delayed response test of working memory to evaluate the consequences of CORT signaling and HPA feedback on cognitive performance in young adult rats ($n = 5$/drug). All drugs were administered via IP injection 30 minutes prior to task performance, using a within-subjects design such that each rat received each dose, with at least a 48 h washout period between successive injections. CORT (0.3-3.0 mg/kg) was used to directly evaluate its effect on working memory ability. Spironolactone (SPIRO; 3-30 mg/kg), a MR antagonist, was used to block HPA negative feedback and elevate circulating CORT. Dexamethasone (DEX; 0.1-1.0 mg/kg), a GR agonist, was used to activate HPA negative feedback and deplete circulating CORT. In Experiment 2, young ($n = 11$) and aged ($n = 16$) rats were characterized for working memory performance on the delayed response task (as in Expt 1) followed by determination of CORT release elicited by 1 hour of restraint stress. CORT was measured in blood samples obtained by tail-bleed during and after restraint. In Experiment 3, two cohorts of young adult rats ($n = 5$-6/drug) were implanted with cannulae directed at the PFC to evaluate the effects of blocking GR (100 ng mifepristone; MIF) or MR (50 ng SPIRO) in the prelimbic (PrL) or infralimbic (IL) subregions of PFC on stress-dependent CORT release (as in Expt 2).
Results: In Experiment 1, 1 mg/kg CORT enhanced working memory performance of young adults. This enhancing effect of CORT on working memory was recapitulated by blocking HPA negative feedback to stimulate CORT release with the MR antagonist SPIRO. Depletion of circulating CORT by activating HPA negative feedback with the GR agonist DEX did not affect working memory. In Experiment 2, individual differences in peak CORT elicited by 1 hour of restraint stress were positively correlated with better working memory performance among aged rats. In Experiment 3, blockade of GR or MR at either the level of the PrL or IL was not sufficient to influence CORT levels during or after recovery from 1 hour of restraint stress.
Conclusions: The present findings suggest that the capacity to dynamically elevate CORT/HPA output is beneficial to working memory in both young adult and aged rats. Our studies show that acutely elevating circulating CORT can enhance working memory that depends on the PFC, though CORT is not necessary for normal working memory. Further, a more robust CORT response elicited by exposure to a novel, stressful stimulus was associated with better working memory function in aged rats; age-matched rats with impaired working memory exhibited a relatively diminished CORT response during stress. Despite the significant effects of CORT on working memory supported by the PFC, modulation of neural activity imparted by GR and MRs within this brain region is not sufficient to influence to HPA output in the context of stress. This latter finding suggests that activity in other regulatory centers (i.e. hippocampus or hypothalamus) is required for the modulation of responses to stress. Future work from our lab will examine the basis for CORT's enhancing effects on working memory and also evaluate the status of CORT receptors in other brain regions that modulate HPA activity in young and aged rats.
Keywords: Corticosteroids, Hypothalamic-Pituitary-Adrenal Axis, Prefrontal Cortex, Acute Stress, Cognitive Aging.
Disclosure: Nothing to disclose.

M3. Leukocyte Telomere Length is Associated With Widespread Grey and White Matter Deficits in Early Aging: Impact of Sex and Menopausal Status

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Background: Leukocyte telomere length (TL) provides an index of cellular age that predicts the incidence of age-related diseases and early mortality in older adults. Leukocyte TL predicts preclinical cognitive decline in the elderly and patients with Alzheimer's disease (AD) exhibit shorter TL cross-sectionally relative to healthy individuals. Evidence from a telomerase-deficient mouse model demonstrated the widespread consequences of telomere attrition on neurodegeneration including reduced proliferation of neural progenitor cells, restricted neurogenesis, and atrophy of white matter tracts. These phenotypes were reversed following reactivation of endogenous telomerase activity. While clinical evidence suggests a correlation between chromosomal and neural aging, direct evidence in humans

is limited. Previous work from our group provided initial evidence extending the animal findings to humans, demonstrating in a sample of midlife women that TL (and the ratio of TL to telomerase) is associated with hippocampal volume (Jacobs et al, JAMA Neurology 2014). In this new, population-based multi-modal brain imaging study, we characterized the relationship between TL, grey/white matter volume and white matter tractography in men and women to determine whether cellular markers of aging reflect age-related structural brain changes.

Methods: Healthy mid-life adults ($N = 208$, 104 men/104 women; mean age = 49.9; age range 45-55) who are part of a 50-year prospective prenatal cohort were enrolled at Brigham and Women's Hospital Outpatient Clinical Research Center, where they completed a fasting morning blood draw followed by a structural and functional MRI scan and clinical and cognitive testing. Menstrual cycle histories and serological evaluations were used to determine women's reproductive stage per STRAW-10 guidelines (premenopause, $n = 32$; perimenopause, $n = 29$; postmenopause, $n = 31$). Automated computational reconstruction of brain surface, cortical thickness maps and segmentation of cortical and subcortical structures was performed on a T1-weighted (MPRAGE) anatomical scan. For the study reported here, regions of interest included the medial temporal lobe (hippocampus, parahippocampus and entorhinal cortex) and rostral middle frontal gyrus. Using Fractional Anisotropy (FA) measures, DTI was used to characterize two white matter fiber connections, the cingulum bundle and the superior longitudinal fascicle II. Leukocyte telomere length was determined by extracting genomic DNA from buffy coats using the QIAmp (Qiagen, Chatsworth, CA) 96-spin blood protocol and a modified version of the real-time PCR-based telomere assay.

Results: We characterized the relationship between leukocyte TL and structural indices of neuronal aging and examined sex-dependent relationships therein. Regression models were adjusted for established risk factors and potential confounders, including age, BMI and smoking status. When examined across subjects, shorter TL was associated with lower white matter volume in the parahippocampus and rostral middle frontal gyrus. However, analyses by sex revealed that this association was exclusive to men and absent in women. In men, shorter TL was associated with an overall reduction in cortical white matter (left hemisphere, $t = 2.68$, $p = .009$; right hemisphere, $t = 2.74$, $p = .007$) as well as regional grey and white matter volume in the parahippocampus (grey matter: left, $t = 2.28$, $p = .025$; right, $t = 2.03$, $p = .045$; white matter: left, $t = 2.94$, $p = .005$; right, $t = 2.79$, $p = .006$), and prefrontal cortex (rostral middle frontal gyrus, grey matter: left, $t = 1.8$, $p = .068$ (trend); white matter: left, $t = 2.74$, $p = .007$; right, $t = 2.79$, $p = .006$). In women, the relationship between TL and neuronal indices was dependent on women's reproductive stage. For premenopausal and perimenopausal women, TL was associated with entorhinal cortex white matter ($t = 2.03$, $p = .05$; $t = 1.9$, $p = .064$ respectively), a relationship that was no longer evident in postmenopause ($t = -.61$, $p = .55$).

Conclusions: Preclinical studies showed that reduced telomerase activity and telomere loss have widespread consequences on neurodegeneration, including restricted neurogenesis and atrophy of white matter tracts. Here, we demonstrate that TL is associated with age-related grey and white matter deficits in humans, findings that are sex-dependent and already present in early midlife. Telomere length was associated with widespread neuronal deficits in men, including overall cortical white matter and grey/white matter reductions in the middle temporal lobe and prefrontal cortex. In women, these associations were dependent on menopausal status. Mounting evidence suggests estradiol helps sustain telomerase, telomere length and PFC/HIPP morphology. Taken together, TL could offer a promising early marker of age-related neural deficits, one that is sensitive to sex-dependent aging trajectories.

Keywords: Aging, Leukocyte Telomere Length, Neurodegeneration, Human Neuroimaging, Sex Differences.

Disclosure: Nothing to disclose.

M4. Decreased FADD Protein is Associated With Clinical Dementia and Cognitive Decline in a Community Sample

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Background: FADD (Fas-associated death domain) adaptor is a crucial protein involved in the induction of cell death but also mediates non-apoptotic actions (cell survival, differentiation and neuroplasticity) via a phosphorylated form (p-Ser194-FADD). Multifunctional FADD works in vivo as a common and major signaling step in the initial activation of structurally different receptors (e.g. neurotransmitter G protein-coupled receptors and receptor channels). Although FADD has a crucial role during embryogenesis/ development, little is known about its expression or functions as the brain ages. This study investigated the possible association of FADD forms with the presence and severity of cognitive decline and increased risk of dementia in an elderly community sample.

Methods: FADD forms were quantified by western blot analysis in dorsolateral prefrontal cortex (DLPFC) samples from a large cohort of participants in a community-based aging study (Memory and Aging Project, MAP), experiencing no-(NCI, $n = 51$) or mild-(MCI, $n = 42$) cognitive impairment, or clinical dementia ($n = 57$). The possible contribution of FADD regulation to the full range of age-related cognitive impairment was also evaluated.

Results: Pro-apoptotic FADD protein was decreased (-42%, $p < 0.01$) while p-FADD was unaltered in the DLPFC of subjects with dementia as compared to NCI subjects. Using linear and logistic regression models taking into account age, education and sex, postmortem interval, APOE genotype, multiple age-related pathologies (i.e., amyloid plaques, tangles, Lewy bodies, cerebrovascular diseases, hippocampal sclerosis), and overall synapse density, higher FADD levels in the DLPFC were associated with decreased likelihood of clinical dementia as well as better global cognitive function in MAP participants ($p < 0.01$ for both associations).

Conclusions: Contrarily to what we initially expected, pro-apoptotic FADD was decreased in an elderly, community-based cohort subjects with dementia. Interestingly, loss of FADD in the DLPFC was associated with cognitive decline and increased risk of dementia in a community sample. The discovery of biomarkers for diagnosis and for a sensitive assessment of the progression of clinical dementia associated with aging is an important area of current research.
Keywords: Biomarkers, Aging and Dementia, Postmortem Human Brain.
Disclosure: Supported by SAF2014-55903-R and 'Ramón y Cajal' Program to M.J.G.-F. (MINECO, Spain).

M5. A Double Blind, Placebo Controlled Study of Neurogenesis Enhancers in Persons With Subjective Cognitive Impairment: Electrophysiologic Results in the First 5 Study Subjects After 2 Years of Treatment and at 6 Month Follow-Up

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Background: Neurogenesis refers to the production of new neurons in the brain. This process was first reported in humans in 1998 (Eriksson, et al, Nat Med). For reasons related to safety, this process could not be confirmed in humans until 2013 (Spalding, et al, Cell). All antidepressant medications are believed to be neurogenesis enhancers. Recent studies have also related serotonergic medication with lower mean cortical binding potential by PET PIB imaging in human brains (Cirrito, et al, PNAS, 2011), and decreased cerebrospinal fluid Aß concentrations (Sheline, et al, Sci Transl Med, 2014).
We hypothesized that antidepressants might slow the progression of decline in persons with subjective cognitive impairment (SCI), a pre-mild cognitive impairment (MCI) stage of eventual Alzheimer's disease beginning ~ 22 years before mild dementia occurs, and lasting for ~15 years prior to the advent of MCI (Reisberg and Gauthier, Int psychogeriatr, 2008; Reisberg et al, Alzheimers and Dement, 2008). Accordingly, we have been conducting a double-blind, placebo controlled, randomized study of two neurogenesis enhancer antidepressant medications, a selective serotonin reuptake inhibitor (SSRI), Lexapro, and a serotonin-norepinephrine reuptake inhibitor (SNRI), Effexor ER (brand medications are used).
Methods: Subjects are treated with identically appearing tablets of Lexapro, 5 mg, Effexor XR, 37.5 mg, or placebo at baseline. Dosing is titrated by a clinician who is blinded to the specific treatments for a two-year period, after which the treatment is tapered and discontinued. Subjects are re-evaluated at 6 months post-treatment. One primary outcome, reported herein, is quantitative EEG (Q-EEG) values. We have previously reported remarkable sensitivity for these measures in predicting outcome in SCI persons over a 7-9 year follow-up. Using logistic regression, an R square of 0.93 ($p<0.001$) was obtained between baseline Q-EEG features and probability of future decline, with an overall predictive accuracy of 90% (Prichep, et al, Neurobiol Aging, 2006). Q-EEG results are reported herein for 6 brain regions, the left (L) and right (R) hippocampus, L and R superior/transverse temporal cortex, and the L and R dorsolateral prefrontal cortex.
Results: We previously reported results for the first 2 study subjects who had been randomized to Effexor XR and treated primarily with 2, 37.5 mg tablets and Lexapro 2, 5 mg tablets, respectively, over the course of the study. The Lexapro treated subject, KR, showed significant ($p<0.01$) improvement on the left side in the 3 brain regions studied at the 2-year follow-up ($p<0.001$ for the L hippocampus and $p<0.00001$ for the L superior/ transverse temporal cortex). This improvement was present in one region at the 6-month post study evaluation, the L superior/transverse temporal cortex ($p<0.001$). No significant changes were observed with Effexor XR. We now report results in 3 placebo treated subjects (JH, LN, and DG). JH and LN did not show significant changes in Q-EEG in any of the 6 brain regions at the 2 year follow up. DG showed significant worsening in the L dorsolateral/prefrontal cortex ($p<0.01$). None of the 3 placebo treated subjects showed significant changes from baseline to 2.5 years.
Conclusions: Subject KR, who received Lexapro, reported at the 2-year visit "Everything seems better … more acute memory… less forgetting… quicker recall." Hence, from electrophysiologic and subjective perspectives, the current results for possible utility of Lexapro treatment of SCI are encouraging.
Keywords: Neurogenesis Enhancers, Electrophysiology, Subjective Cognitive Impairment, Prevention of Alzheimer's Disease.
Disclosure: United States Patent and Trademark Office: Pending Patent, Self; Stringer Foundation: Research Grant, Self; Hagedorn Fund: Research Grant, Self; Louis J. Kay and June E. Kay Foundation: Unrestricted Grant, Self; Mrs. Miriam Glaubach and Dr. Felix Glaubach: Unrestricted Grant, Self; NYU Alzheimer's Disease Center: Provision of Clinical Resources, Self.

M6. Differential Fear Conditioning is Associated With Anterior Insula GABA in PTSD

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Background: Enhanced fear conditioning and deficient differentiation of danger and safety signals have been implicated in the development of posttraumatic stress disorder (PTSD). Functional imaging studies have consistently implicated the anterior insula (AI) in the acquisition of conditioned fear, and there is emerging evidence that the AI may be involved in discriminative fear learning. In PTSD and anxiety-related disorders, the right AI is thought to contribute to anticipating future aversive physiological responses to conditioned stimuli (Paulus and Stein, 2006), and to predicting how the relative value of stimuli might affect bodily states. In this study, we examined whether differential fear conditioning differed between adults with PTSD, trauma-exposed non-PTSD (TENC), and healthy

comparison (HC) subjects. We also tested the hypothesis that individual differences in autonomic indices of differential fear conditioning would be associated with right AI neurochemistry, specifically markers of excitatory (glutamate) and inhibitory (GABA) metabolism using magnetic resonance spectroscopy (MRS).

Methods: This sample included 30 PTSD, 21 TENC, and 26 HC adult participants who completed both single-voxel 3T MRS and a two-day fear conditioning paradigm. All participants were interviewed using the Structured Clinical Interview for DSM-IV, and PTSD and TENC participants also received the Clinician Administered PTSD Scale. MRS spectra were collected from a 2 X 2 X 3 ml right anterior insula voxel using a MEGAPRESS sequence for detection of GABA, and 2DJPRESS editing for glutamate (Glu). Both metabolites were normalized to creatine (Cr). The fear conditioning paradigm involved an acquisition phase with 16 paired presentations of conditioned stimuli (CS+) with the unconditioned stimulus (US, shock), and 16 trials of another conditioned stimulus (CS-) never paired with the US. Skin conductance response (SCR) was recorded as a peripheral indicator of conditioned fear; differential fear conditioning was defined as SCR to each CS+ minus SCR to its temporally closest CS- during the conditioning phase.

Results: PTSD patients had significantly less differential fear conditioning than HC ($t(54) = -2.32$, $p = .02$) but not TENC ($t(49) = -0.76$, $p = .45$) participants. GABA/Cr and Glu/Cr levels in the right AI did not differ significantly between groups. There was a significant correlation of differential SCR with GABA/Cr ($r = 0.36$, $n = 77$, $p = .0013$) in the sample as a whole, such that participants whose SCRs showed stronger differentiation between CS+ and CS- trials had higher right AI GABA. Follow-up analyses identified similar effect sizes when this correlation was examined separately in PTSD patients ($r = 0.35$, $n = 30$, $p = .06$), TENC subjects ($r = 0.31$, $n = 21$, $p = .17$), and HC participants ($r = 0.41$, $n = 26$, $p = .04$). Glu/Cr was not significantly associated with the SCR index of differential fear conditioning ($r = .06$, $n = 77$, $p = .58$).

Conclusions: Higher anterior insula GABA measured by MRS was associated with greater differential fear conditioning in adults with and without PTSD. These findings support a role for the right AI in the detection and encoding of stimulus relevance that is acquired through differential fear conditioning, and are consistent with this region's broader role in the anticipation of aversion.

Keywords: Posttraumatic Stress Disorder, Magnetic Resonance Spectroscopy, Insula, GABA, Fear Conditioning.

Disclosure: Nothing to disclose.

M7. Mother to Infant Transmission of Fear Through Maternal Alarm Calls: The Involvement of the Amygdala, the Glucocorticoid and the Noradrenergic Systems

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Background: Fear and anxiety may be transmitted to subsequent generations and potentiate the emergence of mental disorders. One of the ways fear may be transferred across generations is social fear learning. In social fear learning, children exposed to their caretakers expressing fear, acquire their fear responses. Using a rat model, we previously demonstrated that frightened mother passes fear to her pups though alarm odor (Debiec & Sullivan, 2014; Chang & Debiec, 2016). Here we show that maternal alarm 22 kHz calls paired with a neutral odor produce in rat pups fear responses to this odor. This fear transmission is associated with increased activity of the pup's amygdala and is modulated by glucocorticoid and noredrenergic systems.

Methods: Rats pups at postnatal day 18 were isolated from their mother and presented with 11 pairings of pre-recorded 30 s maternal 22 kHz stress vocalizations (delivered by Ultrasonic Dynamic Speaker Vifa) paired with the peppermint odor delivered by a flow dilution olfactometer controlled by FreezeFrame software (2 L/min flow rate, 1:10 CS odor:air). Controls were pups matched for age that were presented with the equivalent number of peppermint odor exposures without stress vocalizations. On the test day, all pups were re-exposed to the peppermint odor and their freezing responses during the odor presentation were recorded and scored. In a subsequent experiment, we analyzed neural activity of the pup's brain during the 22 kHz stress vocalizations - peppermint odor pairings using the early expression c-Fos gene expression. In a series experiments, using pretraining systemic injections of either saline (controls) or corticosterone synthesis inhibitor metyrapone or beta-adrenergic receptor antagonist propranolol we investigated the role of the glucocorticoid and noradrenergic systems in the mother-child fear transmission using alarm vocalizations. Statistical analysis was performed using Student t test. Differences were considered significant when $P < 0.05$.

Results: Pups exposed to maternal alarm calls paired with a neutral odor ($n = 7$) compared to pups exposed to the neutral odor alone ($n = 6$) were showing higher levels of freezing responses to this odor on the following day ($P < 0.05$). Additional experiments showed that an exposure to maternal alarms calls (but not to the neutral odor alone) was associated with increased c-Fos expression in the amygdala nuclei, auditory thalamus, auditory cortex, hypothalamic regions and the periaqueductal grey. Pretraining infusions of metyrapone or propranolol prevented the transmission of fear using maternal alarms calls.

Conclusions: Our data show that pups may acquire fear responses to the neural odor paired with maternal alarm calls. This transmission is associated with increased amygdala activity and is modulated by the gluococortocid and noradrenergic systems. Elucidating the behavioral, neural and molecular mechanisms of parent-child fear transmission may inform the development of novel therapeutic and preventive methods.

Keywords: Fear, Infancy, Maternal Behavior, Glucocorticoids, Noradrenergic.

Disclosure: Supported by: K08 MH014743-01A1, NARSAD Young Investigator Award by Brain & Behavior Research Foundation and Todd Ouida Clinical Scholar Award in Childhood Anxiety and Depression to JD.

M8. A Roadmap to Medication Assisted Augmentation of Psychotherapy for Posttraumatic Stress Disorder

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Background: Several psychotherapeutic procedures have been identified for the treatment of PTSD, most are exposure based. Their procedures typically overlap and induce traumatic stress in a safe environment. Patients are confronted by increasingly difficult feared situations while applying cognitive restructuring techniques and forms of avoidance strategies. While these therapies are used as first line interventions, a significant number of patients fail to respond optimally to these interventions.
Recently several compounds have been identified to be used in conjunction with this procedure, e.g. the finding that the NMDA receptor complex is critical for associative learning has spearheaded the launch of several new studies in which NMDA antagonists are used to enhance fear extinction employing diverse paradigms. D-cycloserine is one of the compounds since this binds to the glycine site at the NMDA receptor and has therefore clinical potential in augmenting responses to cognitive and behavioural therapies. But also the glucocorticoid and adrenergic signaling systems are targets of novel investigational approaches. These go beyond treatment for the disorder as stand alone treatment, but demonstrated their effect in close conjunction with psychotherapy. Medication provided before or after exposure therapy can enhance outcome by: 1) strengthening learning and memory of fear extinction; 2) disrupting reconsolidation, thereby weakening fear memories; or 3) facilitating engagement in psychotherapy by reducing fear and enhancing openness to experience.
Methods: While this novel medication assisted psychotherapy offers an important opportunity to target these emotional memories and the expression of fear, and there is some validation from clinical studies, there is currently a need for roadmap that will assist in identification and evaluation of these compounds in their efficacy in treatment for PTSD. (This work is part of the Traumatic Stress Network of the European College of Neuropsychopharmacology). Trials are evaluating modulation of neurosteroids, glutamate, GABA, endocannabinoids, oxytocin, neurokinin/Substance P, and dopamine. Yet, for these studies it is not known what the optimal dosing is, as well as what parameters need to be addressed to assess their clinical utility for the treatment of PTSD.
Results: This paper identifies compounds to be used in medication-assisted augmentation of psychotherapy for PTSD. In addition, parameters are identified that need to be monitored in order to assess the efficacy of the intervention. For these parameters, a roadmap is constructed that identifies the essential study design that is needed to assess the efficacy of the intervention: study population, time of administration, mode of administration, dosing, therapeutic strategy, identification of SUDS/emotional involvement during therapy, assessment of outcome and follow-up. The following potential compounds will be discussed: propranolol (adrenergic beta-receptor), hydrocortisone (GR receptor), D-Cycloserin (part agonist at glycine site of NMDA receptor), Ketamine (NMDA receptor antagonist), oxytocine (oxytocine receptors) and MDMA (serotonin and norepinephrine transporters, oxytocine release), LSD (D2 receptor, serotonin receptor).
Conclusions: We conclude that several compounds exist that are or will be investigated in the near future for medication-assisted augmentation of psychotherapy for PTSD. Reconsolidation represents an interesting opportunity to modify or alter fear and fear-related memories. Also compounds that facilitate the engagement in therapy by reducing fear may be targeted for a specific population. These interventions require careful planning, and collaboration with prescribers. This roadmap will assist in moving the field forward in terms of design, dosage as well as effectivity and augmentation strategies for treatment of PTSD.
Keywords: Roadmap, Medication, Psychotherapy, PTSD.
Disclosure: Nothing to disclose.

M9. Neurotensin Differentially Modulates Positive and Negative Associations in Basolateral Amygdala Circuits

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Background: The ability to differentiate between stimuli that predict rewarding and threatening outcomes, and engaging the appropriate behavioral response is critical for survival. The Basolateral Amygdala (BLA) is an almond shaped structure in the brain where different populations of neurons encode positive and negative associations. BLA neurons that differ in their long-range anatomical connectivity play opposing roles in the acquisition of positive and negative associations. BLA neurons orchestrate several behaviors via their long-range connections with diverse structures in the brain. The connection between the BLA and the nucleus accumbens (NAc) has been implicated during positive behaviors, whereas the connection between the BLA and the centromedial nucleus of the amygdala (CeM) has been implicated during negative behaviors. Inputs to NAc- and CeM-projecting BLA neurons (BLA-NAc and BLA-CeM neurons) undergo opposing changes in synaptic transmission after acquiring positive and negative associations. Reward-related associations increase synaptic transmission onto BLA-NAc neurons and decrease synaptic transmission onto BLA-CeM neuron, whereas fear-related associations increase synaptic transmission onto BLA-CeM neurons and decrease synaptic transmission onto BLA-NAc neurons.
However, the mechanisms underlying these opposing changes in synaptic transmission are yet to be elucidated. In this work, we identify that the action of a neuropeptide known as Neurotensin in the BLA has the potential to modulate positive and negative associations through opposing modulation of synaptic transmission onto functionally-distinct BLA neural populations.
Methods: We used RNA-Seq to profile the transcriptomes of BLA-NAc and BLA-CeM neurons.
We used in vivo pharmacology to test the functional role of neurotensin-1 receptor (NTSR1), one of the differentially expressed genes. NTSR1 antagonist was infused into the BLA

prior to the association of a tone with either a sucrose reward or an aversive footshock, and the memory was tested a day later.
We used ex vivo whole-cell patch-clamp electrophysiology and induced long-term potentiation (LTP) either in BLA-NAc or BLA-CeM neurons, both in the presence and absence of NT in the bath.
We searched for potential sources of NT to the BLA using the Allen Brain Atlas, and identified the posterior thalamus as a candidate source of NT to the BLA. We confirmed the existence of functional synapses from posterior thalamic NT neurons in the BLA using ex vivo whole-cell patch-clamp electrophysiology. We expressed Channelrhosopsin-2 (ChR2) selectively in posterior thalamic NT neurons using an NT:Cre mouse, and evoked synaptic release in the BLA using light. Monosynaptic currents were evoked from NT axons in the BLA by blocking action potentials using the sodium channel blocker tetrodotoxin (TTX) and enabling light-evoked synaptic release by blocking the voltage-activated hyperpolarizing potassium channels using 4-Aminopyridine (4-AP).
We used in vivo electrophysiology and identified posterior thalamic NT-expressing neurons using photostimulation in mice expressing ChR2 in posterior thalamic NT neurons. Neural activity was sampled during positive and negative associations.
Results: Profiling the transcriptome using RNA-seq provided a list of candidate genes that were differentially expressed between BLA-NAc and BLA-CeM neurons, including the neurotensin receptor -1 (NTSR1). Upon examination of the functional role of the NTSR1 antagonist, we found that NTSR1 blockade in the BLA selectively impaired reward, but not fear, learning. Indeed, neurotensin differentially modulated transmission, as reflected by EPSC amplitude in BLA-NAc and BLA-CeM neurons. In the presence of NT, spike-timing dependent induction of long-term potentiation onto BLA-NAc neurons was reduced, whereas LTP onto BLA-CeM neurons was enhanced. Monosynaptic currents evoked by posterior thalamic NT axons onto BLA neurons were blocked with a glutamate receptor blocker NBQX, indicating that the posterior thalamic NT neurons co-release glutamate onto BLA neurons. Using single unit recordings, we discovered that posterior thalamic neurons, both NT-expressing and unidentified neurons encoded tone onsets and offsets, port entries to consume sucrose solution, as well as foot shocks. However, during the association of a tone with sucrose reward, posterior thalamic NT neurons showed greater responses to port entries, relative to unidentified neurons in the posterior thalamus.
Conclusions: In conclusion, we have discovered that divergent populations of neurons in the BLA are differentially sensitive to NT, a neuropeptide whose action modulates BLA-mediated learning. Moreover, we identify the posterior thalamus as a source of NT to the BLA, characterize the response properties of these neurons, and provide initial evidence of a circuit mechanism whereby NT-expressing thalamic neurons likely impact the activity of BLA neurons during positive and negative associations.
Keywords: Basolateral Amygdala, Neurotensin, Valence.
Disclosure: Nothing to disclose.

M10. Social Modulation of Conditioned and Innate Fear Responses

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Background: Amongst social animals, the presence of a conspecific companion can have powerful effects on mood and behavior. In particular, the social modulation of fear and anxiety represents a highly conserved trait, as both humans and rodents display decreases in anxiety and stress responses when an affiliative conspecific is present. However, the neural processes underlying this social buffering phenomenon remain largely unknown.
Methods: Our group has found that in mice, conspecific presence decreases freezing in both conditioned and innately fearful contexts. In order to identify neural populations that may contribute to social buffering of fear responses, we used a mouse line in which neurons expressing the immediate early gene, Arc, are indelibly labeled following interaction with a novel conspecific. Using this line, we optogenetically re-activated socially-labelled cells in order to test the hypothesis that these neuronal populations may mediate the social modulation of fear responses.
Results: We identified a subset of cells within the infralimbic prefrontal cortex (ILPFC) of male and female mice that are labeled in response to interacting with a novel, ovariectomized female conspecific but not in response to a toy mouse, novel object, or food reward. Optogenetic activation of conspecific-labeled ChR2-eYFP+ neurons decreases freezing in conditioned and innately fearful contexts, without impacting locomotion, recapitulating the effects of conspecific presence.
Conclusions: These data suggest that activation of neurons associated with an affiliative conspecific in the ILPFC may be sufficient to mediate the effects of conspecific presence on fear responses. Thus, targeting this cell population may provide novel therapeutic opportunities that harness circuits naturally engaged by social interaction in order to treat fear and anxiety-related disorders.
Keywords: Social Buffering, Optogenetics, Fear.
Disclosure: Nothing to disclose.

M11. Intranasal Administration of Neuropeptide Y in Patients With Posttraumatic Stress Disorder: A Single Ascending Dose Study

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Background: Posttraumatic stress disorder (PTSD) is a common and disabling psychiatric condition and current treatments possess only limited efficacy. Safe, more effective, and mechanistically novel medications are urgently needed

for the treatment of PTSD and other stress-related disorders such as major depressive disorder. A large body of work implicates the neuropeptide Y (NPY) system as a critical regulator of stress and fear-related behaviors, and as a promising therapeutic focus for PTSD. Therapeutic development of NPY and other peptides is limited by lack of passage of NPY across the blood-brain barrier, and by the digestion of peptides within the gastrointestinal tract. Prior work suggests that intranasal delivery may represent a viable delivery route for peptide-based therapeutics. The current study, therefore, was designed to assess the safety and initial efficacy of ascending doses of NPY delivered via an intranasal route in patients with PTSD.

Methods: We conducted a proof of concept randomized, double blind, crossover single ascending dose study of NPY in patients with PTSD. The study featured a hybrid dose escalation design in which cohorts of three patients were treated at each dosing level beginning with the lowest study dose (1.4 mg); at the point at which a dose limiting toxicity was observed, the study switched to a continual reassessment method in which subsequent patients were assigned to one of five pre-specified dose levels (1.4 mg, 2.8 mg, 4.6 mg, 6.8 mg, and 9.6 mg) according to a Bayesian dose-toxicity model. NPY (Bachen, Bubendorf, Switzerland) was dissolved in saline and delivered via nasal drug delivery device (Kurve Technology, Bothell, Washington). Each patient underwent baseline assessments and was dosed with NPY or saline placebo on two separate treatment days that occurred one to two weeks apart. On each treatment day, patients underwent a trauma script provocation procedure 30 min following drug dosing and completed assessments throughout the day. Measures of anxiety [Beck Anxiety Inventory (BAI)] and PTSD [Impact of Events Scale-Revised (IES-R)] were acquired and comparison of change from baseline to immediately post-trauma script between NPY and placebo at different dose levels represented the analytic approach to detect efficacy.

Results: Twenty-six patients with PTSD were randomized in the study and received at least one dose of NPY or placebo. Two patients discontinued subsequent to the first treatment day and were not crossed over. Therefore 24 subjects completed the study at the following dosing levels: 1.4 mg ($n = 3$), 2.8 mg ($n = 6$), 4.6 mg ($n = 5$), 6.8 mg ($n = 6$), and 9.6 mg ($n = 6$) as dictated by the dose escalation algorithm. NPY was well tolerated up to and including the highest dose of 9.6 mg; the study did not identify the maximum tolerated dose (MTD). Repeated measures ANCOVAs showed a trend towards a significant drug x time x dose interaction for the BAI [$F(5, 100) = 1.95$, $p = .092$] and a significant drug x time x dose interaction for the IES-R [$F(5, 100) = 2.39$, $p = .042$]. To examine these interactions, we compared change in scores in low dose (1.4 mg, 2.8 mg, 4.6 mg; $n = 13$) and high dose (6.8 mg, 9.6 mg; $n = 11$) groups, respectively. In the high dose group, there were significant increases in BAI [$t(10) = 2.98$, $p = .014$],and IES-R [$t(10) = 2.73$, $p = .021$] under placebo but not NPY, indicating that NPY may have an anxiolytic and stress decreasing effect. In contrast, there were no significant increases in scores for either NPY or placebo in the low dose group. These data are suggestive of a therapeutic signal at the high but not low dose, although the study had low power to detect dose-related efficacy effects.

Conclusions: Herein we report results of a single ascending dose study of intranasal NPY in patients with PTSD. We show that doses up to and including 9.6 mg are well tolerated and without toxicity; the MTD was not identified. We found a preliminary efficacy signal at higher NPY doses. Additional studies exploring the safety, efficacy, and target engagement of NPY in humans are warranted.

Keywords: Traumatic Stress, Posttraumatic Stress Disorder, Resilience, Neuropeptide Y, Early Phase Drug Development.

Disclosure: Nothing to disclose.

M12. Prefrontal Cortical mGluR5 Availability in PTSD: Preliminary Findings From a [18F]FPEB PET Study

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Background: The lifetime prevalence of posttraumatic stress disorder (PTSD) in the US is approximately 8% (Kilpatrick et al. 2013). PTSD is characterized by a constellation of symptoms including intrusion symptoms, avoidance, negative alterations in cognition and mood, and hyperarousal. PTSD is associated with a dysregulation in threat-related processing, namely hyperarousal of the amygdala and reduced inhibitory control of the prefrontal cortex (PFC). However, relatively little is known about the molecular underpinnings of this disorder. The ability of the metabotropic glutamatergic receptors (mGluRs) to modulate neuronal excitability in brain circuits involved in anxiety and stress has made them a promising target for drug development for psychiatric disorders (Krystal et al. 2010). Here we focus on the mGluR5 as the potential target for the reduction of PTSD symptomatology. Blockade of mGluR5 has been found to produce anxiolytic effects in animal models (Swanson et al. 2005) and modulation of mGluR5 has been implicated in fear extinction (Fontanez-Nuin et al. 2011). However, to date the mGluR5 has not been investigated in individuals with PTSD in-vivo. Using positron emission tomography (PET) and the radioligand [18F]FPEB, which has high selectivity and specificity for mGluR5, we aimed to quantify mGluR5 availability in individuals with PTSD. Additionally, in a postmortem sample of individuals with PTSD and controls, we aimed to investigate expression of proteins that have a functional relationship with mGluR5 and glucocorticoid (GC) function, which is implicated in PTSD.

Methods: Ten individuals with PTSD (age = 34 ± 8years) and 10 age-, sex-, and smoking status-matched healthy controls (age = 35 ± 9years) participated in one [18F]FPEB PET scan and a comprehensive clinical assessment. Volume of distribution (VT: the ratio of activity in tissue relative to that in blood) in grey matter PFC regions was computed using a venous input function. The radiotracer was injected as bolus plus constant infusion and subjects were scanned during steady state (90-120mins post-injection). Using postmortem brain tissue from the PTSD Brain Bank, massively parallel RNA-sequencing was performed on Brodmanns Area 25 (part of vmPFC and anterior cingulate)

in 19 individuals with PTSD and 19 matched controls. Transcriptome profiles were generated from the sequencing data and were interrogated for mGluR5 and GC related transcripts.

Results: We observed higher mGluR5 density in individuals with PTSD compared to controls in regions of the PFC [ventromedial PFC (vmPFC; 18% higher; Cohen's d = 0.85), dorsolateral PFC (dlPFC; 19% higher; Cohen's d = 0.86) and orbitofrontal cortex (OFC; 19% higher; Cohen's d = 0.86). There was a positive correlation between mGluR5 density and severity of depressive symptoms (vmPFC r = 0.73, $p = 0.02$, dlPFC r = 0.72, $p = 0.02$ and OFC r = 0.73, $p = 0.02$). In the postmortem sample, we found an increased expression of Shank-1 (1.8 fold increase, $Q < 0.01$) in individuals with PTSD compared to controls. Conversely, we observed decreased SGK1 (-2.3, $Q < 0.01$) and FKB5 (-2.5), $Q < 0.02$) expression in PTSD as compared to controls.

Conclusions: Our in vivo evaluation of mGluR5 availability in individuals with PTSD indicated a nearly 20% higher PFC mGluR5 availability as compared to controls. Of clinical relevance, higher mGluR5 availability was associated with a greater depressive symptomatology in PTSD, which might also reflect a greater severity of PTSD itself. In fact, inverse agonism of mGluR5 has been suggested for preventive treatment of acute- and PTSD (Tronson et al. 2010). Consistent with an upregulation of mGluR5, we found an upregulation of Shank-1 in an independent sample of postmortem brain tissue, a protein which anchors mGluR5 receptors to the cell surface. We also replicated our previous finding of decreased SGK-1 in PTSD (Licznerski et al. 2015). In animals, cortisol upregulates SGK-1 (Anacker et al. 2013) and corticosterone administration has been shown to reduce mGluR5 expression (Besheer et al. 2014). Taking this together with evidence for lower cortisol levels in PTSD (Meewisse et al. 2007), one explanation for our findings is that deficits in cortisol signaling in PTSD could contribute to the upregulation in mGluR5 by upregulating Shank1. While further work is needed to confirm our findings in a larger sample and clarify the relationship between mGluR5 and GCs in vivo, our findings suggest that the mGluR5 may be implicated in the pathophysiology of PTSD and represent a novel target for drug development.

Keywords: PTSD, PET Imaging, Glutamate Receptor Activity.

Disclosure: Nothing to disclose.

M13. Characterizing Extended Amygdala Neuron Populations in Primates as a Basis for Exploring Altered Ce and BST Function in Anxiety

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Background: Alterations in extended amygdala (EA) function have been linked to stress-related psychopathology, including anxiety and depressive disorders. Our laboratory has developed a non-human primate (NHP) model of anxious temperament (AT) as a means to investigate the neural and molecular mechanisms underlying the childhood risk to develop anxiety and depression. In preadolescent rhesus monkeys, we demonstrated that two major nodes of the EA, the central nucleus of the amygdala (Ce) and the bed nucleus of the stria terminalis (BST), are key regions in the neural circuitry mediating AT. The Ce and BST are primarily composed of striatal-like medium spiny GABAergic neurons that can be subdivided into multiple subtypes based on their neuropeptide profile. Importantly, some studies in rodents demonstrate an uneven anterior-posterior (A-P) distribution of neuropeptide expression in the lateral subdivision of the Ce (CeL); however, little is known about the functional significance of this gradient in expression and whether this is a feature of neuropeptide distribution in the EA of NHPs. To further understand the cellular and molecular alterations in the EA that mediate AT it is critical: 1) to understand the extent to which EA GABAergic neurons are similar to or different from striatal GABAergic neurons and 2) to use these data to characterize cell types that are more highly expressed in and across the A-P extent of the Ce and the BST.

Methods: To examine selectivity in EA gene expression, we first used human and monkey microarray data from the Allen Brain Atlas to identify genes that are more highly expressed in Ce compared to striatum (putamen and caudate). We then validated these findings using RNA sequencing (RNA-Seq) in rhesus tissue samples from our NHP brain bank. Because somatostatin (SST) and cholecystokinin (CCK) were both more highly expressed in Ce than the striatum and the previous rodent studies demonstrating a role for these neuropeptides in mediating aspects of threat responding, we focused on these neuropeptides in the NHP EA. In situ hybridization was used in 4 rhesus monkeys (mean age = 9.55, 3 female) to characterize the expression of SST and CCK mRNA within amygdala and BST. Additionally, we characterized the distribution of SST and CCK mRNA across the A-P extents of the CeL and the corresponding dorsal lateral division of the BST (BSTLd). Finally, immunohistochemical staining methods were used to characterize the distribution of protein expression in relation to the mRNA expression studies.

Results: Confirming results from the Allen Brain Institute databases, RNA-Seq analyses identified many neuropeptides with significantly greater expression in the Ce as compared to striatal regions. SST and CCK were among the neuropeptides that were more highly expressed in Ce. In situ hybridization for SST and CCK revealed that SST and CCK are distributed throughout the amygdala. With focus on the EA distribution of these peptides, CeL SST expression was significantly correlated with A-P position, such that higher expression was found in the posterior CeL ($F4, 30 = 19.29$, $p = 0.02$). A similar gradient in SST expression was not observed in the BSTLd. Furthermore, CCK distribution was not significantly associated with A-P position in either CeL or BSTLd.

Conclusions: Using existing databases in combination with our rhesus monkey RNA-Seq data, we characterized genes that are preferentially expressed in the Ce as compared to the striatum. This approach provides a foundation for more in depth investigation of EA GABAergic neuronal subtypes. In addition, these studies demonstrate the value of examining

the distribution of mRNA and protein expression across the A-P extent of the NHP CeL and BSTLd. Understanding the functional relevance of differences in the A-P expression of neuropeptide-expressing populations that are enriched in the primate EA will help direct cellular and molecular studies aimed at understanding the function of these neuropeptides, and guide the development of target-specific novel therapeutics.

Keywords: Anxiety, Extended Amygdala, Neuroanatomy.

Disclosure: Nothing to disclose.

M14. Altered Dynamic Brain EEG Connectivity in Social Anxiety Disorder

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Background: Inspired by the Nash embedding theorems, which showed that any compact Riemannian n-manifold can be isometrically embedded in a Euclidean space of dimension 2n+1, and by Theorema Egregium, which showed that the Gauss curvature of a 2-manifold embedded in 3D depends only on the first fundamental form and is thus invariant when it is bent without stretched or torn (i.e., complete isometric mappings preserve the Gauss curvature), the goal of proposed study was to understand the manifold properties or the intrinsic geometry of the brain's state-space. Furthermore, we explored whether these manifold properties differed in participants with social anxiety disorder (SAD) compared to healthy comparison subjects using dynamic electroencephalography (EEG) connectivity during an emotion regulation task.

Methods: EEG data were collected from 20 healthy participants (age: 26.95 ± 9.64, 8M, 12F) and 20 participants with SAD (age: 26.9 ± 8.43, 8M, 12F) using the Biosemi system (Biosemi, Amsterdam, Netherlands) with an elastic cap with 34 scalp channels. Each participant underwent one session of the Emotion Regulation Task (ERT). During the ERT, participants were asked to look at pictures displayed on the screen, and listen to a corresponding auditory guide. Two types of pictures were on display for seven seconds in random orders: emotionally neutral pictures (landscape, everyday objects, etc.) and negative pictures (car crash, nature disasters, etc.). After the picture was displayed for one second, participants were instructed to "look": viewing the neutral pictures; to "maintain": viewing the negative pictures as they normally would; or to "reappraise": viewing the negative pictures while attempting to reduce their emotion response by reinterpreting the meaning of pictures. EEG data were preprocessed using Brain Vision Analyzer (Brain Products, Gilching Germany), by first segmenting task trials into 7-second segments with a window size of 0.05s (the first and last 5 time points were discarded, resulting in 130 time points per task; resting state data was similarly preprocessed). Frequencies-of-interest were set from 1Hz to 50Hz in increments of 1Hz. The final output of each subject was averaged over trials within the same task. As functional communication between two brain regions result in synchronized or phase-coupled EEG readouts, in this study we used weighted phase lag index (WPLI) computed between the times series of two channels to form EEG connectomes (each of which a symmetric 34 by 34 matrix). In order to learn the intrinsic geometry of a high-dimensional manifold, the EEG connectomes from all subjects at all time points were used to sample possible states of the manifold that is shared among all subjects. Then, graph dissimilarity space embedding is used to represent each connectome as a point in a very high-dimensional space (number of dimensions equal to the number of connectiomes for each subject at each timepoint). This is then followed by 1) manifold learning via local neighborhood reconstruction and 2) manifold embedding into a lower dimensional Euclidean space using nonlinear dimensionality reduction (NDR). Once this is achieved, thought chart of any given individual can be constructed by tracing the trajectory of the time-dependent connectome of that subject for any given task. Manifold properties can be quantified by measuring the trajectory length of a given subject's thought chart or the "spread", the area occupied by a given subject's thought chart in the state-space. We examined group differences for trajectory length and spread in the state-space during the ERT and correlations with measures of anxiety severity (Liebowitz Social Anxiety Scale: LSAS and Hamilton Rating Scale for Anxiety: HAM-A).

Results: Participants with SAD had significantly greater trajectory lengths and spread with all aspects of the ERT (neutral, maintain, reappraise) ($p < .0001$). Furthermore, trajectory length and spread associated with all aspects of the ERT correlated with anxiety severity scores in the total sample (length x HAM-A: neutral $r = .64$, $p < .001$, maintain $r = .68$, $p < .001$, reappraise $r = .69$, $p < .001$; length x LSAS: neutral $r = .83$, $p < .001$, maintain $r = .88$, $p < .001$, reappraise $r =$). Within the SAD group, trajectory length and spread during the maintain phase of the ERT correlated with LSAS scores ($r = .54$, $p = .015$; $r = .54$, $p = .01$).

Conclusions: The present study is the first-ever demonstration of altered brain network dynamics in SAD using unsupervised manifold learning applied to dynamic EEG connectivity. Future work will examine whether brain state-space dynamics could be used as a platform for neurofeedback applications.

Keywords: EEG Biomarkers, Social Anxiety, Connectome, Dynamic Connectivity.

Disclosure: Nothing to disclose.

M15. Mechanisms by Which the Prefrontal Cortex Distinguishes Ventral Hippocampal From Mediodorsal Thalamic Inputs

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Background: The prefrontal cortex (PFC) serves as a hub for cognitive and affective behaviors including spatial working memory and anxiety. Two major sources of information to the PFC are provided by the ventral hippocampus (vHipp) and the mediodorsal thalamus (MD), both of which provide glutamatergic input to this structure. Despite this

convergence of input, optogenetically silencing either the vHipp to PFC or MD to PFC inputs produces distinct behavioral responses. For example, silencing vHipp to PFC terminals decreases anxiety (Padilla-Coreano N et al, Neuron, 2016) and selectively impairs encoding of spatial working memory (Spellman T et al, Nature, 2015) while silencing MD to PFC terminals does not affect anxiety and instead selectively impairs maintenance of spatial working memory (Bolkan et al, in preparation). However, the mechanisms by which the prefrontal cortex is able to selectively distinguish between seemingly identical neurotransmitter inputs to generate behaviorally distinct responses is unknown.

Methods: We used Channel-rhodopsin (ChR2) in combination with slice electrophysiology in the PFC to assess the efficacy of synaptic transmission for both projections under different input frequencies. We are currently using a combination of red-shifted and blue-light activated ChR2 to investigate whether vHipp and MD cells project to the same PFC neurons and whether synaptic transmission from these two projections is differentially affected by input frequency.

Results: In mice with either their vHipp or MD bilaterally injected with ChR2, we have found that all layer III/V PFC cells sampled receive MD or vHipp input. vHipp to PFC inputs seem optimally tuned to respond at lower frequencies (8 Hz), as the response amplitude to repeated 5-ms stimulations at this frequency produced consistent, high-fidelity responses, while the responses to repeated stimulation at 20 Hz displayed significant attenuation and increased failure rate. In contrast, MD to PFC inputs, while responding to a single 5-ms light pulse with a comparable amplitude to that of vHipp to PFC inputs, showed less attenuation to subsequent stimulations at 20 Hz and no failures. Experiments probing MD-PFC and vHipp-PFC inputs in the same animals are ongoing and will be presented at the meeting.

Conclusions: Our current data are consistent with a model whereby MD and vHipp inputs broadly project to overlapping pyramidal cell populations, but may be distinguished by optimal synaptic transmission at distinct frequencies.

Keywords: Prefrontal Cortex, Mediodorsal Thalamus, Ventral Hippocampus, Optogenetics, Electrophysiology.

Disclosure: Nothing to disclose.

M16. Ketamine for Social Anxiety Disorder: A Randomized Placebo-Controlled Crossover Trial

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Background: First-line treatments for Social Anxiety Disorder (SAD) are only partially effective. Many SAD patients experience little or inadequate symptom relief with available treatments. Ketamine has robust short-term anti-depressant effects, and is particularly effective in depressed patients with comorbid anxiety. Ketamine is a potent NMDA receptor antagonist and represents an agent with a potentially novel mechanism of action for the treatment of anxiety disorders. We sought to determine the short-term efficacy of ketamine compared to placebo in the treatment of social anxiety disorder.

Methods: We conducted a double-blind, randomized, placebo-controlled crossover trial in 18 adults with SAD and compared the effects of intravenous ketamine (dosed 0.5 mg/kg) on social anxiety symptoms to placebo (normal saline). All participants met DSM-5 criteria for SAD and endorsed a fear of public speaking. Ketamine and placebo infusions were administered in a random order with a 28-day washout period between infusions. The primary outcome of the trial was average Lebowitz Social Anxiety Scale Score for the first 3 days following infusion. All participants were asked to give a 10-minute impromptu speech in front of a small audience at baseline and 24 hours after the intravenous infusion. Anxiety during the speech was self-rated by patients on a visual analog scale (VAS) scored 0 ("not at all anxious") – 100 ("as anxious as you could possibly be") during the impromptu speech. Additionally, blinded raters used the Clinical Global Impression-Improvement Scale (CGI-I) scored 1 ("very much improved") to 7 ("very much worse") to measure overall improvement at 24 hours after each infusion.

Results: Of the 18 participants, 17 completed both infusions and 3 met criteria for current DSM-5 Major Depressive Disorder at enrollment. The CGI-I score at 1 day following infusion was significantly reduced in the ketamine group compared to placebo (paired t-test $t = 2.78$, $p = 0.015$). Eleven of 18 subjects receiving ketamine were rated as minimally improved or better on the CGI 1 day following infusion compared to 2 of 17 receiving placebo. The only two responders to treatment ($CGI < 3$) received ketamine.

Conclusions: Ketamine resulted in a significantly greater reduction in social anxiety when compared to placebo as assessed by CGI completed by blinded raters at 1 day following infusion. A significant limitation to the self-rated anxiety during the impromptu speech is that 17 of 18 patients correctly identified when they received ketamine, suggesting that the use of saline (placebo) as a control for ketamine led to inadequate blinding. Analysis of change in depression symptoms, performance on the speech, social anxiety symptoms, and period effects over the full 2 weeks of symptom monitoring is pending and will be presented at the poster session.

Keywords: Social Anxiety, Clinical Trial, Ketamine, Anxiety Disorders, Mood and Anxiety Disorders.

Disclosure: Nothing to disclose.

M17. Adolescent Social Anxiety is Associated With Dysregulated Neural Response During the Anticipation and Receipt of Peer Evaluation

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Background: Social anxiety (SA) increases during adolescence, when the salience of peer-based social evaluation

peaks. Prior neuroimaging studies show that in socially anxious adolescents, peers elicit dysregulated engagement in key brain regions implicated in social cognition and affect processing (Guyer et al, 2005, Lau et al. 2012). However, SA symptoms are differentially triggered by distinct social contexts. Specifically, uncertain, rather than predictable social situations, commonly elicit SA symptoms. Thus, peer evaluation in the presence of uncertainty may be associated with distinct, yet unknown patterns of brain function that promote the affective symptoms and social biases common to SA. We recently developed an ecologically valid fMRI-based paradigm optimized to test for the effects of social evaluative uncertainty during ongoing social interactions with peers in a virtual school setting (Jarcho et al, 2013, 2016). In the present study, we utilized this paradigm to determine whether neural response in SA, compared with healthy adolescents, varied during the anticipation and receipt of predictable and unpredictable peer acceptance and rejection feedback.

Methods: After undergoing a clinical diagnostic interview (KSADS-PL), gender, and IQ-matched healthy ($n = 21$) and SA ($n = 21$) adolescents (12.36 ± 2.36 years) were told they would visit a Virtual School and interact with purported "Other Students". Before completing the fMRI paradigm, participants learned each student had a reputation for being nice, mean, or unpredictable. While scanning, participants entered classrooms with the Other Students. Unpredictable peers provided 50% positive and negative social evaluation, while nice and mean peers respectively provided 100% positive or negative social evaluation. Whole brain analyses ($p < .005$; cluster threshold > 50 voxels) tested whether SA and Healthy adolescents had different patterns of neural response as they anticipated, and then received predictable and unpredictable positive and negative peer evaluation.

Results: Participants were able to learn Other Student reputations prior to interacting at the Virtual School and subsequently reported believing these interactions involved real peers. During anticipated peer evaluation, extensive group differences in brain function emerged in medial prefrontal cortex (9, 37, 8; 678 voxels) dorsal anterior cingulate (-9. 20, 43; 64 voxels), superior temporal gyrus (57, -27, 5; 76 voxels), putamen (29, 9, -1; 73 voxels), and ventral striatum (6, 6, -1; 54 voxels), key regions implicated in social cognition and affective processing. For each activation cluster, SA adolescents had heightened engagement relative to healthy adolescents. Additionally, more severe symptoms of anxiety, measured by the Screen for Child and Anxiety Related Disorders, and more fear during the scan, were associated with greater activity. During the receipt of peer evaluation, a distinct pattern of group differences emerged in the right temporal pole (49, 16, -30; 51 voxels), a brain region implicated in mentalizing and binding affective meaning to social experiences. SA adolescents had less activity when they received negative social evaluation from unpredictable relative to mean peers, $t(18) = -3.39$, $p < .005$; healthy adolescents exhibited the opposite pattern $t(18) = 3.29$, $p < .005$. Neither group differentiated between positive feedback from unpredictable compared with nice peers.

Conclusions: These results demonstrate that anticipating peer evaluation, regardless of the valence of its expected outcome, elicits heightened engagement in socio-affective processing networks among SA adolescents. Moreover, they suggest that peer-related uncertainty may play a more central role in potentiating neural dysreguation in SAD than social rejection alone. Results also suggest further nuances in the relationship between brain function and experiences of social acceptance in normatively developing adolescents.

Keywords: Adolescence, Anxiety, Social Interaction, Functional MRI (fMRI), Fronto-Striatal networks.

Disclosure: Nothing to disclose.

M18. A Reproducible Inbred Mouse Model of Post-Traumatic Stress Disorder Symptoms That Identifies Susceptibility

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Background: A history of stress increases the likelihood that an individual will develop post-traumatic stress disorder (PTSD) . The disorder is defined by persistent recollections of a traumatic event that are accompanied by hyperarousal, avoidance of trauma-related cues and negative changes in mood and cognition3. The limited neurobiological understanding of PTSD has been attributed, in part, to the need for improved animal models.

Methods: We employed a stress enhanced fear learning (SEFL) paradigm that combines restraint stress with fear conditioning to precipitate traumatic memories in mice. Extinction profiles of male and female mice after SEFL were examined. Further characterization of behavioral and molecular patterns induced by SEFL in male mice were assessed with anxiety tests, Fos immunohistochemistry and RNA-sequencing.

Results: This SEFL paradigm recapitulates several hallmark features of PTSD, including differential vulnerability to the effects of stress. The stress susceptible subgroup displays persistently elevated, extinction-resistant fear memory, hyperarousal, generalization, dysregulated corticosterone and altered Fos activation in PTSD-associated brain regions, the infralimbic cortex and basolateral amygdala (BLA), following remote memory retrieval. Importantly, behavior during SEFL training was predictive of stress susceptible and resilient animals, enabling molecular studies without the typical confound of additional behavioral phenotyping. Transcriptome-wide analysis of the BLA revealed divergence between these two subgroups, including dysregulated transcription of genes that have been implicated in PTSD by polymorphisms specific to PTSD patients. Finally, key behavioral outcomes of the model, including susceptibility, were easily replicated in an independent laboratory.

Conclusions: This SEFL model provides a readily available tool for development of PTSD therapeutics that is construct and face valid, as well as compatible with the growing number of mouse-specific research resources.

Keywords: Fear Extinction, Traumatic Stress, Resilience.

Disclosure: Nothing to disclose.

M19. HIV Alters PTSD Symptomology and Psychophysiology in Traumatized Individuals

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Background: HIV-infected individuals are exposed to high rates of trauma that can lead to the development of posttraumatic stress disorder (PTSD). The incidence of PTSD in people living with HIV (PLWH) has been reported to be as high as 30% in both women and men. Importantly, low socioeconomic status (SES) among disadvantaged African Americans living within urban environments is strongly associated high levels of trauma exposure and PTSD, as well as increased risk for HIV infection. Exposure to stressors and increased stress reactivity associated with PTSD increases antiretroviral therapy (ART) non-compliance, care drop out, and engagement in transmission risk behaviors. While PTSD in PLWH has been associated with attenuated levels of ART compliance, hastened disease progression and decreased quality of life, it still remains unclear how HIV infection influences PTSD presentation in trauma-exposed individuals. Furthermore, the lack of knowledge surrounding how HIV status influences the presentation of PTSD may limit the generalizability of behavioral and pharmacological treatment strategies to PLWH and PTSD. Thus, we undertook the current study to characterize how HIV infection influences PTSD presentation (symptoms and psychophysiological hyperarousal) in trauma-exposed PLWH. Furthermore, because HIV has been linked to cognitive deficits, we tested the hypothesis that PLWH would show deficits in physiological learning as assessed in a fear-conditioning paradigm.
Methods: All participants ($n = 42$, 25 without HIV, 17 PLWH) were between 18 and 48 years old recruited from Grady Memorial Hospital in downtown Atlanta, GA and provided informed consent. All subjects participated in a clinical interview conducted by a trained clinician on all psychological assessment instruments. Lifetime trauma history was determined by the 14-item Traumatic Events Inventory (TEI), which assesses for experiencing and witnessing traumatic events. Childhood trauma history was assessed via the Childhood Trauma Questionnaire (CTQ). The PTSD Symptom Scale (PSS) was used to determine current overall PTSD symptoms, as well as avoidance, re-experiencing, and hyperarousal sub-clusters. A sub-set of subjects also participated in a fear-potentiated startle (FPS) paradigm to assess for psychophysiological hyperarousal previously described to be present in PTSD, as well as deficits in physiological learning. FPS was measured by the relative increase in the acoustic startle reflex in the presence of conditioned stimuli that have been paired with aversive unconditioned stimuli. The FPS consisted of an initial habituation phase wherein conditioned stimuli (CS) were presented without any reinforcement. The fear acquisition phase consisted of three blocks with four trials of each type of CS (reinforced conditioned stimulus, CS+; non-reinforced conditioned stimulus, CS-; noise probe alone, NA) for 12 trails per block and a total of 36 trials. Both CSs were colored shapes (i.e. blue square, purple triangle) presented on a computer monitor for six seconds each and counterbalanced across subjects. The unconditioned stimulus (US; aversive stimulus) was a 250-msec air blast of 140-psi intensity to the larynx.
Results: PLWH exhibited higher levels of re-experiencing ($F = 12.19$, $p = 0.001$) and avoidance ($F = 8.12$, $p = 0.007$) PTSD symptoms compared to individuals without HIV, after controlling for trauma exposure. PLWH also showed impaired fear conditioning in the FPS paradigm compared to the traumatized controls (interaction effect of HIV and Trial Type, $F = 7.34$, $p = 0.03$). While individuals without HIV showed a typical increase in startle magnitude to the CS + that was previously paired to an aversive stimulus ($F = 23.75$, $p = 0.003$), PLWH exhibited a deficit in fear conditioning, even after controlling for trauma exposure ($p > 0.05$). Specifically, PLWH did not show FPS to the danger signal (CS+).
Conclusions: Taken together, these preliminary data indicate that HIV is associated with altered PTSD symptom presentation and fear learning deficits in traumatized individuals. Furthermore, these data suggest that HIV is associated specifically with symptoms of re-experiencing and avoiding the traumatic event; rather than more general trauma-related psychopathology such as hyperarousal. Given that these symptoms promote avoidance of PTSD treatment, these data have high clinical significance for treating PLWH. The finding that PLWH also show psychophysiological fear learning deficits also has treatment implications for PLWH, as the most effective evidence-based treatments for PTSD are based on fear learning mechanisms. Ongoing analyses are being performed to directly assess the effects of HIV on fear extinction in traumatized individuals.
Keywords: PTSD, HIV, Psychophysiology.
Disclosure: Nothing to disclose.

M20. Identification of a Novel Genetic Candidate for Fear Extinction in Mice

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Background: Although stress and psychological trauma affects many people, some people are more susceptible due to inherited factors and some people are in high risk due to their environment. In our previous studies we identified that inbred mice strain 129S1/SvImJ (S1) has impaired fear extinction compared to normally extinguishing C57BL/6J (B6) mice. In this study we aimed to identify gene candidates that would be critical in the extinction of traumatic memories and associated behaviors.
Methods: In order to identify phenotypic and genetic differences across mouse strains to locate genomic regions associated with variation in trauma-relevant behaviors we performed a quantitative trait loci (QTL) associated with fear extinction in a population generated from crossing these inbred mouse strains. We used Pavlovian fear conditioning

and extinction protocol to test our mice. Using Palkovits punch techniques we collected basolateral amygdala (BLA) after test and used the tissue punches for biochemical and genetic analysis. Immunohistochemistry was used to identify the perineuronal nets in BLA. We used in situ hybridization for detecting the distribution, neuronal subtype-specific expression of our gene of interest in BLA. Stereotaxic injections were used for lenti viral based functional modifications in BLA.

Results: First, we found these strain differences to be resistant to developmental cross-fostering and associated with anatomical variation in BLA perineuronal nets, which are developmentally implicated in extinction. Next, we performed BLA expression-profiling on genes located within an extinction-associated QTL and nominated Ppid (peptidylprolyl isomerase D, a member of the tetratricopeptide repeat (TPR) protein family) as an extinction-related candidate gene. Subsequently, we showed that the extinction-impaired mouse strain had reduced BLA Ppid and GR (glucocorticoid receptor) gene expression, but retained the extinction-facilitating effects of a systemic GR agonist. Then, using a virus-based approach to directly regulate Ppid function, we demonstrated that downregulating BLA-Ppid was sufficient to impair extinction, while upregulating BLA-Ppid produced facilitation of extinction coupled to changes in in vivo neuronal extinction-encoding.

Conclusions: Collectively, our results identify Ppid as a novel gene involved in regulating extinction possibly via modulations on downstream GR signaling. Identification of Ppid as a novel gene critically involved in fear extinction in mice could potentially help us to understand more on the genetic and pathophysiological mechanisms underlying risk for trauma-related disorders.

Keywords: Fear Extinction, Basolateral Amygdala, QTL, GR Chaperone, Mouse Behavior.

Disclosure: Nothing to disclose.

M21. Neural Response During Reward Anticipation Differs in Tanner-Staged Children and Adolescents as a Function of Task Difficulty and Reward Probability

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Background: Changes in the neural underpinnings of reward processing during adolescence may contribute to observed increases in risk-taking behavior during this developmental period, but the existing literature regarding differences between children and adolescents in brain activation during reward processing has provided inconsistent information. For example, studies have found both hyperactivation and hypoactivation in the striatum and related inter-connected regions (including the amygdala, the hippocampus, and the anterior cingulate) during reward anticipation, and even those investigations employing similar task designs do not always yield equivalent results. These contradictory findings may result from different ages and/or developmental (pubertal) stages of participants, cohort effects, or differential task demands. Here, we compared the neural processes underlying reward anticipation during variable task difficulty and reward probability in adolescents and pre-pubertal children, with pubertal (Tanner) stage ascertained by clinicians.

Methods: Typically developing children (17 girls, mean age = 8.6, range = 8.1-9.1; 24 boys, mean age = 8.7, range = 8.3-9.4; all Tanner Stage 1) and adolescents (12 girls, mean age = 13.1, range = 12.3-14.6; 18 boys; mean age = 13.0, range = 12.2-14.0; Tanner Stages 2-5, median = 4) performed a modified monetary incentive delay (MID) task in a 3T fMRI scanner. The task included trials with potential monetary reward and two levels of predictable difficulty (depending on required response time), as well as baseline trials where no monetary gain was possible. Anticipatory background cues indicated whether the imminent trial would be a potential reward trial (and its predicted difficulty) or a non-reward trial. A green background cue indicated a reward trial with a 67% chance of being easy (up to 1250 ms given for the response, thus requiring little effort and having likely success), whereas a red background cue indicated a reward trial with a 67% chance of being 'difficult' (response required within 175 ms, thus requiring high effort and having less likely success). A gray background cue signaled a baseline control trial with no possibility of reward and a 50:50 distribution of required response times. At the first level, for each trial we modeled the anticipation phase (three levels: red, green, or gray cues) as well as reward outcome (two levels: successful or unsuccessful monetary outcome), and the contrast of interest was defined as the anticipation phase for the red (67% difficult) versus green (67% easy) trials for each participant. The Artifact Detection Tools (ART) software package was used to remove TR's with more than 1.5 mm displacement. These first-level contrasts were then entered into a second-level, whole-brain voxel-wise analysis using a full factorial model to test for effects of pubertal status and sex, and their interaction, in SPM5.

Results: The number of TR's removed did not differ between sexes or pubertal groups and averaged 5%. Whole-brain voxel-wise analysis examining the main effect of pubertal status revealed several regions where neural response in pre-pubertal children differed from adolescents as a function of task difficulty during reward anticipation; these included the caudate (voxel-level $p = 0.0005$, uncorrected), anterior cingulate ($p = 0.001$, uncorrected), amygdala ($p = 0.002$, uncorrected), and hippocampus ($p = 0.002$, uncorrected). Post-hoc analyses showed that in all of these regions, children had greater neural response during easy trials with more likely success than during difficult trials, whereas adolescents showed the opposite pattern. No significant main effect of sex or interaction between pubertal status and sex were observed at $p < 0.005$, uncorrected.

Conclusions: In the present study, we measured brain activation associated with task difficulty and reward probability during reward anticipation by independently manipulating reward-predicting anticipatory background cues. Our data provide evidence for differential neural recruitment in adolescents and pre-pubertal children in several regions previously associated with reward processing. The results emphasize the importance of considering differential task

demands in interpreting brain responses to reward-predicting stimuli. In addition, our determination of pubertal stage by clinician-performed Tanner staging provides a foundation for future longitudinal examination of the role of hormonal changes in reward-related neurodevelopment.
Keywords: Children and Adolescents, Reward Neural Circuitry, Striatum, Puberty, Brain Development.
Disclosure: Nothing to disclose.

M22. Age-Related Changes in Atypical Structural Covariance Networks in Males With High-Functioning Autism Spectrum Disorder

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Background: Autism spectrum disorder (ASD) is a neurodevelopmental disorder associated with atypical structural and intrinsic functional connectivity in the main brain networks. Network-level brain structural organizations may reflect not only structural and functional connectivity but also the prevention of functional signal bias (Alexander-Bloch et al, 2013). Limited ASD studies investigated structural covariance networks based on variable seed definitions, in heterogeneous cohort with a wide age range and mixed sex, thus still yielding inconsistent findings. As age and sex effects on brain structures and functions may be distinct in ASD from typically developing controls (TDCs), we aimed to directly explore structural covariance networks comparable to intrinsic functional networks in a more homogeneous sample with ASD, regarding male-only participants and narrower age subsamples.
Methods: We assessed 230 male participants (115 ASD, and 115 TDCs) with clinical evaluation and structural MRI scans on Simens Magnetom Trio 3 T system. Participants were subgrouped into children (54 ASD, age range 7-12.5 years, mean ± SD 10.8 ± 1.2 years; 54 age-matched TDC), adolescents (33 ASD, age range 12.5-18 years, mean ± SD 14.6 ± 2.4 years; 33 age-matched TDC), and adults (28 ASD, age range 18-30 years, mean ± SD, 21 ± 2.6 years; 28 age-matched TDC). Surface-based morphometry analysis was implemented using FreeSurfer ver. 5.3.0. Structural covariance analysis was applied to characterize structural relationships of cortical thickness (a seed-based approach). The seeds were derived from the hubs of Yeo's 7-networks (2011) based on functional parcellation, including the salience network, default mode network, dorsal attention network, control network, visual network, somatomotor network and limbic network.
Results: In children with ASD, we identified that there was significant structural covariance within the salience, default mode, dorsal attention, control and limbic networks as compared with TDC children. TDC children had significant visual and somatomotor structural covariance networks, but these covaried relationships were not identified in children with ASD. In both ASD and TDC adolescent groups, there was no significant structural covariance network identified from the seeds based on Yeo's 7-networks. In young adults with ASD, we identified only limited regions showing significant structural covariance with the key hubs of the brain networks subserving high cortical functions, as compared with TDC adults. Between-group comparisons, in terms of the significant group by seed interactions, showed that TDC adults, relative to adults with ASD, had higher structural covariance within these networks involved in high cortical functions. ?In young adults with ASD, we identified the prominent structural covariance between homologous lateral prefrontal regions, concurring with earlier resting-state fMRI reports (Hahamy et al, 2015), whereas this covariance was not identified in TDC adults.
Conclusions: Our results extend prior results of age-related neurostructural atypicality in ASD to structural covariance networks. Overall, we identified increased structural covariance of the major networks in children with ASD, whereas reduced covariance in adults with ASD was found. These age-specific differences echo earlier literature of resting-state functional MRI in ASD, further endorsing the speculation that structural organizations may reflect intrinsic functional connectivity. Future work to investigate the replication and generalization issues to other subgroups on the spectrum is warranted.
Keywords: Autism Spectrum Disorder, Structural Covariance, Networks, Age Effects.
Disclosure: Nothing to disclose.

M23. Identifying Convergent Biological Pathways in Autism Using a Zebrafish Model

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Background: Autism spectrum disorders (ASD) are a group of devastating neurodevelopmental syndromes that affect up to 1 in 68 children. The zebrafish is a model vertebrate system well-suited for conducting small molecule screens to uncover convergent biological pathways in ASD. We have shown that zebrafish mutants of the ASD risk gene, Contactin Associated Protein-like 2 (CNTNAP2), display forebrain GABAergic deficits, and we identified estrogenic compounds as suppressors of the cntnap2 mutant behavioral phenotype. Here we investigate morphological and behavioral phenotypes in zebrafish mutants of Chromodomain helicase DNA binding protein 8 (CHD8), one of the strongest ASD risk genes identified to date, to illuminate neurochemical pathways underlying ASD.
Methods: To investigate the function of Chd8, we generated two lines of chd8 mutants in zebrafish using TALENs that target regions early in the gene and an exon containing a nonsense mutation in an individual with ASD. We analyzed inhibitory and excitatory neuronal populations in chd8 mutants during early brain development. To predict neural pathways that are disrupted in mutants, we conducted quantitative pharmaco-behavioral profiling of chd8 mutant larvae and compared the mutant behavioral fingerprint to a previously reported dataset of the behavioral profiles of wild-type larvae exposed to 550 psychoactive compounds. Finally, we conducted pharmacological screens to identify phenotypic suppressors.

Results: We found that zebrafish chd8 mutants display GABAergic deficits in the forebrain, though the deficit is less than in cntnap2 mutants (10-20% versus 34%). There were no significant regional differences in glutamatergic neurons. Head and brain size are unaffected in homozygous chd8 mutants during early developmental stages, though homozygous mutants are smaller on average as adults and show increased mortality. High-throughput quantitative behavioral profiling revealed a robust and reproducible phenotype of decreased periods of inactivity during the daytime in both lines of chd8 mutants. In addition, we found that estrogenic compounds modulate the chd8 mutant behavioral phenotype.
Conclusions: Pharmaco-behavioral profiling of zebrafish mutants highlights the strength of this system to investigate common morphological and pharmacological pathways involving ASD risk genes. These results illuminate a novel neurochemical pathway with relevance to ASD.
Keywords: Autism Spectrum Disorder, Zebrafish, Genetics, Translational Neuroscience, Pharmacology.
Disclosure: Nothing to disclose.

M24. Characterization of the Intrinsic Functional Connectivity of the Habenula in Children With ADHD

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Background: The habenula is a small brain region adjacent to the thalamus that directly influences striatal monoaminergic circuitry through its connections with the ventral tegmental area (VTA) and substantia nigra compacta. These striatal circuits have been implicated in reward processing and motor function, and show abnormalities in individuals with Attention-Deficit/Hyperactivity Disorder (ADHD; Swanson et al, 2007). This suggests that habenula function, as well as its functional connections, may be disrupted in ADHD. Initial support for this comes from animal work demonstrating that habenula lesions result in hyper-locomotion, impulsivity, and attention deficits (Lee & Goto, 2011). The present study takes an initial step towards evaluating the role of the habenula in pediatric ADHD by examining the intrinsic functional connectivity (iFC) of this region. While we predicted that children with ADHD would exhibit alterations in habenula iFC with the VTA and striatum, whole-brain analyses were conducted due to our limited knowledge of broader habenula iFC networks, particularly in children.
Methods: Children (ages 5- 9 years old) were recruited across two groups: children with a diagnosis of ADHD (ADHD; $n = 40$) and children without any current Axis I disorder (healthy comparisons, HC; $n = 38$). Diagnoses were determined by parent interview using the Schedule of Affective Disorders and Schizophrenia for Children, Present and Lifetime Version (KSADS-PL; Kaufman et al, 1997). Following this assessment, children attended an MRI scan session during which they completed a 6-minute resting state scan (e.g., lie still with eyes open) and a high-resolution anatomical scan for registration. All neuroimaging preprocessing and group level analyses were conducted using an alpha version (0.3.9) of the Configurable Pipeline for the Analysis of Connectomes (C-PAC; http://fcp-indi.github.io/). Seed-based intrinsic functional connectivity (iFC) was assessed using habenula regions of interest (ROIs) identified individually by inspection of normalized T1-weighted images. Two 2mm radius spherical ROIs were created that encompassed the left and right habenulae for all but 3 participants, who were excluded. A time series was obtained for each ROI and then correlations were calculated between these time series and every other voxel in the brain resulting in individual correlation maps which were then converted to Z-value iFC maps using Fisher's r-to-z transformation and transformed into MNI152 2mm standard space. This analysis produced subject-level maps of voxelwise correlations with the time-series of the left and right habenula ROIs. Group-level iFC analyses were conducted using a random-effects, ordinary least-squares model, including two group mean predictors (ADHD vs. HC) and three nuisance covariates (demeaned age, sex, framewise displacement); all were GRF corrected at $p < 0.05$, $Z > 2.3$.
Results: Groups did not differ in age, sex, or movement during the resting state scan. Direct comparison of ADHD vs. HC children elicited two significant findings. First, HC evidenced significant positive iFC between the left habenula and bilateral putamen that was absent for the ADHD group. Second, group differences were observed for iFC between the right habenula and the precuneus. Specifically, HC showed positive iFC while the ADHD group showed negative iFC between these two regions.
Conclusions: These findings point to possible disruptions in habenular functional networks in children with ADHD. Specifically, the absence of typical iFC between left habenula and the putamen may reflect ADHD-related dysfunction of striatal dopamine circuits. Further, children with ADHD exhibited altered iFC between the right habenula and the precuneus, suggesting disruption of broader habenula-based networks encompassing the default mode network. Of note, ADHD-related alterations in habenula iFC differed for the left and right habenulae, which is consistent with previous work suggesting differential functions of these homotopic regions.
Keywords: Habenula, ADHD, Resting State Functional Connectivity, Children.
Disclosure: Nothing to disclose.

M25. The Causes of Intellectual Disability in Autism

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Background: Autism spectrum disorders (ASD) frequently present with moderate to severe cognitive impairment. Despite substantial research, a fundamental question about the origin and links between ASD and low intelligence (IQ) still remains. ASD displays a complex pattern of inheritance. Previously, noninherited rare mutations were shown to

contribute to ASD and low IQ in individual families, but in the majority of cases causes remain unknown.
Methods: This study examined whether the lowered IQ in ASD is caused largely from an unlucky combination of the genetic and environmental risk factors distributed within their sibship or from a qualitatively different process.
Results: Non-ASD comparisons with low IQ (IQ < 80) had siblings with IQ scores that were intermediate between them and the population (IQ = 91.4, $p < 0.0001$). Strikingly different results were observed for siblings of individuals with ASD. This group had IQ scores indistinguishable from the population distribution, and even slightly higher mean score (IQ = 102.5).
Furthermore, while siblings of ASD probands were more likely have a diagnosis of ASD (OR = 11.53, 95% CI = 9.23-14.40), psychotic disorders (OR = 3.38, 95% CI = 2.84-4.02), and mood disorders (OR = 2.41, 95% CI = 1.93-3.02) compared to siblings of matched controls, the siblings with psychiatric disorders themselves were more likely to score in the top 10% of IQ distribution (OR = 1.55, 95% CI = 1.39-1.72).
Conclusions: The causes of intellectual impairment in ASD are not the same as the ones influencing IQ in their family members. It is likely that a pathogenic developmental insult of substantial effect size preventing normal cognitive development in ASD is either de-novo genomic or environmental.
Keywords: Autism, Genetics, de-Novo, Giftedness.
Disclosure: Nothing to disclose.

M26. Mistimed Developmental Trajectories of Cortical Plasticity Upon Inflammation

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Background: Childhood and adolescent critical periods of heightened neuroplasticity are essential for development of normal brain function and behavior. Disruption of these periods of developmental plasticity may alter the neurodevelopmental trajectory and may confer risk for psychiatric and neurodevelopmental disorders. Identification of disruptors of developmental plasticity windows is essential for prevention and therapeutic intervention.
Methods: We developed and applied an integrative bioinformatics approach to systematically match plasticity to 436 disease signatures, to yield a ranked list of diseases most likely to dysregulate plasticity signature genes. We applied a novel Disease Leverage Analysis across the ranked disease list to identify shared pathophysiology that may disrupt developmental plasticity. Informatics-driven hypothesis was then experimentally validated using the ocular dominance model of experience-dependent cortical plasticity.
Results: Through a novel computational assessment of transcriptional signatures of various diseases and multiple signatures of neuroplasticity, we found inflammation as a common pathological process central to a broad range of diseases predicted to dysregulate transcriptional signatures of plasticity. To experimentally test the hypothesis that inflammation would disrupt developmental plasticity in vivo, we used the ocular dominance model of experience-dependent cortical plasticity, and found that systemic lipopolysaccharide suppresses postnatal cortical plasticity by accompanying transcriptome changes in a specific set of molecular regulators of plasticity.
Conclusions: Together with our previous study demonstrated that late adolescent redox dysregulation can lead to an open ended critical period for cortical plasticity (Morishita et al,Biol Psychiatry 2015), our study suggests inflammation in children and adolescence may have unexpected differential negative consequences on the postnatal developmental trajectory than previously realized by disrupting neuroplasticity during critical windows of development. Mistimed developmental trajectories of brain plasticity may underlie, in part, the pathophysiology of schizoiphreania and other psychiatric disorders associated with inflammation.
Keywords: Cortical Plasticity, Inflammation, Transcriptomics, Bioinformatics, Adolescence.
Disclosure: Nothing to disclose.

M27. Allometry of the Developing Human Brain

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Background: How does the human brain's shape vary with its size? This basic question has received little study, despite being pivotal for understanding the developmental origins of adult brain organization and accurately detecting regional brain changes in neurological and psychiatric disorders. Allometry – the study of scaling relationships – provides an empirical framework for testing if and how brain shape varies with brain size. In phylogenetics, allometry can quantitatively reconstruct how inter-species differences in neuroanatomy arose from patterned investments (hyperallometry – disproportionate expansion) and divestments (hypoallometry – lack of proportional expansion) in the size of specific structures relative to the whole brain. In contrast, few studies have quantified neuroanatomical scaling in humans, and no studies have addressed allometry in development. Here, we use two independent large-scale structural neuroimaging datasets to characterize the spatial patterning, temporal stability, and reproducibility of human brain allometry. We also test if brain allometry (i) aligns with spatial patterns of cortical gene expression, and (ii) proves useful in the clinical-science setting of quantifying brain shape variation amongst patients with varying degrees of sex chromosome aneuploidy (SCA).
Methods: We utilized two independent structural magnetic resonance imaging (sMRI) brain scan samples spanning childhood through to early adulthood: (i) a "discovery dataset" [NIH Intramural Brain Development Study (NIBDS)] comprising 1552 longitudinally acquired 1.5T sMRI brain scans in 794 typically developing individuals

(403 male), and (ii) a "replication dataset" [Philadelphia Neurodevelopmental Cohort (PNC)] comprising 1586 cross-sectionally acquired 3T sMRI scans (755 male). Both datasets were processed with established automated pipelines (cortex: CIVET, subcortex:MAGeT) for measuring global (total bilateral) and local (vertex-wise) estimates of surface area (SA) across the cortical sheet, amygdala, hippocampus, pallidum, striatum, and thalamus. For each structure, generalized additive models (multi-level for NIBDS, and single-level for PNC) were used to model vertex-wise SA as function of total SA, age, and sex. By log10-transformation of SA measures, the estimated coefficients linking differences in global size to local area provided a continuous scaling metric to distinguish regions that become disproportionately large (hyperallometry, values >1), small (hypoallometry, values <1), or unchanged in their relative size (isometry, values ~ 1) with increasing brain size. Permutation methods quantified the spatial similarly between cortical allometry maps in NINBS and PNC data. We integrated these allometric maps with Allen Brain Atlas measures of gene expression to test for gene sets showing differential expression between hyper- and hypoallometric regions of the cortical sheet. Finally, we tested the practical utility of allometric norms by applying them to quantify the brain-size independent shifts of brain shape in humans with diverse SCAs (XXX, XXY, XYY, XXYY, XXXXY).

Results: Analysis of the "discovery" NIBDS dataset revealed significant changes in both cortical and subcortical shape with normative variations of overall structure size. These allometric maps were bilaterally symmetric, developmentally fixed across the age-range examined, and fully reproducible in the "replication" PNC dataset. In the cortex, primary sensory and motor, ventral occipito-temporal and insular regions are significantly hypoallometric, whereas the temporo-parietal junction, precuneus, and medial/dorsolateral prefrontal cortices are significantly hyperallometric. Hyperallometric cortices show an up-regulation of genes encoding proteins that (i) localize to mitochondria and synapses, and (ii) influence respiratory electron transport chain and potassium channel functioning. The spatial patterning of subcortical allometry recapitulates that of the cortex according to the known topography of cortico-subcortical connectivity. Finally, an allometrically-informed analysis of brain shape in SCA shows that mounting chromosome count in humans leads to an increasingly severe remodeling of brain shape that is independent of brain size.

Conclusions: Our study reveals a coordinated reorganization of human brain shape with normative variations in brain size. The rules regulating this process appear to be established before mid-childhood, and remain unaltered into early adulthood. These allometric maps echo the brain's functional and transcriptomic topography. Specifically, increasing brain size leads to a disproportionate expansion of, default mode and multimodal association, cortices, but a lack of proportionate expansion in the insula, ventral stream, and sensori-motor cortices. The biological investment signaled by preferential growth of hyperallometric regions is likely mirrored by an energetic investment given their high expression of genes involved in oxidative phosphorylation. Finally, by using allometric norms to reveal dose-response effects of gene-dosage on brain shape in SCA, we demonstrate the practical utility of allometry for clinical science. Collectively, our findings detail the power of allometric analysis for revealing the contours of neuroanatomical variation in health, and their targeted in disruption disease.

Keywords: Allometry, Brain, Gene Expression.
Disclosure: Nothing to disclose.

M28. Common and Dissociable Regional Cerebral Blood Flow Differences Associated With Dimensions of Psychopathology Across Categorical Diagnoses

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Background: The high comorbidity among neuropsychiatric disorders suggests the possibility of common neurobiological phenotypes. Regional cerebral blood flow (rCBF) is one brain phenotype that primarily reflects trait-like regional brain function and may be a useful biological marker of psychopathology. Abnormalities in rCBF are present in many neuropsychiatric disorders and emerge relatively early in development. Most studies on rCBF differences focus on comparing individuals with diagnoses to healthy controls. Few studies examine associations between rCBF and dimensional measures of psychopathology, and fewer still do so in youth. Such work could provide important information about developmentally-informative biomarkers of dimensional psychopathology.

Methods: To investigate abnormalities in rCBF common across psychiatric disorders, we utilized a sample of 1274 youths who completed cross-sectional imaging as part of the Philadelphia Neurodevelopmental Cohort. We quantified perfusion on a voxelwise basis using arterial spin labeled MRI at 3T. A generalized additive model with penalized splines was used to study the relationship between rCBF and psychopathology. A dimensional measure of psychopathology was constructed using a bifactor model of item-level data from a psychiatric screening interview. This bifactor model yielded orthogonal dimensions of psychopathology that included both shared, overall psychopathology as well as specific factors corresponding to anxious-misery, psychosis, behavioral (externalizing), and fear.

Results: Overall psychopathology was associated with elevated perfusion in the dorsal anterior cingulate cortex (ACC). Furthermore, several clusters of elevated perfusion were associated with specific dimensions of psychopathology. Anxious-misery was related to elevated perfusion in the frontal pole, while psychosis symptoms were associated with elevated perfusion in superior parietal cortex. Elevated perfusion in the precuneus was associated with behavioral symptoms, whereas fear symptoms were associated with higher perfusion in a network of frontal and parietal regions.

Conclusions: The results of this study demonstrate common and dissociable rCBF abnormalities across neuropsychiatric disorders in youth. In particular, elevated perfusion was a common feature of overall psychopathology. Given that the dorsal ACC is central to numerous processes including executive function, reward decision making, and affect regulation, results suggest a common circuit relevant for psychopathology across categorical clinical diagnoses.
Keywords: Developmental Psychopathology, Cerebral Blood Flow, Anterior Cingulate Cortex.
Disclosure: Nothing to disclose.

M29. Children With ADHD and Symptoms of Opposite Defiant Disorder Improved in Behavior When Treated With Methylphenidate and Adjuvant Risperidone, Though Weight Gain was Also Observed

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Background: Children with ADHD often also show symptoms of oppositional defiant disorders (ODD). We investigated the impact of adjuvant risperidone (RISP) to a standard treatment with methylphenidate (MPH) in children with ADHD and symptoms of ODD.
Methods: Inclusion criteria were as follows: 1. Diagnosis of ADHD (DSM-IV: 314) and ODD (DSM-IV 313.81); 2. Within the first 4 weeks: initial treatment of 1mg MPH/kg/d with no/very small symptoms improvements; 3. initial family counseling and psychoeducation with no improvements in behavior. Of the 172 children approached, 84 children met the inclusion criteria mean age: M = 8.55; 73.8% males) and took part in a double-blind, randomized, placebo-controlled, clinical trial lasting eight weeks. Participants were randomly assigned either to the MPH+RISP (1.1mg/kg/d + 1mg/d) or to the MPH+PLCO (placebo) (1.1mg/kg/d) condition. Symptoms of ADHD and ODD, weight, height, and blood pressure were assessed at baseline, and at weeks 2, 4, 6 and 8.
Results: Symptoms of ADHD and ODD decreased over time, though the significant Time by Group interaction showed that symptoms improved more in the MPH+RISP than in the MPH only condition. In the MPH+RISP condition weight and waist circumference also increased over time.
Conclusions: Data suggest that adjuvant RISP improved symptoms in children with ADHD and co-occurring ODD, but weight gain and higher prolactine levels were also observed. This may become an issue, once children become adolescents, a period of life in which body shape and body self-image are closely linked to self-confidence and peer acceptance.
The ethical committee of the Hamadan University of Medical Sciences (Hamadan, Iran) has approved the study, which was conducted in accordance with the ethical standards laid down in the Declaration of Helsinki. All participants and their parents signed the written informed consent.
Keywords: Attention Deficit Hyperactivity Disorder, Opposite Defiant Disorder, Methylphenidate, Risperidone, Weight Gain.
Disclosure: Nothing to disclose.

M30. From Behavior to Biomarkers in Dup15q Syndrome: Moving Towards Targeted Treatments for a Genetically Defined Neurodevelopmental Disorder

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Background: The surge in clinical genetic testing for children with neurodevelopmental disorders, particularly Autism Spectrum Disorder (ASD), has facilitated the identification of causative rare genetic variants and, with the ascertainment of subgroups with shared variants, the identification of clinically meaningful genetic syndromes (Jeste and Geschwind, 2014). Insights into syndrome specific developmental profiles and genetically informed biomarkers has lagged behind genetic testing, leaving considerable uncertainty regarding prognosis and recommended treatments after a diagnosis is made. Duplication of 15q11.2-q13.3, or Dup15q syndrome, represents one of the most common copy number variants associated with ASD (Hogart, 2010). Several genes critical for brain development are located in this 15q region, including UBE3A and a cluster of GABAA receptor genes. Moreover, case reports have described an electrophysiological pattern of excessive beta band oscillations in clinical EEGs of these children that may reflect overexpression of these GABAA receptor genes. Over the last two years, the behavioral and electrophysiological features of Dup15q syndrome, particularly as they may inform future clinical trials, are being elucidated through collaborative efforts facilitated by the national Dup15q alliance and the UCLA Intellectual and Developmental Disabilities Research Center. The first objective of this multidisciplinary study has been to identify patterns of social communication, adaptive, and cognitive skills in children with Dup15q syndrome compared to those with nonsydromic ASD (DiStefano et al, 2016). The second objective has been to quantify spontaneous beta oscillations in children with Dup15q syndrome and to examine the relationship between this EEG biomarker and clinical characteristics (Frohlich et al, 2016).
Methods: We recruited 13 children from the UCLA Dup15q clinic and compared them to 13 IQ and age matched cohort of children with ASD. We then collected data from an additional 25 children with Dup15q syndrome at the national Dup15 Alliance Family meeting in Orlando, Florida. Participants were assessed for verbal and non-verbal cognition, ASD characteristics [Autism Diagnostic Observation Schedule (ADOS)], adaptive function [Vineland Adaptive Behavior Scales (VABS)], and psychiatric comorbidities [Child Behavior Checklist (CBCL)]. High density EEG (128 channel, EGI inc) was recorded while children watched an abstract video, with an additional typically developing comparison group undergoing EEG testing. Relative power in delta (1 – 4 Hz), theta (4 – 8 Hz), alpha (8 – 12 Hz), low beta (12 – 20 Hz), high beta (20 – 30 Hz), and gamma (30 – 48 Hz) was calculated. For clinical measures, group comparisons were performed between diagnostic groups: Dup15q and ASD, and within the Dup15q group based on duplication type and epilepsy status. For EEG measures, group comparisons were performed between Dup15q, ASD

and TD. Within the Dup15q group, predictors of beta power were investiated, including age, duplication type, cognitive fucntion and epilepsy.
Results: All children with Dup15q syndrome met criteria for ASD, but severity scores were lower in children with Dup15q syndrome with strengths in discrete social behaviors that required less motor skills. Children with Dup15q syndrome demonstrated significantly more impairment in motor function (Gross motor DQ: Dup15q M = 35.38, ASD M = 70.21; t = 5.9, $p < .001$; Fine motor DQ: Dup15q M = 30.03, ASD M = 66.5; t = 5.2, $p < .001$) and daily living skills (Dup15q M = 53.18, ASD M = 63.82; t = 2.41, $p = .03$). Within the Dup15q group, children with epilpesy demonstrated significantly lower cognitive and adaptive function than those without epilepsy ($p < 0.01$ for all measures). Relative beta power was significantly higher in Dup15q syndrome than in the TD ($p <$ 1.0 10-4, FDR corrected, Cohen's d = 1.7) and ASD ($p <$ 1.0 10-4, FDR corrected, Cohen's d = 1.3) groups. Beta power was highest in children without epilepsy.
Conclusions: We have identified behavioral and neurophysiological features that distinguish a genetically defined subgroup within the autism spectrum. The relative strength observed in social interest and responsiveness in the context of impaired motor skills represents an important avenue for intervention, including aggressive treatment of epilepsy, early and consistent focus on motor skills, and intervention targeting joint attention and language within a play context, in order to build on social interest to further develop social communication abilities. As genetic testing in ASD and related neurodevelopmental disorders becomes clinical gold standard, an increasing number of children are diagnosed with genetic variants that not only will elucidate causal mechanisms but also therapeutic targets. The identification of these targets necessitates quantifiable biomarkers that relate directly to genetic mechanisms. Studies in Dup15q syndrome provide a promising path towards this mission, as the elucidation and quantification of an electrophysiological biomarker could improve diagnosis and prognostication, as well as measurement of target engagement and outcomes in clinical trials, a model that can inform similar investigations in the quickly expanding number of high-risk genetic syndromes associated with neurodevelopmental disorders.
Keywords: Autism Spectrum Disorders, Biomarkers, Genetics, Electrophysiology, Epilepsy.
Disclosure: Nothing to disclose.

M31. Change in Adiponectin Predicts Weight Gain During Initial Antipsychotic Exposure in Youth

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Background: Adiponectin is an adipocyte-derived hormone that actively regulates energy homeostasis in humans, with plasma levels inversely correlated with body mass index (BMI). Low circulating levels of adiponectin may precede insulin resistance leading to dysglycemia and diabetes. 1 2 Decreases in adiponectin have been reported during olanzapine treatment in adults, 3 4 with reports of less weight gain with higher baseline adiponectin in antipsychotic-treated youth. 5 However, prior studies have not used direct measures of adiposity, confirmed use of high molecular weight (HMW) adiponectin, or evaluated patients with no prior antipsychotic exposure. Here, we report the association between whole body adiposity and plasma adiponectin in youth ages during an initial course of antipsychotic treatment.
Methods: An antipsychotic-naïve cohort of youth ages 6-18 were randomized to 12 weeks of treatment with aripiprazole, olanzapine or risperidone (MH72912). Youth were metabolically well-characterized via hyperinsulinemic euglycemic glucose clamps using stable isotopomer methodology to measure tissue-specific insulin sensitivity, Dual Energy X-ray Absorptiometry (DEXA) to evaluate body composition, and numerous plasma measures, including High Molecular Weight (HMW) Adiponectin. ANOVA and ANCOVA were used to evaluate the main effect of time on DEXA total % fat, with separate assessments of the interactions of time and both baseline adiponectin and change in adiponectin, controlling for race and gender.
Results: In this subset of 106 study completers, the mean age was 11.3 years (SD = 2.6), with 71.7% male ($n = 76$) and 46.2% African American ($n = 49$). During 12 weeks of treatment, adiponectin decreased by 2115 ng/mL (SD = 3472) ($F[1,105] = 39.35$, $p < 0.0001$), and DEXA total % fat increased by 2.6% (SD = 3.2) ($F[1,105] = 69.34$, $p < 0.0001$). A significant interaction between time and baseline adiponectin predicted DEXA % fat outcome ($F[1,104] = 6.16$, $p = 0.02$), explained by differences in fat increases across different baseline adiponectin values. A significant interaction between time and change in adiponectin also predicted DEXA % fat outcome ($F[1,104] = 7.80$, $p = 0.006$), explained by differences in fat increase in relation to different levels of change in adiponectin during treatment. Neither race nor gender significantly influenced results of the analyses.
Conclusions: Adiponectin is a known early indicator of diabetes risk, and a potential predictive biomarker for metabolic dysregulation during antipsychotic treatment. These results indicate that adiponectin levels prior to treatment, in addition to adiponectin changes during treatment, may modulate changes in adiposity during initial antipsychotic exposure. Future studies should further assess the role of adiponectin as a mediator or moderator of change in adiposity during antipsychotic treatment.
Keywords: Antipsychotic Induced Weight Gain, Children and Adolescents, Adiponectin.
Disclosure: Otsuka America Pharmaceuticals, Inc.: Grant Support, Self; Amgen, Inc.: DSMB membership, Self; Reviva Pharmaceuticals: Consultant, Self; Sunovion: Consultant, Self; NIMH: Grant Support, Self; Sidney R. Baer, Jr. Foundation: Grant Support, Self; Center for Brain Research in Mood Disorders (CBRIMD): Grant Support, Self.

M32. Evidence for a Relationship Between TMS Measures of Cortical Excitability and Inhibition and Glutamate in Depressed Adolescents

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Background: Converging evidence from transcranial magnetic stimulation (TMS) studies and proton magnetic resonance spectroscopy (1H-MRS) research points to the role of glutamate in the pathophysiology of major depressive disorder (MDD). However, few have used TMS and 1H-MRS methods in conjunction to examine the relationship between neurochemistry and neurophysiology, and to our knowledge no prior studies have investigated TMS-MRS relationships in psychiatric populations or in a pediatric sample. This study aimed to examine relationships between TMS measures of cortical excitability and inhibition and concentrations of glutamatergic metabolites as measured by 1H-MRS in a sample of depressed adolescents.
Methods: Twenty-four youth (aged 11-18 years) with MDD underwent TMS testing, which included measures of the resting motor threshold (RMT), cortical silent period (CSP), short-interval intracortical inhibition (SICI) at 2 and 4 ms interstimulus intervals (ISIs), and intracortical facilitation (ICF) at 10, 15, and 20 ms ISIs. Fourteen participants from the same sample also completed 1H-MRS in a 3T MRI scanner after TMS testing. Neural metabolite concentrations, including those of glutamate (Glu) and glutamate + glutamine (Glx), were obtained in medial anterior cingulate cortex (ACC) and left primary motor cortex voxels during a TE-PRESS sequence. Metabolite concentrations were corrected to cerebrospinal fluid (CSF) after tissue segmentation. Spearman rank-order correlations were calculated to assess relationships between TMS measures and 1H-MRS-measured glutamatergic metabolites. Additionally, multivariable linear regressions were performed, with age, sex, and depression severity as covariates.
Results: In the medial ACC, ICF amplitude at the 10-ms ISI had significant positive correlations with both [Glu] ($\rho = 0.6364$, $p = 0.0261$) and [Glx] ($\rho = 0.6364$, $p = 0.0261$). Significant regression models were found for ICF amplitude at the 20-ms ISI with [Glu] ($F(2,11) = 10.0202$, $p = 0.0051$, adjusted $R2 = 0.6212$) and [Glx] ($F(2,11) = 8.4227$, $p = 0.0087$, adjusted $R2 = 0.5744$) as independent variables. In the left primary motor cortex, RMT and [Glx] had a significant positive correlation ($\rho = 0.63$, $p = 0.0158$), while SICI amplitude at the 4-ms ISI and [Glu] also had a significant positive correlation ($\rho = 0.5385$, $p = 0.0470$). Significant regression models for both RMT ([Glu]: $F(3,13) = 17.7345$, $p = 0.0003$, adjusted $R2 = 0.7943$; [Glx]: $F(3,13) = 34.1264$, $p < 0.0001$, adjusted $R2 = 0.8843$) and SICI amplitude at the 4-ms ISI ([Glu]: $F(4,13) = 8.1078$, $p = 0.0047$, adjusted $R2 = 0.6862$; [Glx]: $F(4,13) = 6.7389$, $p = 0.0086$, adjusted $R2 = 0.6384$) were found.
Conclusions: These data support the role of glutamate in cortical excitatory and inhibitory processes that are measured by TMS. Further research aimed at examining the relationship between glutamatergic metabolite concentrations measured through MRS and the excitatory and inhibitory physiology measured through TMS is warranted. Combined MRS-TMS methods show promise for future investigations of the pathophysiology of MDD in adults as well as in children and adolescents.
Keywords: Transcranial Magnetic Stimulation, Proton Magnetic Resonance Spectroscopy, Glutamate, Major Depressive Disorder (MDD), Children and Adolescents.
Disclosure: Pfizer, Inc.: Research Grant (Investigator-Initiated), Self; Mayo Clinic Foundation: Research Grant (Departmental Small Grant Program), Self.

M33. Ethanol Exposures During the Developmental Period Increase Microglia Sensitivity to a Stress Challenge During Adulthood: A Possible Cause for the Stress Hyperresponse in Fetal Alcohol Exposed Offspring

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Background: In the healthy central nervous system (CNS), microglia, the immune cells of the CNS, quickly detect a stimulus, become activated and elicit the proper response. Once the stimulus is removed and homeostasis is restored, microglia revert to their inactive ramified state and returns to their surveillance duties. Recently it has been shown that a single exposure to Escherichia coli (E. Coli) activates microglia in rat neonates, and causes long-term alterations in microglia response and behavior in adulthood. No previous investigation on the long-term effects of ethanol induced microglia activation during the neonatal period in rats, a period equivalent to the third trimester of human pregnancy, has been reported. In this study, we investigate the effects of neonatal ethanol on microglia and their influence in regulation of the hypothalamic-pituitary-adrenal (HPA) axis response to an immune stress challenge during adulthood.
Methods: Neonatal rat pups were fed by oral gavage a milk formula containing 11.34% ethanol (vol/vol), yielding a total daily ethanol dose of 2.5 g/kg (AF), or isocaloric control (PF), or they were left in the litter with the mother (AD) for 5 days (postnatal days 2-6). Some of the AF rats additionally received a minocycline pre-treatment one hour prior to the first feeding on each day by subcutaneous injection of minocycline solution (45μg/kg bodyweight). Two hours after the last feeding, some of the pups were sacrificed and hypothalamus was dissected for microglia separation by differential gradient centrifugation using OptiPrep gradient and characterization by measuring production of various activation markers and cytokines. The rest of the pups were maintained in the controlled condition until 90 days of age when they were used for immune stress challenge (Lipopolysaccharide, LPS; 100ug/kg bodyweight i.p.) and microglia characterization and HPA axis responses by measuring plasma levels of corticosterone and adrenocorticotrophin (ACTH). Animal surgery and care were performed in accordance with institutional guidelines and complied with the National Institutes of Health policy.

Results: We found binge-like ethanol exposures during the postnatal period increased microglial activation markers (e.g., IBA1, CX3CR1), inflammatory cytokine (e.g., TNF-α), an inflammatory signal receptor (TLR-4), and inflammatory cell signaling molecules (IKBA) in microglia isolated from the hypothalamus. Comparable results were also obtained following a known activator of microglia, LPS. In association with the microglial activation, we found increased proopiomelanocortin (POMC) neuronal (a neuronal population known to regulate the HPA axis function) apoptosis in the arcuate nucleus of the hypothalamus. Treatment with minocycline, an inhibitor of microglia activation, blocked ethanol effect on POMC neuronal apoptosis. Neonatal ethanol also promotes the adult response of the microglia to an immune challenge. In addition, neonatal ethanol increased the response of the HPA axis, plasma corticosterone and ACTH, following LPS challenge, while neonatal minocycline prevented ethanol action on the stress hormone responses. Neonatal ethanol altered the expression of various proteins related to Dnmts, HDACs and MeCp2 and increased histone acetylation and decreased DNA methylation in microglia.
Conclusions: These data suggest that neonatal exposure to a high dose of ethanol activates microglia by producing various inflammatory cytokines leading to killing of POMC neurons and a deficiency in feedback regulatory control over the stress axis function. Additionally, neonatal ethanol exposures increase microglia sensitivity to the subsequent stimuli by possibly altering epigenetic mechanisms. In conclusion, this work provides important insights into the effects of both immediate and long-term effects of ethanol induced microglia activation during third trimester gestation. In our model, ethanol induced microglia activation leads to long-term alterations in microglia responses to subsequent stimuli that contribute to changes in HPA response in adulthood.
Keywords: Prenatal Ethanol, Stress Abnormalities, Proopiomelanocortin Neurons, Microglia Priming.
Disclosure: Supported by NIH grant R37AA08757.

M34. Reward-Related Neural Activity and Structure Predict Future Substance Use in Dysregulated Youth

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Background: Substance use is a common risk-taking behavior in youth. Predicting those youth who may engage in future substance use could facilitate early identification of substance use disorder vulnerability. The propensity for risky behaviors such as substance use in youth may be related to a delay in the development of reward circuitry, specifically, prefrontal cortical regions implicated in cognitive control, alongside the emergence of increased dopaminergic activity in subcortical regions, during puberty (Steinberg et al, 2008). Reward circuitry comprises a widespread neural network, including ventral striatum, amygdala, and insula, and specific prefrontal cortical regions: ventrolateral prefrontal cortex (vlPFC;BA47), dorsal anterior cingulate (dACC; BA24/32), and medial prefrontal cortex (mPFC;BA10). Identifying in youth reward function and structure predictors alongside clinical and demographic predictors, would not only provide markers to identify risk of substance use disorders, but would also provide targets to ultimately guide early intervention and treatment choice. We aimed to identify biomarkers that predicted future substance use in psychiatrically unwell youth.
Methods: We examined neuroimaging measure of reward circuitry activity in 73 behaviorally and emotionally dysregulated youth mean aged 13.9 (standard deviation = 2.0) 30 of whom were female. These youth were from three clinical sites (University of Pittsburgh Medical Center, University Hospitals Case Medical Center, and Cincinnati Children's Hospital Medical Center) in the Longitudinal Assessment of Manic Symptoms (LAMS) study. We used the GLMNET package and Least Absolute Shrinkage and Selection Operator (LASSO) regression for variable selection along with k-fold cross validation, to predict substance use 24.3 months after neuroimaging assessment. Predictor variables included neural activity during a reward task, whole brain cortical thickness, clinical and diagnostic measures, and demographic variables. SPM8 and Freesurfer were used to analyze the neural data. We then quantified the predictive relationships of the selected variables using hierarchical logistic regression and post hoc sensitivity analyses.
Results: Future substance use was associated with higher left medial prefrontal cortex activity to win versus control and lower left insula activity to loss versus control during a card guessing reward task. Additionally, thicker left caudal anterior cingulate cortex, higher depression and lower mania scores, as well as not using antipsychotic medication, more parental stress, and older age were associated with future substance use. This combination of variables explained 60.4% of the variance in future substance use and accurately classified 83.6% of participants as either using or not using alcohol or drugs.
Conclusions: Substance use was predicted by a combination of reward-related neural activity, cortical thickness, age, mood symptoms, parent stress, and medication. These variables explained a large portion of the variance and were useful identifiers of future substance use. In addition, this analysis highlighted the value of combining multiple domains to provide a comprehensive understanding of substance use development in youth. Limitations include absence of measures of pubertal status, sibling and peer substance use, and parental monitoring. This may be an important step toward identifying neurobiological measures characterizing youth at risk of substance use, and provides promising neural targets for future therapeutic interventions.
Keywords: Prediction, Functional MRI (fMRI), Youth, Adolescent Alcohol.
Disclosure: Curemark, Forest, Lilly, Neuropharm, Novartis, Noven, Shire, Supernus, YoungLiving (as well as NIH and Autism Speaks): Funding, Self; Arbor, Gowlings, Ironshore,

Neuropharm, Novartis, Noven, Organon, Otsuka, Pfizer, Roche, Seaside Therapeutics, Sigma Tau, Shire, Tris Pharma, Waypoint: Consulting and Advisory Boards, Self; Noven: Travel Support, Self.

M35. Oxytocin vs. Placebo for the Treatment of Hyperphagia in Children and Adolescents With Prader-Willi Syndrome: Preliminary Results

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Background: Oxytocin (OXT) has been implicated in the pathophysiology of Prader-Willi Syndrome (PWS). Studies have shown that the number and size of OXT neurons is significantly reduced in individuals with PWS. Further, animal studies have demonstrated that a reduction in OXT neurons leads to overeating, which reverses after OXT administration. OXT administration in individuals with ASD impacts issues relevant for children with PWS such as repetitive behaviors, rigidity, and social communication skills. The few clinical trials of OXT in PWS have yielded some promising results, including improvements in eye gaze, trust in others and reduction in anxiety, although higher doses of OXT (40iu) yielded an increase in tantrums, perhaps via cross-binding to V1aR. To date, there have been no double-blind placebo-controlled trials in children with PWS that examined the effects of OXT over the course of more than 5 days. PWS is a homogeneous orphan population in which oxytocin therapeutics is matched to the underlying mechanism of the disorder to obtain greater drug-placebo separation.
Methods: We conducted an 8-week double-blind placebo-controlled treatment study of intranasal OXT (IN-OXT) in 24 children with PWS. Patients received a low OXT dose of 16 IU per day (2 puffs per nostril/4 IU each). The primary outcome measure was hyperphagia measured by the Revised-Dykens Hyperphagia Questionnaire. Secondary and exploratory measures assessed repetitive behaviors (RBS-R and Y-BOCS), social communication (ABC-SW and SRS), disruptive behaviors (ABC-I), quality of life (Caregiver Strain Scale), hypotonia, oxytocin levels (oxytocin receptor genotype and salivary oxytocin), gut hormone measurements, dietary diary and BMI.
Results: Demographics of the sample are described, including age, gender, and subtype (i.e., PWS by deletion, UPD and imprinting). Interim-analysis for completers are reported. The Revised-Dykens Hyperphagia Questionnaire demonstrated good separation over time (over 8 weeks; starting at week 2) in responders vs non-responders for hyperphagia ratings. There is also good separation over time for compulsivity measures (YBOCS, RBS-R) and quality of life (Caregiver Strain) measures. Of interest, improvement in Caregiver Strain measures correlated with improvement in measures of hyperphagia, compulsivity, rigidity, and irritability, but not social communication measures. There were no significant adverse events reported, and treatments were well tolerated.
Conclusions: Interim analyses demonstrate good sensitivity to change on measures of hyperphagia, compulsivity, rigidity, and caregiver strain, but not social communication measures. Improvement in hyperphagia, compulsivity, rigidity, and irritability are associated with improvement in the QOL Cargiver Strain Scale over 8-weeks of intranasal oxytocin administration versus placebo in children 5-18 years old with PWS. Larger scale phase 2b studies are required to replicate these interim pilot data.
Keywords: Prader Willi Syndrome, Oxytocin, Human Clinical Trial.
Disclosure: Nothing to disclose.

M36. CSF Amyloid and APOE ϵ4 Related Decline in Episodic Memory Over 12 Months Measured Using the CANTAB in Individuals With Amnestic MCI: Results From the European-ADNI Study

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Background: Accumulation of beta amyloid (Ab) in the brain and carriage of the apolipoprotein E (APOE) e4 allele have both been reported to independently and interactively linked with greater neurodegeneration, cognitive decline and incipient Alzheimer's disease (AD). However, as most studies have observed these effects over 18-months or more, the effect of Ab and e4 on cognitive decline over 12-months remains unknown. Detection and monitoring of subtle cognitive decline over shorter time periods is critical for diagnosis of patients at risk for dementia and monitoring the therapeutic effects of symptomatic and disease modifying treatments. In this study we examined the effects of Ab and e4 on rates of cognitive change assessed using the CANTAB over 12-months in aMCI patients. We hypothesized that Ab+ individuals would show a greater decline in episodic memory and this would be increased in APOE e4 carriers.
Methods: 145 participants with aMCI were recruited from the European-ADNI study. They were aged between 55-90, had a $\geq$1SD deficit in episodic memory and fulfilled a clinical diagnosis of aMCI. Participants underwent cognitive assessment using the CANTAB at baseline, 6 and 12 months and lumbar punctures for CSF measurement of Abeta42. Individuals were divided into Ab+ (CSF-POS) ($<$550pg/ml) ($n=55$) and Ab- (CSF-NEG)($>$550pg/ml)($n=90$).
Results: Ab+ aMCI individuals (relative to Ab-) showed faster decline in paired associates learning (PAL) ($p=0.02$, ES = 0.4) over 12 months. Ab+ aMCI individuals who were APOE e4 carriers (compared to non- carriers) had a greater decline in PAL ($p<0.01$, ES = 0.6). No other cognitive domain including attention and executive function were affected by Ab positivity and/or APOE e4 carriage.
Conclusions: Ab+ individuals with aMCI declined at a faster rate over 12-months on the CANTAB-PAL task of episodic memory (i.e. learning of an association between object and location). The decline in memory in Ab+ individuals with aMCI was exacerbated by the presence of the APOE e4 allele. Other cognitive domains including psychomotor speed, sustained attention, working memory and executive function were not affected Ab positivity and/or APOE e4 carriage. These findings suggest a memory decline in Ab+ individuals

with aMCI can be detected as early as 12 months using sensitive computerised tests of episodic memory.
Keywords: Episodic Memory, Alzheimer's Disease, Beta-amyloid Peptide 1-42, APOE, Mild Cognitive Impairment due to AD.
Disclosure: Nothing to disclose.

M37. The Alzheimer's Prevention Initiative Generation Study: A Preclinical Trial in APOE4 Homozygotes

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Background: The Alzheimer's Prevention Initiative (API) was established to evaluate preclinical Alzheimer's disease (AD) treatments in cognitively unimpaired people who, based on age and genetic background, are at imminent risk for developing symptoms of AD. The API APOE4 Trial, also known as the Generation Study, is evaluating the effects of two amyloid targeted therapies (CAD106 and CNP520) in cognitively normal people who, on the basis of age and being apolipoprotein (APOE) E4 allele homozygotes, are at particularly elevated risk of developing symptoms of AD. CAD106 is an active immunotherapy against Aβ; CNP520 is a Beta-site-APP cleaving enzyme-1 inhibitor. We hypothesize that Aβ-lowering therapies might be most effective in the preclinical stages of AD, prior to development of extensive pathology. Identification of APOE4 homozygotes is employed as a prognostic enrichment strategy to select individuals likely to show cognitive decline in the near future. APOE4 homozygotes are at elevated risk of developing symptoms of late–onset AD: by age 85, the risk of symptomatic AD reaches 51% for male homozygotes and 60-68% for female homozygotes (Genin et al, 2011). About 70-80% of APOE4 homozygotes age 60-75 will have extensive fibrillar Aβ deposition (Jansen et al, 2015).
Methods: Under the auspices of the Alzheimer's Prevention Registry, we developed a novel, trial-independent APOE recruitment registry known as GeneMatch to support enrollment into this and other studies. The Generation Study employs two primary outcomes: time to diagnosis of MCI due to AD and decline on the API preclinical composite cognitive (APCC) test. The APCC was developed as a sensitive tool to detect and track cognitive decline in individuals at risk for progression to the clinical stages of AD. The effects of therapy on various biomarkers will be assessed as will the extent to which treatment biomarker effects could predict clinical benefit. Importantly, the impact of disclosing APOE4 genotype and associated risk information to older adults will be assessed.
Results: The global trial, funded by NIH, philanthropy and Novartis/Amgen, has just launched. GeneMatch serves as the primary recruitment mechanism in the US; over 4,000 volunteers have been genotyped thus far, of whom nearly 5% are APOE4 homozygotes. APOE4 homozygotes and a random sample of non-homozygotes who consent and appear to meet basic trial eligibility criteria for the Generation study are invited to trial sites for additional screening and disclosure of APOE genotype and associated risk of developing symptoms due to AD.
Conclusions: Trial progress to date will be summarized. We anticipate that the GeneMatch program, which will also be summarized, will identify a large pool of prospective participants for the Generation Study and future trials.
Keywords: Alzheimer's Disease, Genetic Testing, Aging and Dementia.
Disclosure: Novartis, Amgen: Research Support, Self.

M38. Can Neuroimaging Measures of Hippocampal Structure Predict Alzheimer's Vs. FTLD Neuropathologies?

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Background: Despite advances in the development of biomarkers for early detection of Alzheimer's and frontotemporal dementias, accurate ante-mortem diagnosis remains a challenge. Few studies using structural MRI to classify dementia have the ability to confirm with neuropathologic indices. We performed a neuroimaging study to directly correlate ante-mortem hippocampal shape with postmortem Alzheimer's and TDP-43 neuropathology burden.
Methods: Ante-mortem T1-weighted MRI were collected from 42 subjects from the Rush Alzheimer's Disease Center's Memory and Aging Project and Religious Orders Study. Postmortem neuropathologic burden was quantified for PHFtau tangles, β-amyloid, and TDP-43 by immunohistochemistry. The mean age at imaging was 87.6 years with interval between imaging and death of 2.7 years. In the ante-mortem MRI, we generated hippocampal surfaces using multi-atlas FS-LDDMM pipeline. We then computed a population average surface and vertex-wise deformation for each subject from this average. At each vertex, we applied generalized linear model using surface deformation to predict neuropathology, accounting first for ante-mortem covariates (i.e., age at imaging, gender), and then for post-mortem covariates (i.e., age at death, indices of arteriolosclerosis, cerebral atherosclerosis, cerebral infarctions, hippocampal sclerosis, DLB). We mapped the standardized predictor coefficients onto the surface to visualize distribution patterns.
Results: When covarying for ante-mortem covariates, significant relationships existed between higher global PHFtau tangle burden and inward surface shape deformation in CA1 and subiculum, between higher β-amyloid burden and inward subiculum deformation, and between more severe TDP-43 neuropathology and inward CA1 deformation, with differing patterns along the hippocampal surface. These relationships persisted when covarying for post-mortem covariates.
Conclusions: Our findings of PHFtau tangles, β-amyloid and TDP-43 neuropathology burdens relating to specific regional hippocampal shape with distinct patterns along the surface provide support that patterns of hippocampal atrophy may

be biomarkers for specific AD and FTLD neuropathologies. These results need to be further validated in larger samples.
Keywords: Hippocampal Subfields, Postmortem Human Brain, Antemortem.
Disclosure: Nothing to disclose.

M39. Maternal Immune Activation: Association With Fetal Neurobehavior and Neonatal Connectivity of the Salience Network

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Background: During pregnancy, maternal immune activations (MIA) arise from infection, environmental stress, and poor physical health and, in preclinical models, have proximal and long lasting impact on offspring.
Translational human studies are just beginning to consider MIA with alterations in the brain and associated behaviors. In infants, three studies found an association with MIA and head circumference, an indirect measure of the brain. Epidemiological studies have associated MIA with increased risk of psychiatric disorders. Nevertheless, there remains a paucity of human research investigating the role of MIA in altered neurodevelopment. To date, no studies have investigated the effects of MIA on functional connectivity, or the temporal correlation between synchronous fluctuations in brain activity.
Numerous preclinical models investigating MIA with rodents or non-human primates provide templates to inform human studies. MIA has been associated with altered development in a widespread and non-specific set of brain regions including the hippocampus, the prefrontal cortex, the mid-temporal lobe, the parietal lobe, the insula, and the cingulate cortex. However, behavioral deficits are more specific to emotion, inhibition, and attention regulation. In humans, these behaviors have been related to functional connectivity patterns of the salience network, a large-scale brain network anchored in the insula and dorsal anterior cingulate (dACC). Together, these converging results suggest that the insula and anterior cingulate represent good candidate regions for investigations of functional connectivity patterns associated with MIA.
Here, we test the hypothesis that higher levels of MIA as measured by maternal interleukin (IL)-6 during the 3rd trimester will be associated with greater functional connectivity of the insula and dACC in neonates. In turn, the strength of connectivity will be associated with fetal neurobehavioral measures.
Methods: Thirty-two pregnant women, aged 14 to 19, were recruited from Columbia University Medical Center. They received routine prenatal care and had no major health problems. At 34-37 weeks of gestation, the women underwent diagnostic evaluations and blood draws, and fetal assessments included fetal heart rate (FHR), heart rate variability (FHRV), and correlation of movement (FM) and FHR (FM-FHR coupling). IL-6 was measured using the enzyme-linked immunosorbent assay. For the neonates, resting-state functional MRI data were acquired on a GE Signa 3T scanner. Standard seed connectivity from the dACC, and the right and left insula was performed. IL-6 was correlated with connectivity while controlling for post-menstrual age (PMA) at scan and sex.
Results: All neonates were appropriate for gestational age (birthweight: 3243.9 ± 593.8 kg, PMA at birth: 39.3 ± 1.4 weeks) and were scanned at 42.4 ± 1.7 weeks PMA. The majority were female (72%). With higher levels of maternal IL-6, the neonates exhibited greater connectivity between the left insula and medial prefrontal cortex (mPFC). With higher levels of maternal IL-6, the neonates exhibited weaker connectivity between the dACC and mPFC. No significant correlations between maternal IL-6 and connectivity from the right insula were observed. The strength of connectivity between the left insula and mPFC correlated positively with FHR and FM-FHR coupling. The strength of connectivity between the dACC and mPFC correlated positively with FHRV and FM-FHR coupling.
Conclusions: Using maternal IL-6 concentration measured during the 3rd trimester and neonatal functional connectivity, we show for the first time that MIA is associated with individual differences in salience network connectivity to the mPFC. The network connectivity is associated with increases in fetal neurobehavioral indices of autonomic and motor nervous system development. The brain regions of the salience network are critical for performing many cognitive behaviors, emotion regulation, and autonomic functions and are consistently implicated in neuropsychiatric disorders, suggesting a pathway for MIA to increase psychiatric risk. Future studies should relate MIA and these brain regions to postnatal measures of cognitive and emotion regulation in human offspring.
Keywords: Infant, Resting State Functional Connectivity, Fetal Neurobehavior, Maternal Immune Activation.
Disclosure: Nothing to disclose.

M40. Altered Inhibitory Control in Women Remitted From Anorexia Nervosa When Hungry and Fed

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Background: Anorexia nervosa (AN) is characterized by extreme caloric restriction that often results in a dangerously low body weight, a relentless drive for thinness, and body image disturbance. Enhanced cognitive control in AN, and/or the capacity to ignore homeostatic signals regulating eating, may contribute to an ability to restrict food intake even when hungry. While healthy individuals are highly motivated by hunger, as shown by an increased preference for high-calorie foods and reduced cognitive control, AN individuals maintain high levels of inhibitory control and are unmotivated to consume foods. Determining whether this ability to avoid eating is due to increased inhibitory control when hungry, or whether it is due to an impaired ability to interpret the salience of homeostatic signals that motivate behavior is critical to improve treatments for this deadly disorder. To address these questions, we examined whether

AN is associated with abnormal response in inhibitory control neural circuitry when hungry vs. fed.
Methods: Women remitted from AN (RAN; $N=25$) and control women (CW; $N=22$) completed a parametric Stop Signal Task during two counterbalanced fMRI visits: when hungry (after a 16-hour fast) and after a standardized meal (30% of daily caloric needs). Women remitted from AN were studied to avoid the confounding effects of malnutrition on brain response. This task requires the participant to inhibit a motor button-press response after it is initiated and activates cognitive control circuitry. To examine the influence of metabolic state on neural activation associated with successful inhibition, BOLD fMRI data for correct inhibitory trials were analyzed using Group (RAN, CW) x Visit (Hungry, Fed) x Difficulty (Easy, Hard) linear mixed effects analyses (LMEs) computed in R. Hard trials were defined as those where the Stop signal occurred shortly (0-200 ms) prior to a subject's prescan mean reaction time (MRT), and easy trials were those where there was a longer period (300-500 ms) between the Stop signal and MRT. Primary analyses were conducted in three, bilateral ROIs associated with inhibitory control: lateral prefrontal cortex, cingulate-insula, and striatum. Each ROI was treated as a search region. Exploratory voxelwise analyses were also conducted. Correction for multiple comparisons was determined with Monte Carlo simulations using AFNI's 3dClustSim (ROI-wise and voxelwise corrected $ps<0.05$ for all comparisons). Post-hoc pairwise comparisons were FDR corrected for multiple comparisons.
Results: Groups performed similarly on the task, regardless of metabolic state. During correct inhibitory responses, a Group (RAN, CW) × Visit (Hungry, Fed) × Difficulty (Easy, Hard) interaction was found in the left anterior insula. Post-hoc analyses showed CW had increased activation during inhibition of hard trials when fed compared to hungry, suggesting CW used greater neural resources to inhibit responses when fed. In contrast, RAN had increased inhibitory activation for hard trials when hungry compared to when fed. When fed, RAN relative to CW showed decreased inhibitory activation for hard trials, although no group differences were found when hungry.
Conclusions: In the context of equivalent task performance, our findings suggest that RAN may require fewer neural resources to successfully inhibit responses when fed, suggestive of cognitive efficiency, but greater effort to maintain performance when hungry, suggesting they may be less sensitive to the adaptive and motivating influence of hunger. This is consistent with prior findings of increased inhibitory control in AN, and suggests that AN women may not experience reduced inhibitory control when hungry, which could support successful dietary restriction. Results were strongest in the anterior insula, a region important for evaluating interoceptive signals (e.g., hunger) and integrating salient cues with motivational/emotional processes to guide decision-making and response inhibition. Taken together, these findings suggest that the influence of metabolic state on cognitive processing is altered in AN. Characterizing the neurocircuitry contributing to AN symptoms may directly inform new therapeutics, and results support the development of interventions that address altered influence of metabolic state in AN.

Keywords: Anorexia Nervosa, Functional MRI (fMRI), Inhibitory Control, Hunger and Satiety.
Disclosure: Nothing to disclose.

M41. Role for Hypothalamic Projections to Habenula in Obesity

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Background: Rates of obesity are on the rise worldwide, resulting in a growing threat to public health. Pharmacotherapies that safely reduce body weight in obesity remain elusive. Likely contributing to this failure is our poor understanding of the complex neuronal mechanisms that influence preference for palatable high-calorie "obesogenic" food relative to healthier lower calorie (and less palatable) alternatives. Similarly, we know little about the mechanisms by which consumption of palatable food can transition from controlled to compulsive, thereby driving the development of obesity. The lateral hypothalamus (LH) is considered a critical node in the neurocircuitry that maintains energy homeostasis, at least in part by regulating the rewarding properties of food. Classic experiments demonstrated that electrical stimulation of LH is positively reinforcing and can induce feeding behaviors in satiated rodents. Conversely, LH lesions induce profound anorexic responses that can result in starvation and death.
We found that the development of obesity in rats is associated with the emergence of a profound brain reward deficit, measured by elevated LH self-stimulation thresholds in rats. In addition, obese rats demonstrate markedly reduced willingness to work for food rewards. However, precisely how LH influences brain reward function and the value of food, and by extension its role in obesity, remains unclear. The habenula is a brain region located at the dorsomedial extreme of the thalamus linking forebrain and midbrain structures and is divided into two principal parts termed the medial habenula (MHb) and lateral habenula (LHb). The LHb has been described as a "preference center" which exerts a negative influence over motivated behaviors through inhibition of midbrain dopamine neurons. A major input to the LHb originates in the LH, providing a potential mechanism by which the LH can influence brain reward function and the motivational value of food. Here, we tested the hypothesis that LH projections to LHb play an important role in feeding behavior, food preference and the development of food-relevant motivational deficits in obese rats.
Methods: To visualize LHb signaling dynamics during discrete aspects of feeding behavior we expressed the genetically encoded intracellular calcium indicator GCaMP6m in LHb of rats and chronically implanted a gradient refractive index lens (GRIN) which could be coupled with a microendoscope. We directly modified the activity of the LH- > LHb pathway by delivering an AAV2/5-Cre-eYFP virus into the LHb, which travels in a retrograde fashion to express Cre recombinase in cell bodies of LHb-projecting LH neurons. A Cre-inducible diphtheria toxin (DTA) was then delivered to the LH, selectively ablating LH neurons that project to LHb. In a separate set of experiments,

we delivered Cre-inducible excitatory (hM3Dq) Designer Receptors Exclusively Activated by Designer Drugs (DREADD) to LH instead of Cre-inducible DTA. This allowed for stimulation of the LH->LHb circuit via administration of clozapine-N-oxide (CNO), which exclusively stimulates DREADDs.

Results: We found we found that LHb cellular firing abruptly decreased when a hungry rat given access to food began to eat. These observations support the hypothesis that feeding-relevant information is conveyed from LH to the LHb inputs and that the LHb likely plays a role in regulating the motivational properties of food. When we directly modified activity of the LH->LHb pathway we found that ablation of the LHb projecting LH neurons decreased the motivational value of standard food pellets, as measured by decreased willingness to work for standard chow pellets. Conversely, chemogenetic stimulation of the LH->LHb pathway increased the motivational value of standard food pellets in rats. Interestingly, ablation of LH->LHb pathway increases consumption of palatable energy-dense food whereas DREADD-mediated stimulation of the LH->LHb pathway decreased consumption of palatable food. These data suggest that the LH->LHb system plays an important role in regulating the motivational value of, and preference for, palatable food. Intriguingly, we found that lesion of the LH->LHb pathway triggered compulsive-like responding for food rewards reflected by persistent responding even when food-seeking responses resulted in the contingent delivery of noxious footshocks. Furthermore, lesioned animals displayed a strong trend towards increased responding for sucrose pellets compared to standard chow pellets in the face of punishment. Similarly, ablation of the LH->LHb lesion greatly attenuated hyponeophagia in rats when they were presented with novel calorically dense palatable food as reflected by reduced latency to consume such.

Conclusions: Together, these data reveal that lesioning the LH->LHb pathway induces a switch in food preference away from standard chow and toward palatable food and triggers compulsive-like palatable food responding in a manner similar to that seen in obese rats with a history of extended access to a cafeteria-style diet.

These findings identify the LH->LHb pathway as an important brain circuit involved in regulating feeding behavior and food choice. Currently, we are seeking to identify LHb neurons that transduce food-relevant information and explore adaptive responses in this pathway that may occur during the development of obesity.

Keywords: Obesity, Lateral Hypothalamus, Lateral Habenula, Food Preferences.

Disclosure: Nothing to disclose.

M42. Predicting Relapse in Bulimia Nervosa: Neural and Behavioral Response to Catecholamine Depletion

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Background: Bulimia nervosa (BN) is a severe psychiatric disorder characterized by recurrent binge eating episodes followed by inappropriate compensatory behavior such as purging or excessive exercise. Frank proposed in his model of eating disorders that BN is associated with a desensitized dopamine system (Frank, 2016). However, direct investigations on the causal effect of a hypofunction of the dopamine system on neural activity, bulimic and depressive symptoms, and on the course of the illness are still missing. Catecholamine depletion induced by alpha-methyl-paratyrosine (AMPT) is an instructive paradigm to investigate directly the relationship between dopaminergic neurotransmission and the symptoms and course of BN. In our previous behavioral study, experimental catecholamine depletion provoked mild eating disorder symptoms in fully remitted BN (Grob et al, 2015). The purpose of this study was to examine the effect of catecholamine depletion on neural activity in BN. Furthermore, we were interested in the relationship between this effect and the risk for relapse.

Methods: In a randomized, double-blind, crossover design, catecholamine depletion was achieved using the oral administration of AMPT over 24 hours in 18 remitted bulimic (rBN) and 22 healthy (HC) female participants. Cerebral blood flow (CBF) was measured using a pseudo continuous arterial spin labeling (pCASL) sequence. AMPT-induced mood and eating disorder symptoms were examined using the Montgomery-Åsberg Depression Scale (MADRS) (Schmidtke et al, 1988), the Eating Disorder Examination-Questionnaire (EDE-Q) (Hilbert and Tuschen-Caffier, 2006), and the vigor subscale of the Profile of Mood States (POMS) (McNair et al, 1981). Bulimic relapse was assessed in a follow-up telephone interview (latency varied between 18 and 42 months) after study participation.

Results: RBN participants revealed no increases of eating disorder symptoms following AMPT administration. However, AMPT reduced POMS vigor in both groups, and this effect was stronger in rBN participants. Furthermore, in rBN participants, AMPT decreased CBF in the pallidum and posterior midcingulate cortex (pMCC), whereas in HC participants, we did not find AMPT-induced alterations in CBF in these brain regions.

AMPT-induced depressive symptoms and reductions in CBF in the hippocampus/ parahippocampal gyrus predicted relapse in rBN participants. In contrast, AMPT-induced CBF increase in the hippocampus/ parahippocampal gyrus predicted remission.

Conclusions: We demonstrated that AMPT decreased CBF in the pallidum and pMCC in rBN participants. In the context of the Frank model (Frank, 2016), these regions can be considered as neural correlates of the desensitized dopamine system in BN.

In contrast to our previous study (Grob et al, 2015), we did not observe an AMPT-induced increase of eating disorder symptoms. However, our earlier investigation was carried out in a controlled environment, without food cues and with regular, standardized meals (Grob et al, 2015). The uncontrolled environment in which this study was conducted might have overridden the effect of AMPT on eating disorder symptoms. In rBN participants, AMPT reduced vigor more strongly than in healthy individuals. This vigor reduction might trigger eating disorder symptoms to counteract dopamine deficiency and the related depression-like mental state.

AMPT-induced depressive symptoms and CBF reduction in the hippocampus predicted bulimic relapse. Binge eating was

reported to have an anti-depressive and dopamine elevating effect (Jahng et al, 2012). Therefore, dopamine deficiency and a dysfunctional hippocampus activity might trigger inappropriate behavior, such as binge eating to reduce negative emotions and anhedonia. Our findings expand Frank's model of eating disorders (Frank, 2016), and emphasize the importance of depressive symptoms and the stress system in the course of bulimia nervosa.

Keywords: Bulimia Nervosa, Catecholamine Depletion, Relapse, Cerebral Blood Flow, Neuroimaging.

Disclosure: Nothing to disclose.

M43. Neural Correlates of Value Across Eating Disorders and Obesity

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Background: Studies utilizing the temporal difference model of reward learning in eating disorders and obesity have demonstrated involvement of ventral striatum, putamen, and orbitofrontal cortex, and have suggested a continuum of prediction error signaling. For instance, patients with increased episodic or continuous food consumption behaviors (bulimia nervosa, obese) have a weaker responsiveness than controls in neural circuitry associated with prediction error, and patients who restrict (anorexia nervosa) have a stronger activation. The data begs the question of whether reward value is also processed differently between groups. We sought to answer this question by utilizing the temporal difference model which has been previously characterized in both animal and human studies to assess functional imaging correlates of value signal across groups with various types of eating disorder pathology. We further wanted to test whether value signal would be related to treatment success in AN as measured by BMI change during treatment.

Methods: Adult female participants (total $N = 143$; mean age 26 years old, range 18-45 years old) with varied eating pathology participated in the study: anorexia nervosa (AN), both – ill (AN-ill) ($N = 30$), and recovered (AN-rec) ($N = 24$), bulimia nervosa (BN) ($N = 23$), obesity (OB) ($N = 19$), and healthy controls (HC) ($N = 47$). Clinical variables including age, BMI, and sensitivity to reward and punishment questionnaires were collected on all participants. Data on length of treatment (in a structured eating disorders program) was available for AN-ill subjects. A temporal difference learning task, which is based on reinforcement learning models, involves initially training participants to associate a visual stimulus with an unconditioned taste stimulus, and subsequently violating the learned contingencies. Prediction error signal can be measured, as can expected value signal for the unconditioned stimulus, both of which change over the course of the task. The task was performed in the MRI scanner. Following preprocessing of images, the value signal regressors across all trials were generated for each subject utilizing the temporal difference equation and convolved with the hemodynamic response function. A second level random effects whole brain group analysis using the general linear model including age, mood and anxiety comorbidity, and medication use was performed with $p < 0.001$ and $k = 10$ thresholds to test for group effect. Regions surviving small volume family-wise error correction ($p < 0.05$) were extracted and analyzed in SPSS in ANOVA to test for group differences. Post hoc analyses utilized Dunnett's T3 test if variances were unequal. Secondary whole brain regression analyses with similar statistical thresholds examined relationships of value signal to change in BMI over the course of treatment.

Results: As expected based on differences in pathology, there were differences in mean BMI on the date of scan between groups: AN-ill mean = 16.1 (SD 1.0); AN-rec mean = 20.8 (SD 2.4); HC mean = 21.8 (SD 1.7); BN mean = 22.8 (SD 5.6); OB mean = 34.8 (SD 4.9)). Average length of treatment for AN-ill was available when participants had completed their treatment program: (mean = 89 days; SD 32 days; range 39-137 days). Whole brain analysis utilizing value signal regressor revealed four areas with between-group differences: (1) left putamen ([-26, -14, 12]; $k = 211$, $pFWE < 0.01$); (2) right anterior cingulate (ACC) ([4 26 18]; $k = 14$, $pFWE < 0.05$); (3) left posterior insula ([-42 0 -6]; $k = 22$, $pFWE < 0.05$); (4) right superior frontal gyrus (SFG) ([14 -6 76]; $k = 78$, $pFWE < 0.01$). Group differences in left putamen and the right SFG were driven by higher signal in AN-ill and AN-rec vs. BN and OB. Post hoc testing in right ACC revealed a group difference driven by the AN-ill group having higher signal than all other groups (including AN-rec). Left posterior insula group difference was driven by AN-ill having higher signal than all other groups with the exception of AN-rec, which did not differ from other groups. Whole brain analysis in the AN-ill group utilizing value regressor revealed a negative relationship between BMI change and value signal in right ventral anterior insula (MNI coordinates: [46 8 -6]; $k = 11$, $pFWE < 0.05$)). Extracted right ventral anterior insula value signal was negatively correlated with BMI change over the course of treatment in the subset of AN-ill patients ($R = -0.47$, $p = 0.008$). Additionally, BMI change (discharge - admission) was correlated with length of treatment ($R = 0.62$, $p < 0.001$) in this group of patients. Sensitivity to reward or punishment did not correlate with the value signal in any of the groups.

Conclusions: Value signal differed by eating pathology along a continuum in that patients with restrictive eating behaviors (AN, AN-rec) had higher signal than patients with non-restricting (BN, OB) behaviors. In the context of this task, value is conceptualized as a salience signal, and does not necessarily connote a positive emotional valence. In particular, the results suggest two types of differences, trait and state-related. Higher value signal in AN-ill and AN-rec vs. BN and OB in left putamen and right SFG are suggestive of a trait marker, distinguishing between restrictive and non-restrictive eating patterns, in that this marker is present across both ill and recovering AN. By contrast, differences in right ACC and left posterior insula were more suggestive of a state marker. Value signal in right ventral anterior insula was negatively correlated with BMI change, and thus may be a candidate biomarker for illness severity in patients with AN. Overall, these preliminary findings suggest that patients with restrictive eating behaviors may have higher salience signal related to taste stimuli compared to patients with non-restrictive eating behaviors.

Keywords: Eating Disorders, Reward System, Functional MRI (fMRI).

Disclosure: Nothing to disclose.

M44. Bidirectional Effects of Insulin on Excitatory Transmission in the Nucleus Accumbens are Lost After High-Fat Diet Consumption

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Background: Recent studies show that insulin modulates the function of brain reward and motivation systems. For example, infusion of insulin directly into the NAc increases lever pressing for food (Figlewicz et al, 2008), and insulin receptor activation enhances dopamine release in the nucleus accumbens (NAc) but reduces excitatory transmission in the VTA and hippocampus (Labouebe et al, 2013; Stouffer et al, 2015; Man et al, 2000). Excitatory transmission in the NAc plays critical roles in motivation for food. However, insulin's effects on NAc excitatory transmission are unknown. In addition, consumption of foods high in fat alters motivation, peripheral sensitivity to insulin, and excitatory transmission in the NAc (Brown et al, 2015; Oginsky et al, in press). Therefore here, we determined the effect of insulin on excitatory transmission in medium spiny neurons in the NAc core in control and high-fat diet fed adult rats. Furthermore, given that insulin can activate insulin receptors (IR) and insulin-like growth factor receptors (IGFR), we also determined the role of these two receptor populations in the effects of insulin using antagonists selective for each receptor.
Methods: Whole-cell patch clamp recordings were made from medium spiny neurons in the NAc core of adult male rats before and after bath application of insulin (30, 50, 100, and 500 nM). Evoked excitatory post-synaptic currents (eEPSCs) and mini EPSCs (mEPSCs) were measured at a holding potential of -70 mV in the presence of the GABAA receptor antagonist picrotoxin (100 μM). The contribution of IRs and IGFRs to insulin's effects on eEPSCs were determined using the IGFR antagonist, PPP (500 nM) or IR antagonist, HNMPA (300μM), respectively. mEPSC frequency and amplitude were analyzed using MiniAnalysis (Synaptosoft, Inc.) and paired-pulse procedure were used to examine effects of insulin on the probability of glutamate release. For studies of the effect of high-fat diet, recordings were made in age-matched rats after free access to either high-fat diet (60% fat, Open Source Diets D12492; 8 weeks) or standard lab chow (Lab Diet 5001). Insulin levels were determined from fasted plasma blood samples (16hr) collected after 7 weeks of high-fat diet or chow. Insulin levels were determined by a double-antibody radioimmunoassay. In addition, body weight and food intake were monitored throughout and fat mass was determined by NMR (Minispec LF9011, Bruker Optics).
Results: We found that low concentrations of insulin (30 nM) increased excitatory transmission via activation of insulin receptors and increases in pre-synaptic glutamate release. Conversely, moderate to high concentrations of insulin (100-500 nM) resulted in activation of IGFRs and an opposing decrease in excitatory transmission that was mediated by a reduction in pre-synaptic glutamate release. Furthermore, insulin-receptor mediated increases in excitatory transmission were lost in high-fat diet fed rats. In addition, the loss of IR mediated responses occurred in the absence of overt changes in fasted circulating insulin levels, but in the presence of moderate increases in fat mass.
Conclusions: Our data show that insulin bi-directionally alters excitatory transmission in the NAc, with IR-activation enhancing excitatory transmission and IGFR-activation decreasing excitatory transmission. Furthermore, these effects of insulin are mediated by changes in pre-synaptic glutamate release. In addition, our data suggest that increases in fat mass, even in the absence of elevations in fasted insulin levels, are sufficient to reduce IR-mediated effects on brain function. These data shed light on potential mechanisms by which insulin may influence food-seeking and eating behavior and on how diet-induced obesity may alter insulin's effects on NAc function.
Keywords: Nucleus Accumbens, Glutamatergic Transmission, Insulin, Diet Induced Obesity, Insulin-like Growth Factor 1.
Disclosure: Nothing to disclose.

M45. An Anatomy-Guided Discovery of a Connection Hub in the Dorsal Anterior Cingulate Cortex: Implications for Psychiatric Disorders

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Background: One of the biggest challenges for neuroscience is understanding how the billions of neurons in the human brain work together to generate well coordinated behaviors. Network theory suggests that the complex brain is at its best computation efficiency when organized into segregated modules that integrate information through connectional hubs: a special set of brain regions that have extensive connections with different modules. Neuroimaging studies have utilized connectivity patterns over the whole brain to locate these hubs. The dorsal anterior cingulate cortex (dACC), a region involved in complex cognitive processing, is in a prime position for integrating across functional modalities. It has extensive connections with several prefrontal cortical regions, premotor areas, multisensory areas including the insula, and the amygdala. This places the dACC in an ideal position for integrating cognitive processing for goal-directed behavior, decision making and socially driven interactions. Indeed, abnormalities in the dACC are associated with several in psychiatric diseases.
However, neuroimaging is an indirect tool for measuring brain networks and whether anatomic hubs exist within the dACC remains unknown. Anatomic studies demonstrate an anterior-posterior topography of inputs to the dACC, such that anterior regions receive inputs from anterior prefrontal cortex (PFC), while more posterior areas are more closely linked to posterior PFC and premotor. Our hypothesis is that, within this organization of the dACC, there exist regions that receive a broader input than what would be predicted by a general topographic organization.
Using tract tracing experiments in nonhuman primates (NHP), we first identified the inputs to different subparts along the longitudinal axis of dACC. Using diffusion MRI (dMRI) in NHPs, we then tracked probabilistic streamlines from cortex to dACC to determine whether dMRI

methodology could identify the correct connections seen in the tracing studies. Finally, we used dMRI in normal human subjects to test whether a similar pattern of dACC connectivity existed in humans. Our findings provide a detailed picture of the location and connectivity profile of a hub within dACC. This hub maybe a key region within the dACC for integrating information across modalities and thus, an area associated normal and abnormal functioning.
Methods: Retrograde tracers were injected into equal-spaced sites along the longitudinal axis of areas 24 and 32. Cell labeling was quantified using stereology. The number of cells in each area was normalized by the total number in all areas. The distance from labeled cells to the injection site was quantified by atlas-based coronal distance.
Diffusion weighted images were acquired from postmortem monkey brains, submerged in Fomblin solution, on a 4.7 T Magnetic Resonance Imaging (MRI) system. Diffusion images from the Human Connectome Project were used for correspondent analysis in humans.
The gray matter of the diffusion images was divided into areas according to standard atlases. Seed masks were put on each area one at a time, and probabilistic tractography was performed between the seed and the dACC (area 24 and 32). Regions with high probability of tract terminals were identified through a clustering algorithm.
Results: Consistent with the literature, gradual change of connectivity was observed along the dorsal-ventral and rostral-caudal axes, with the ventral and rostral injection sites receiving more inputs from the frontal pole, the ventral medial PFC and area 25, and the dorsal and caudal sites more from the orbitofrontal cortex, the dorsal lateral PFC, the premotor and motor areas (Morecraft, 1998). Interestingly, a rostral dACC site violated this rule: this region receives inputs from all of the afore-mentioned cortical divisions. In addition, it also receives input from the ventral lateral PFC, the multisensory temporal cortex and the amygdala. Importantly, this site receives 50% of its inputs from 7 different cortical areas, whereas the other sites receives input from only 2–4.
Probabilistic tractography on the monkey diffusion data showed consistency with the tracing result. The regions of significant tract terminals from all seeds converged to the rostral area 32 of dACC. In the human tractography, the convergent zone was at the rostral tip of Brodmann area 24.
Conclusions: Our findings demonstrate anatomic evidence for a region within the dACC that serves as a connection hub. It is characterized by the large number, long distance and substantial diversity of inputs this location receives compared to other dACC areas. Moreover, the detailed connectivity profile found at the dACC connection hub provides critical information about the functions it is likely to integrate, including goal-directed control, emotion, motivation, and multisensory integration. Such information paves a new way of looking at dACC's multi-functionality: different parts of dACC may specialize in a particular function, while the connection hub may act as an area that is involved in flexibly switches between functional modalities and facilitating information exchange among the specialized parts. In this perspective, future clinical studies may seek to test the potential links between abnormalities within hubs and psychiatric disorders.

Keywords: dACC, hub Analysis, Large Scale Networks, Neuroanatomy, Diffusion Weighted Imaging.
Disclosure: Nothing to disclose.

M46. Cognitive Deficits Following Adolescent Alcohol Use may be Attributable to an Alcohol-Induced Arrested Development of Dopamine Network Dynamics

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Background: Adolescent alcohol use remains a major public health concern due in part to well-established findings implicating the age of alcohol use onset with the development of alcohol use disorders and persistent decision-making deficits in adults. However, therapeutic treatment options remain limited. We have demonstrated with a preclinical model that alcohol exposure during adolescence promotes maladaptive, risky choice behavior in adulthood. This impairment represents a unique vulnerability of the developing brain as we have also shown that adults given identical exposure do not show this deficit. In addition, we have established neural correlates of risk bias within the ventral striatum where phasic dopamine release in response to risky options is increased by adolescent alcohol exposure and is positively correlated with risk preference. These findings suggest that changes in striatal dopamine transmission, as a consequence of adolescent alcohol exposure, could bias choice by assigning greater value to risky options, but the underlying circuits and mechanisms involved remain unknown. Therefore, we hypothesized that midbrain circuitry, including specific inputs to the mesolimbic dopamine system, may be corrupted by early life alcohol exposure. Our most recent published work supports this hypothesis and points to a mechanism whereby alcohol is able to disrupt the normal development of DA network dynamics. Indeed, an appealing theoretical approach has been to link maturational changes in DA systems with behavioral changes in decision making and to posit that early life alcohol use confers a neurobiological profile that promotes persistent maladaptive decision making into adulthood. However, to address this hypothesis we must first have an adequate understanding of the normal development of these systems and their contribution to a decision making profile in adolescence that has been proposed, based on observations in humans, to be biased toward risk taking.
Methods: To examine this hypothesis experiments were conducted with four separate approaches. In approach one; adolescent rats were assessed on the probability discounting task beginning on postnatal day (PND) 49. The task involves the choice between a "safe" lever associated with the certain delivery (1.00) of 2 high pellets, and the "risky" lever was associated with the probabilistic delivery (1.00, 0.75, 0.50, 0.25, or 0.00) of 4 high fat pellets. Risky decisions are those where a large but uncertain reward is favored over a smaller certain reward of equal expected value. In approach two; we coupled the decision making task with real time measurement of phasic dopamine activity in response to safe and risky cues with fast-scan cyclic voltammetry (FSCV). We first conducted this in adolescent animals to ensure the

viability of multiple day measurements in the same animal with subjects of this age group. In a third experimental approach we utilized an anesthetized preparation to probe dopaminergic circuitry at three age points (PND 30, PND 50, PND 120) to assess function throughout this developmental period. Finally, in a fourth approach we collaborated with another lab to assess the degree that function is reflected in structure of dopamine neurons by measuring dendritic maturity throughout these same time points.

Results: Through the analysis of choice behavior on this task, our results confirm that adolescent rats demonstrate a standard probability discounting curve where decreasing probability of large reward delivery leads to a decrease in the choice of this "risky" option (Figure 1). Interestingly, we further demonstrate that the probability discounting curve of adolescent rats closely resembles the probability discounting curve of adult rats exposed to alcohol during adolescence. Using this task, we have previously shown that adult rats with adolescent alcohol intake make more risky decisions compared to controls, and these preliminary findings suggest that adolescent rats may be similarly risk-taking. These data lend support to our hypothesis that adolescent alcohol intake arrests the developing adolescent brain "locking in" an adolescent phenotype of risk taking behavior. Ongoing studies will directly compare naïve adolescents to adults on this probability discounting task. In addition, we establish here that we can acquire reliable voltammetric signals in animals this age over the entire range of the probability discounting tasdk so that we may establish and compare neural correlates of risk preference in all groups. We next examine the time course throughout adolescence of stimulated dopamine release. The underlying hypothesis for these experiments is that this pattern is dynamic throughout this period of development and that alcohol use during this period may alter this course. We find that stimulated dopamine release peaks in mid to late adolescence and returns to a pre-adolescent pattern in adulthood.

Conclusions: The general goal of these experiments was to test the hypothesis that a behavioral and neurobiological profile exists during adolescence that is maintained through adulthood in animals that consume alcohol during this time window. However, before testing this hypothesis we needed to establish an adolescent profile of decision making and dopamine signaling. Here, we provide evidence that adolescent animals display probability discounting and that the specific pattern of discounting is similar to adult animals that had alcohol during adolescence (risk preferring). In addition, we show that peak stimulation-evoked dopamine release occurs in mid to late adolescence and is similar to previously demonstrated elevated dopamine release in adult animals with prior adolescent alcohol exposure. Combined, these data lend support to the hypothesis that adolescent alcohol exposure may lock in a dopaminergic profile that contributes to heightened risk-taking behavior seen both in adolescence and in adult animals with prior adolescent alcohol use.

Keywords: Dopamine, Adolescent Alcohol, Reinforcement-Based Decision-Making.

Disclosure: Nothing to disclose.

M47. Cortical Maturation Delays Characterize ADHD in a Large-Scale Mega-Analysis Across the Life-Span Performed by the ENIGMA-ADHD Working Group

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Background: Neuroimaging studies show structural alterations of various brain regions in children and adults with ADHD. However, these studies are often underpowered and heterogeneous in their methods. After studying subcortical structures (Hoogman et al, 2015, Biological Psychiatry. 77:105S), the ENIGMA-ADHD Working Group now aims to study cortical measures across the life-span in the largest cross-sectional sample of participants with ADHD and controls to date.

Methods: A total of 34 sites from around the world are part of the ENIGMA-ADHD Working Group, a preliminary analysis was performed in data from 32 sites that had run the fully automated and validated segmentation software (FreeSurfer) to segment cortical thickness and cortical surface area of the areas listed in the Desikan-Killiany atlas. Case-control mega-analysis was carried out on all brain regions, taking age, gender, and site as covariates in the model. We also ran the same model adding Mean Thickness (in the thickness analyses) and Mean Surface area (in the surface area analyses) as a covariate. To understand the effects of age on cortical measures, stratified mega-analyses were carried out for children (age $<$ 15years) and adults (age $>$ 21years). *P*-values corrected for multiple comparisons using the Benjamini-Hochberg procedure (false discovery rate of 5%) are reported.

Results: In the combined analysis of all subjects, the results of 2055 participants with ADHD and 1821 controls showed significantly reduced thickness values in the temporal pole (Cohen's d: -0.16, pFDR = 4.28x10-5), fusiform gyrus (Cohen's d: -0.14, pFDR = 0.0003), precentral gyrus (Cohen's d: -0.12, pFDR = 0.002), entorhinal cortex (Cohen's d: -0.11, pFDR = 0.008), parahippocampal gyrus (Cohen's d: -0.09, pFDR = 0.04), and paracentral lobule (Cohen's d: -0.09, pFDR = 0.04). The stratified analysis in children (n ADHD = 1084, n controls = 1070) implicated the same regions as the combined analysis, only with larger effect sizes (e.g. temporal pole Cohen's d = -0.20 and fusiform gyrus Cohen's d = -0.21). Additional structures differing between childhood cases and controls were the lateral occipital cortex and the insula (Cohen's d -0.13 and -0.12, respectively). The stratified analysis in adults (nADHD = 549, ncontrols = 415) did not reveal any regions in the cortex showing significant differences between cases and controls. However, borderline significant effects were found in the lateral occipital cortex and the superior parietal cortex, with these regions appearing thicker in patients; Cohen's d: +0.19, pFDR = 0.08, and Cohen's d: +0.19, pFDR = 0.08, respectively. Including 'mean thickness' in the model did not change the results.

The analysis for surface area revealed a strong global case-control effect of mean surface area, Cohen's d = -0.21, pFDR = 4.67x10-9, with no additional regional effects. When we performed the analysis in children only, the effect became

stronger, Cohen's d = -0.29, pFDR = 2.00x10-9. In contrast, in adults, no effect of case-control status was found on mean surface area.

Conclusions: We found six areas in the cortex to be thinner in cases compared with controls. These areas are mainly located in the temporal and frontal lobes. The strongest effects were present in the temporal pole and the fusiform gyrus. Both of these structures have strong connections with the amygdala, which matches with results of our first study, in which the largest effect size was for a volume decrease in the amygdala of patients with ADHD, highlighting the importance of emotional processing in ADHD (Hoogman et al, 2015, Biological Psychiatry. 77:105S). Our results are also in line with previous work, showing links with impulsivity symptoms (McLaughlin et al, 2014, Biol Psychiatry. 76:629-638) and faster thinning in ADHD children in a longitudinal study for the fusiform gyrus (Shaw et al, 2011, Am J Psychiatry. 168:143-151), as well as increased activity (Wilbertz et al, 2015, World J Biol Psychiatry.Epub) and thinning in children with ADHD (Fernández-Jaén et al, 2014, Psychiatry Res. 224:8-13) for the temporal pole. The apparent normalization observed in adults in this cross-sectional study provides additional evidence for cortical maturational delays in ADHD patients earlier described in an earlier longitudinal study (Shaw et al, Proc Natl Acad Sci USA. 104:19649-19654).

Keywords: Attention Deficit Hyperactivity Disorder, Neuroimaging, Cortical Maturation Delay, ENIGMA Working Group, Mega-Analysis.

Disclosure: Nothing to disclose.

M48. Prefrontal Cortex to Accumbens Projections in Sleep Regulation of Reward

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Background: Seeking reward drives the life of animals forward, while abnormally enhanced or compromised reward processing is implicated in several detrimental psychiatric illnesses. In both humans and experimental animals, sleep is a prominent factor regulating reward processing of the brain. Following acute sleep deprivation (SD), amplified positive emotion and enhanced motivation for reward have been consistently observed across multiple modalities, including high calorie food wanting, monetary reward and risk taking, drug and alcohol intake, as well as intra-cranial self-stimulations. On the bright side, SD has been used as a fast-acting alternative treatment for the broadly defined depressive syndrome. However, the neural circuit mechanisms underlying sleep regulation of reward remain elusive.

The nucleus accumbens (NAc) is considered a limbic-motor interface that integrates reward and motivational inputs and translates them for motor outputs. Excitatory glutamatergic inputs carrying information of context, cues, and behavioral control from the prefrontal cortex (PFC), hippocampus, basolateral amygdala (BLA), thalamus, and other regions converge onto the NAc. Alterations in glutamatergic transmission or the balance between excitatory and inhibitory inputs (E/I) to the NAc lead to a variety of reward-malfunctioning including overeating and drug abuse. Whereas the glutamatergic inputs all come from brain regions that dynamically respond to sleep, it is not known whether and how their synaptic contacts on NAc neurons are affected by sleep or sleep loss. The present study aims to characterize whether and how SD affects the glutamatergic projections to NAc neurons and the overall E/I balance, and determine how these SD-induced alterations affect reward-elicited behaviors. We use a multi-disciplinary approach including in vivo EEG recordings, sleep interventions, in vitro brain slice electrophysiology, behavioral tests, and in vivo optogenetic manipulations.

Methods: Young adult mice (8 – 12 weeks old) were trained to self-administer sucrose pellet until a stable baseline was achieved. On SD day, they underwent acute SD by gentle handling for 6 hr during the first half of the light phase, during which they had full access to food and water. They were then tested for sucrose self-administration at the same time of day as on baseline days. EEG and EMG signals as well as mice behaviors were recorded to monitor the effectiveness of SD. Separate groups of mice were used for electrophysiological recordings from brain slices containing the NAc following normal sleep or SD. For in vivo optogenetics, mice received intra-mPFC injection of AAV2-SSFO-mCherry, and bilateral guide cannula implantation just above the NAc. The mice then underwent sucrose self-administration training and tests over the following 6 – 9 weeks under four different conditions in a counter-balanced manner: control (with brief anesthesia and optic fiber insertion), control with light stimulation (LS), SD (with brief anesthesia and optic fiber insertion), SD with LS. Mice usage was in accordance with protocols approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

Results: We found that after acute SD, mice had an increase in sucrose seeking and consumption ($F_{2,30} = 19.45$, $n = 16$, $p < 0.001$, one-way RM ANOVA; $p < 0.001$ control vs. SD, $p = 0.887$ control vs. recover) but not food intake, suggesting a selective enhancement of motivation for reward. In the NAc, a key brain region regulating emotional and motivational responses, we observed a decrease in the ratio of the overall excitatory over inhibitory synaptic inputs onto NAc principle neurons after SD (AMPA/GABA ratio, $F_{2,61} = 15.74$, $n = 17$-24 cells per group from 8-11 mice each, $p < 0.001$, one-way ANOVA; $p < 0.001$ control vs. SD, $p > 0.999$ control vs. recover). The shift was partly mediated by reduced glutamatergic transmission of presynaptic origin. Further analysis revealed that there was selective reduction of the glutamate release probability at the medial prefrontal cortex (mPFC)-to-NAc synapses (control, 0.60 ± 0.02, $n = 17/4$; SD, 0.53 ± 0.02, $n = 16/6$, $p < 0.05$, t test), but not those from the hippocampus, thalamus, or the basal lateral amygdala. To reverse this SD-induced synaptic alteration, we expressed the stabilized step function opsin (SSFO) in the mPFC; optogenetic stimulation of SSFO at mPFC-to-NAc projection terminals persistently enhanced the action potential-dependent glutamate release (LS x time interaction, $F_{52,988} = 1.466$, $p < 0.05$; main effect of LS, $F_{2, 38} = 303.9$, $n = 9 – 19$, $p < 0.001$, two-way RM ANOVA). Intra-NAc optogenetic stimulation of SSFO selectively at mPFC-to-NAc

terminals restored normal sucrose seeking in mice after SD (SD x LS interaction, F1, 56 = 5.291, $n = 15$ each group, $p < 0.05$, two-way ANOVA; control vs. SD, $p < 0.01$, SD vs. SD + LS, $p < 0.001$, control vs. control + LS, $p = 0.284$, control + LS vs. SD + LS, $p > 0.999$, Bonferroni posttest) without affecting food intake (SD x LS interaction, F1,9 = 0.098, $n = 10$ each group, $p = 0.761$; main effect of SD, F1,9 = 2.939, $p = 0.121$; main effect of LS, F1,9 = 0.231, $p = 0.642$; control vs. control + LS, $p > 0.999$; SD vs. SD + LS, $p > 0.999$, Bonferroni posttest, two-way RM ANOVA).

Conclusions: These results provide a circuit-based understanding about sleep-mediated regulation of reward seeking, highlighting the mPFC-to-NAc projection as a key circuit-based target for sleep to regulate reward-motivated behaviors.

Keywords: Sleep, Reward, mPFC, Accumbens, Self-Administration.

Disclosure: Nothing to disclose.

M49. Sex Differences in Persistent Changes in Hippocampal Gene Expression and Memory After a Subchronic Immune Challenge

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Background: Systemic inflammatory events, including major illness and injury, have long lasting consequences on emotional regulation and cognitive processes. Post-operative cognitive dysfunction and memory deficits after heart attack can persist for months and years after resolution of immune-related signaling. Whereas acute effects of immune activation on depression-like behaviors and memory tasks, and the molecular mechanisms that mediate these changes are increasingly studied, the long-lasting effects of immune challenge remain unknown. We have previously demonstrated sex-specific patterns of memory deficits 8-weeks after surgical induction of myocardial infarction in mice, or after a subchronic lipopolysaccharide injection protocol. Here we aimed to examine the persistent changes in hippocampal gene expression after a subchronic, systemic immune challenge in both male and female mice.

Methods: Subjects: Male and female C57Bl6 mice (Envigo Laboratory, Indianapolis) 9 weeks old were acclimated to the colony room (12:12hour light:dark cycle; ad lib food and water) for one week prior to immune challenge. Systemic immune challenge: 5 i.p injections of LPS (250ug/kg) or vehicle were given over 13 days. Gene expression: 8 weeks after the final LPS injection, hippocampi were dissected and RNA isolated for Illumina RNA sequencing of hippocampus. RNAseq data was analyzed by the University of Michigan Bioinformatics Core. Western blotting: Standard immunoblotting techniques were used to determine levels of proteins for signaling pathways with altered gene expression.

Results: Both males and females show altered gene expression in hippocampus 8 weeks after a subchronic immune challenge. Surprisingly, LPS-treated males show much many more changes in gene expression (~230genes) than LPS-treated females (26 genes), with very few genes up- or down-regulated in both sexes. Specifically, males showed persistent dysregulation of gene expression associated with growth factors, immune pathways, and synaptic plasticity. In contrast, females showed alterations in genes associated with catecholaminergic signaling.

Conclusions: Transient immune activation causes long lasting dysregulation of gene expression in the dorsal hippocampus. These persistent changes are both qualitatively different in males and females, with more changes in males compared with females; and quantitatively different, with genes from different signaling pathways affected in males and females. Future research will assess (a) the role of epigenetic alterations in mediating long lasting changes in hippocampal gene expression after immune challenge, and (b) sex-specific roles of identified genes and signaling pathways in memory deficits and affective dysregulation that persist long after major illness or injury.

Keywords: Neuroimmune, Synaptic Plasticity, Epigenetic, Gene Expression, Learning and Memory, RNAseq, Mood and Anxiety Disorders.

Disclosure: Nothing to disclose.

M50. GLYX-13 (Rapastinel), a Non-Psychotomimetic Rapid Antidepressant Reverses Loss of Synaptic Connectivity in Medial Prefrontal Cortex (mPFC) Caused by Chronic Stress

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Background: GLYX-13, an NMDA receptor modulator, produces a rapid antidepressant effect in major depression without inducing the initial psychotomimetic effects of ketamine. The antidepressant effect of ketamine has been attributed to its ability to stimulate synaptogenesis, thereby repairing the loss of critical synaptic connections underlying depressive illness (1). Ketamine also completely reverses CUS induced depression-like behaviors and reduction in EPSC responses to 5-HT and hypocretin in layer V pyramidal cells in the medial prefrontal cortex (mPFC). Recently we reported that GLYX-13 shares many mechanistic actions with ketamine (2). For example, within 24 hours both drugs, via the mTORC1-dependent synaptogenic pathway, increase the density of synaptic spines in the apical dendritic tuft of layer V pyramidal neurons in the mPFC. Moreover, both drugs increase the frequency EPSCs in response to hypocretin activation of thalamo-cortical inputs, but only ketamine increases 5-HT activation of cortico-cortical inputs. The present study extends this work and investigates whether GLYX-13 is able the rapidly reverse the effects of CUS.

Methods: Recordings were performed in mature rats ~8-12 weeks in age (~300-350g). Mature rats were chosen to exclude any possible development changes as seen in immature rats (see 2). Whole cell patch clamp recordings were made from layer V pyramidal cells in brain slices of the anterior cingulate (AC) and prelimbic subregions of mPFC. During recording cells were passively filled with Neurobiotin to label and visualize cells. Both EPSC and IPSC responses were recorded after bath application of activators of two

different inputs to layer V cells: cortico-cortical by serotonin (5-HT) and thalamocortical by hypocretin/orexin (hcrt). The PSC responses were tested in brain slices 24 hrs following administration of GLYX-13 (3 mg/kg, i.v.) or ketamine (10 mg/kg, i.p.); these doses were selected based on our prior studies (2). Slices were later fixed and treated with Strepavidin-594 to amplify the fluorescence signal. Images were taken throughout the apical dendritic field using 2-photon laser or confocal microscopy. Apical dendrites were then analyzed for spine density and spine morphology.

Results: Twenty fours after infusion of GLYX-13, in an undifferentiated population of layer V pyramidal cells in mPFC, we found that there was reversal of the CUS decrease in 5-HT and hypocretin induced EPSCs. To investigate these changes in more detail we also distinguished between the two main populations of layer V pyramidal cells: the thick tufted, long-projecting Type 1 cells and the less numerous thin tufted, local and callosal projecting Type 2 cells. We found that Type 1 cells, but not Type 2 cells were largely responsible for the GLYX-13 mediated reversal of the marked decrease in 5-HT and hypocretin-induced EPSCs after CUS. In addition, our preliminary data indicates that the CUS-deficits in 5-HT- and hypocretin-induced IPSCs in Type 1 cells may also be reversed by GLYX-13. In contrast, while ketamine was effective in reversing the CUS-deficits in EPSCs in Type 1 cells it failed to increase IPSCs in Type 1 cells in control or CUS animals.

Conclusions: The main findings of this study are that GLYX-13, like ketamine is highly effective in reversing the CUS-induced reduction in EPSC responses to 5-HT and hypocretin, especially in Type 1 cells. Studies are underway to confirm the reversal by GLYX-13 of CUS-induced reductions in 5-HT- and hypocretin-induced IPSCs, and to examine the developmental effects on GLYX-13 and 5-HT-induced responses. These findings could be relevant to the concept that in schizophrenia deficits in interneurons result in impaired screening of thalamo-cortical inputs as well as inhibitory regulation of mPFC local circuits. The initial psychotomimetic effects of ketamine may similarly be caused by a failure of this drug to up-regulate IPSCs in proportion to enhancement of EPSCs. Our data indicate that GLYX-13 can produce a proportional increase in both IPSCs and EPSCs, putting it in contrast with ketamine. We conclude that the high efficacy of the GLYX-13-mediated restoration of the excitatory/inhibitory balance could account for the fact that, unlike ketamine, it does not produce initial psychotomimetic effects in depressed patients.

Keywords: Depression, Glutamate, GABA.

Disclosure: Nothing to disclose.

M51. The Antidepressant Actions of Tianeptine Require the Mu-Opioid Receptor

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Background: While the majority of pharmacological agents used for the treatment of depression target the serotonin system, selective serotonin reuptake inhibitors (SSRIs) are not ideal because only a fraction of patients achieve remission. Novel targets are needed for the development of new antidepressant drugs for this 'treatment resistant' population and additionally, for patients who suffer from intolerable side-effects stemming from SSRI administration. The recent discovery that the effective antidepressant tianeptine (Stablon) is an agonist at the mu-opioid receptor (MOR) and delta-opioid receptor (DOR) has revealed potential novel targets for drug development.

Methods: Our studies were aimed at understanding the neural circuits through which tianeptine exerts its antidepressant effects. Using mouse models, we assessed the acute and chronic effects of tianeptine on depressive and other opioid-related behaviors. Additionally, using genetic and pharmacological models we tested whether the behavioral effects of tianeptine are mediated by MOR and/or DOR.

Results: Chronic tianeptine administration resulted in antidepressant behaviors measured in the forced swim and novelty-suppressed feeding assays. Classic opioid-like effects were also seen following acute administration including analgesia, conditioned place preference, hyperactivity, and decreased feeding. Interestingly, following chronic administration of tianeptine, there was no tolerance to the analgesic effect of tianeptine or naloxone-precipitated withdrawal response, which is in sharp contrast to the effects of other MOR agonists such as morphine. We found no behavioral response to tianeptine in MOR knockout (KO) mice. The effects were also abolished by pretreatment with an MOR antagonist. Although tianeptine also activates the delta-opioid receptor (DOR), albeit with reduced potency, all responses to tianeptine were unaffected in DOR KO mice. Tissue-specific MOR KO mice are now being used to dissect the circuitry that underlies the opioid-dependent antidepressant action. Preliminary evidence suggests that the antidepressant phenotype is dissociable from the opioid-induced hyperactivity phenotype.

Conclusions: The antidepressant response to tianeptine in mice is dependent on MOR, but not DOR, activation. Overall, our results point to a novel entry point to understand the neural circuits underlying depression, and a potential avenue for the development of a new class of antidepressant drugs.

Keywords: Antidepressant, Tianeptine, Opioid System, Drug Discovery - New Approaches, Mu-Opioid Receptors.

Disclosure: Servier: Consultant, Self; Lundbeck: Consultant, Self.

M52. Whole Blood Gene Expression Profiling Confirms the Importance of Interferon Signaling Pathways in Defining the Phenotype of an Inflammatory Depressive Episode

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Background: Inflammation can be an important driver of depression based on experimental models in animals

and humans. Human models on inflammation-induced depression have focused on interferon-alpha (IFNα) as this cytokine is used therapeutically and chronic treatment leads to depression in up to half of cases. However, it is clear that depression may also be caused by factors other than inflammation, making the relative importance of inflammation in typical depressed patients unclear. An additional limitation to application of these models has been lack of findings showing differences in IFNα between depressed patients and healthy subjects, so that the generalizability of IFNα based human studies to clinical populations is not certain. To help close this gap in translation we conducted immune phenotyping in adults with depression in order to determine which immune pathways are dysregulated. Based on the hypothesis that inflammation defines a subgroup of depression, we empirically divided subject according to whole blood gene expression patterns.

Methods: 40 adults in a SCID-validated current depressive episode (either Major or Bipolar Depression) were phenotyped and provided whole blood RNA samples. Samples were ribosomal RNA and hemoglobin mRNA depleted before being converted to cDNA libraries and sequenced. After quality control and alignment, gene expression counts were calculated for the sample using the HTSeq package. Cluster analysis was used to divide the subjects into groups according to their gene expression signatures. Differential gene expression analysis using the EdgeR package used a false discovery rate (FDR) adjusted threshold of 0.05 for significance of differential expression. Gene set enrichment analysis (GSEA) was performed using the MiSigBD database (Broad Institute) was performed to determine differences between the two groups in immune activity, also using a FDR adjusted significance threshold of 0.05.

Results: The best cluster solution used two groups (N1 = 23 and N2 = 17). There were 133 genes differentially expressed between the two groups. 70 genes had higher mean expression in group 1 compared to group 2 and 58 genes showed the reverse pattern. The most significant pathway was IFN-gamma response ($p < 0.001$), with 11 genes all of which were expressed more highly in group 2 (CCL2, CMPK2, IFI44, IFI44L, IFIT1, IFIT2, IFIT3, OAS3, PTGS2, RSAD2, TNFAIP6). Of these genes, 6 are also activated by IFN-alpha. 6 genes encoding targets of the transcription factor family E2F also formed a significant pathway ($p < 0.001$) with greater expression in group 1 (BIRC5, CDC20, E2F8, MKI67, SPC24, RRM2). 6 genes which partially overlapped with the INF-gamma pathway significantly represented targets of the transcription factor Nf-kb ($p < 0.001$) when activated by the cytokine Tumor Necrosis Factor-alpha (ABCA1, CCL2, IFIT2, PTGS2, TNFAIP6, TUBB2A). Genes up regulated and down regulated by kRas protein activation were also found to be significantly enriched (both $p = 0.006$) (PCSK1N, PTGS2, LY96, PDCD1LG2 up-regulated) (IFI44L, RSAD2, TFCP2L1, KRT5 down-regulated) with expression higher in group 2 for 7 of the 9. Clusters did not differ by age, gender, race, or body mass index.

Conclusions: Our results demonstrate that out-patients with current depression can be divided into empirical clusters according to pattern of immune gene expression and that genes regulated innate immunity, in particular, the interferon response, account for the difference between them. This result supports the importance of these pathways in defining inflammation associated depression and provides a starting point for further characterization and eventual treatment development for this important sub-type.

Keywords: Depression, Inflammation, Genomics.

Disclosure: Nothing to disclose.

M53. The Effects of Long-Term Psychosocial Adversity on Dopaminergic Function and the Acute Stress Response

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Background: Many major mental illnesses including depression, schizophrenia and addictions are associated with chronic exposure to psychosocial stressors (PSS). In parallel, dopaminergic dysfunction has been associated with these disorders and there is evidence that the dopamine system may mediate the link between PSS and mental illness. For example, elevated urinary dopamine and metabolites have been reported in girls with a history of sexual abuse compared to those without (De Bellis et al. 1994). In an [11C]-Raclopride study by Pruessner et al. (2004) ventral striatal dopamine release was increased in response to PSS in humans who reported insufficient early life maternal care. Similarly, a further [11C]-raclopride study (Wand et al. 2007) found stress-induced cortisol levels were positively associated with amphetamine-induced DA release in the striatum. Using the radioligand [11C]-PHNO, a D2/3 agonist, Mizrahi et al. (2011) found increased PSS-induced dopamine release in the striatum of antipsychotic-naïve subjects with schizophrenia and those at clinical high-risk of the illness compared to matched healthy controls, possibly indicative of a sensitised dopaminergic stress response. Whilst these findings support acute dopamine release in response to stress in humans, it is unknown if long-term psychosocial adversity has the same effect. We therefore sought to examine whether long-term exposure to psychosocial adversity was associated with alterations in striatal dopamine synthesis capacity as well as psychological and physiological responses to acute PSS.

Methods: We compared dopamine synthesis capacity in $n = 17$ human participants with a high cumulative exposure to psychosocial adversity ('HA' group) with $n = 17$ participants with low cumulative psychosocial adversity exposure ('LA' group) who were age- and sex-matched. Participants were excluded if they met DSM-IV-TR criteria for mental illnesses and/or drug dependency or had an immediate family history of a severe mental illness. No participant was taking psychiatric medicines during the study. HA participants were required to have a history of long-term exposure to psychosocial adversity in childhood and as an adult. Psychosocial adversity was further operationalised as at least two of: upbringing and/or current living in inner-city London, a history of first or second generation migration, a history of childhood (before age 16 years) adversity and/or psychological trauma (Parental separation/divorce or death

of a first-degree relative or physical/sexual abuse or neglect or foster care/adoption OR major disaster OR war), and/or current adult adversity (e.g. living alone for over a year, lacking a confidant, being unemployed). Dopamine synthesis capacity (indexed as the influx rate constant Kicer) was measured with [18F]-DOPA PET. We used the Montréal Stress Task to induce acute PSS and measured the subjective and physiological stress responses with visual analogue scales and salivary amylase and cortisol, respectively.

Results: HA had reduced dopamine synthesis capacity in the striatum (effect size d = .80, t = 2.27, $p = .03$) and its limbic (effect size d = .95, t = 2.64, $p = .01$) and associative subdivisions (effect size d = .81, t = 2.28, $p = .03$) compared with LA. HA had increased levels of depression, anxiety, schizotypy and aberrant salience compared with LA. Across all participants limbic striatal dopamine synthesis capacity was negatively associated with depressive symptoms (r = -.42; $p = .02$). HA had a reduced amylase (F = 4.6, $p = .04$) and attenuated cortisol response (t = 2.18, $p = 0.04$), yet a potentiated subjective response (t = 2.08, $p = 0.05$) to acute stress. There was a relationship between striatal dopamine synthesis capacity and the subjective response to acute stress in LA (r = .63, $p = .03$), which was not present in HA.

Conclusions: Long-term exposure to psychosocial adversity is associated with reduced striatal dopamine synthesis capacity. Our finding of a negative relationship between depressive symptoms and limbic dopamine synthesis capacity suggests that long-term exposure to psychosocial adversity may increase the risk of mental illness by dampening the dopamine system. Likewise, our finding that in participants with a high exposure to psychosocial adversity acute stress results in blunted physiological yet potentiated subjective responses to acute psychosocial stress suggests that chronic exposure to psychosocial adversity disrupts these mechanisms. These findings are highly relevant to understanding the biological mechanisms linking exposure to psychosocial adversity and a range of common and severe mental illnesses including affective disorders, addictions and schizophrenia.

Keywords: Psychosocial Stress, PET Imaging, Psychosis, Depression, Acute and Chronic Stress.

Disclosure: Nothing to disclose.

M54. Rapastinel (Glyx-13), a Rapid Acting Antidepressant, Does not Increase Extracellular Levels of Dopamine and Glutamate in Rat Medial Prefrontal Cortex

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Background: Rapastinel (Glyx-13) is a novel NMDA receptor modulator with glycine-like partial agonist properties. Rapastinel is in late-stage clinical development as an adjunct therapy for major depressive disorder. Ketamine, a noncompetitive NMDA receptor channel blocker, is known to produce rapid and sustained antidepressant effect in treatment-resistant patients with major depression. However, ketamine, a schedule II controlled substance, is abused frequently and is known to produce addictive and psychotomimetic effects in animals and humans. Both ketamine and rapastinel produce antidepressant-like effects in rodent models of depression but rapastinel does not exhibit ketamine-like CNS adverse effects. For example, rapastinel does not produce ketamine-like sedation and rewarding effects or disrupt sensorimotor gating in rodents (Burgdorf et al, 2013). In the present study, we evaluated acute effects of rapastinel and ketamine on extracellular levels of dopamine and glutamate in rat medial prefrontal cortex (mPFC) using intracerebral microdialysis. The onset and the duration of antidepressant-like effects of rapastinel and ketamine were evaluated in the rat forced swim test (FST).

Methods: All experiments were carried out in adult male Sprague Dawley rats. Microdialysis experiments were carried out in freely moving rats as described in Kehr et al,2001. Briefly, guide cannulae were surgically implanted into mPFC using standard stereotaxic coordinates. On the day of dialysis, a 3-mm semipermeable probe (molecular weight cut off: 50 kDa) was inserted into the guide cannula and mPFC was dialyzed with artificial CSF (flow rate: 1 uL/min) for at least 2 hr. Following this, baseline dialysate samples were collected for the next 60 min and then for additional 6 hr following drug administration. Dopamine and glutamate concentrations in the dialysates were measured using HPLC and LC-MS/MS methods. Rat FST was carried out using a modified version of Porsolt (Porsolt, 1979) at 1-hr, 2-day and 7-day postdose.

Results: Both rapastinel (3-30 mg/kg) and ketamine (3-30 mg/kg) decreased immobility time in the rat FST when tested at 1-hr postdose. This antidepressant-like effect of both drugs persisted at day 2 and at day 7. In the dialysis assay, ketamine significantly elevated extracellular levels of dopamine and glutamate in the mPFC within the first 60 min of dosing. By contrast, rapastinel did not increase dopamine or glutamate levels in mPFC at any of the doses tested.

Conclusions: These data provide a potential neurochemical basis for rapastinel's favorable CNS adverse effects compared to ketamine. Increase in brain dopamine and glutamate levels by ketamine may play a significant role in its high abuse potential and psychotomimetic-like effects.

Keywords: Forced Swim Test, Glutamate, Dopamine.

Disclosure: Allergan, Inc.: Employee, Self.

M55. Anterior Cingulate Cortex Morphology Predicts Remission From Major Depression Following Internet-Based Cognitive Behavior Therapy

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Background: Function and morphology of the rostral and subgenual anterior cingulate cortex (ACC) have been shown to predict symptom improvement following pharmacological and cognitive behavioral therapy for depression. To our knowledge, no study has yet investigated neuroimaging predictors of outcome following internet-based CBT (iCBT). In this study, we examined whether greater baseline thickness and volume of the rostral and subgenual ACC

predict subsequent clinical remission in adults with MDD receiving iCBT as part of a randomized clinical trial (RCT).
Methods: In a parallel-group RCT, adult MDD participants (18-45 years) were randomized to ten weeks of iCBT ($n = 37$) or to a monitored attention control condition ($n = 40$). At baseline, participants were interviewed using the Structured Clinical Interview for DSM-IV and the Hamilton Rating Scale for Depression (HRSD) and underwent magnetic resonance imaging (MRI) at 3T. After the intervention phase, patients received a post-treatment HRSD interview, administered by a rater blind to treatment group. Using Freesurfer, we derived cortical thickness and volume measurements for the rostral ACC (gyrus and sulcus) and subgenual ACC (subcallosal cortex). Repeated measures analyses of covariance (ANCOVA) compared thickness and volume of rostral and subgenual ACC between treatment remitters (post-treatment HRSD $\leq$ 7) and non-remitters in the iCBT group, using hemisphere as a within-subject factor, and covarying for baseline depression as well as average cortical thickness or intracranial volume as appropriate.
Results: Twenty-nine participants who received iCBT had complete MRI data; 18 were classified as remitters and 11 as non-remitters at the post-treatment visit. In the ANCOVA comparing baseline rostral ACC thickness in remitters and non-remitters, the main effect of remission was statistically significant ($F(1,25) = 6.27$, $p = .02$), and the interaction of remission by hemisphere was not significant, reflecting greater left and right rostral ACC thickness in remitters. In the ANCOVA of rostral ACC volumes, only the interaction of remission by hemisphere was statistically significant ($F(1,25) = 7.10$, $p = .01$), reflecting larger volumes of the right rostral ACC in remitters. Subgenual ACC thickness and volume did not differ significantly between remitters and non-remitters.
Conclusions: MRI measurements of rostral ACC anatomy may serve as predictive biomarkers of clinical remission to iCBT for depression. Internet-based and face-to-face CBT should rely on similar neural mechanisms due to their shared therapeutic content, and despite differences in treatment delivery. These findings await replication with independent and larger samples.
Keywords: Major Depressive Disorder (MDD), Cognitive-Behavioral Therapy, Anterior Cingulate Cortex, Treatment Outcome Prediction, Technology.
Disclosure: Nothing to disclose.

M56. Serial IV Ketamine Infusions are Effective for the Treatment of Comorbid Post-Traumatic Stress Disorder and Treatment-Resistant Major Depression

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Background: Comorbid post-traumatic stress disorder (PTSD) and major depressive disorder (MDD) are common sequelae of trauma and associated with a more severe clinical presentation than either disorder alone. Single administrations of ketamine have been shown to be effective for PTSD, and single and series administrations of ketamine have been shown to be effective for MDD, but there have been no studies of serial administrations of ketamine in a comorbid PTSD/MDD population. For the present study, we hypothesized that serial ketamine infusions would improve symptoms of PTSD and depression in a comorbid population and that serial infusions would extend the durability of response beyond that observed for a single infusion.
Methods: Sixteen individuals with MDD and PTSD received six IV infusions of 0.5 mg/kg ketamine over 40 minutes on a Monday-Wednesday-Friday schedule during a 12-day period. Outcome measures included the Montgomery-Asberg Depression Rating Scale (MADRS) and the PTSD-symptom Checklist (PCL-5). Outcomes measures were collected prior to each infusion and 24-hours after each infusion. Subjects were followed weekly for 8 weeks following the sixth and final infusion.
Results: Comparing baseline to 24-hours after the final infusion, a significant decrease in depression symptoms, as assessed by mean total MADRS scores, was observed (mean within-subject change [SD] = 27.1 [6.6]; $t = 16.4$; $df = 15$; $p = 0.000$) and associated with a large effect size (Cohen's $d = 4.1$). Likewise, a significant decrease in PTSD symptoms, assessed using mean total PCL-5 scores, was also observed (mean within-subject change [SD] = 33.2 [18.5]; $t = 7.0$; $df = 14$; $p = 0.000$) and associated with a large effect size (Cohen's $d = 1.80$). For subjects whose depression symptoms responded to the infusion series, the mean time to relapse for depression (mean days to relapse [SE] = 29.5 [5.9]) was superior to previous reports of single-infusions for depression. Similarly, for individuals in remission from PTSD at the conclusion of the infusion series, the mean time to relapse (mean days to relapse [SD] = 44.5 [7.0]) was superior to a previous study of single ketamine infusions for PTSD.
Conclusions: This is the first open-label study describing improvement in PTSD and depression symptoms following serial ketamine infusions in a comorbid population. Although limited by small sample size, improvements in depression and PTSD outcomes were significant and robust with both demonstrating large effect sizes. Further, repeated ketamine infusions extended the period of response for both disorders beyond that reported for a single-infusion. This report suggests that serial ketamine treatments are relevant for the treatment of PTSD and depression.
Keywords: Ketamine, Treatment Resistant Depression, Posttraumatic Stress Disorder, Major Depression, Post-Traumatic Stress Disorder, Trauma and Stress Disorders.
Disclosure: Nothing to disclose.

M57. Immune to Insults? Inflammatory Cytokine Modulation of Serotonin Signaling as a Determinant of the Translation of Early-Life Stress to Adult Anxiety and Depression

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Background: Early-life stress (ELS) is an important risk factor for adult cognitive disability and mood disorders, including major depression and anxiety. The first line of treatment for adult mood disorders are the selective

serotonin (5-HT) reuptake inhibitors (SSRIs) that block the 5-HT transporter (SERT) and thereby prolong 5-HT signaling. We hypothesized that changes in 5-HT signaling, and SERT function specifically, may arise in the context of ELS and thereby impact lifelong risk for mood disorders. In addition, based on the considerable evidence that ELS induces alterations in immune function, we hypothesized that changes in 5-HT signaling might arise from plasticity changes in immune function. We have shown previously that peripheral immune system stimulation induced by the bacterial endotoxin lipopolysaccharide (LPS), as well as the proinflammatory cytokine interleukin-1β (IL-1β), rapidly activates SERT activity (Zhu et al, 2010) via a p38 MAPK signaling pathway (Zhu et al, 2006). Furthermore, the depressive and anxiety-inducing effects of LPS are lost in interleukin-1 receptor knockout (IL-1R1 KO) mice. Here we report ongoing studies designed to test the hypothesis that ELS-induced changes in behavior are dependent upon IL-1R/p38 MAPK signaling in 5-HT neurons.

Methods: Heterozygous IL-1R1 (Jackson Laboratories) were bred to produce IL-1R1 KO and WT littermates (C57Bl/6J background). To investigate a role for inflammatory cytokine signaling to CNS 5-HT neurons as a requirement for long-lasting behavioral effects of ELS, we implemented the construct valid model of maternal separation. In this paradigm, newborn mice were maintained with the dam (normally reared, NR) or separated (MS) for 3 hours daily from P1-P14, with all animals weaned at P21. Behavioral tests, namely the tail suspension and forced swim tests, as well as open field and elevated plus maze, and biochemical experiments were performed at 8-10 weeks of age. Brains and spleens were harvested for evaluation of cytokine levels via qPCR, plasma corticosterone (CORT) levels were measured using ELISA. Plasma 5-HT levels were assessed using HPLC. To ascertain whether changes in 5-HT parameters arise as an immediate result of ELS, a separate cohort of MS/NR animals was sacrificed at P15, and brain, spleens, and blood were harvested for qPCR and HPLC analyses. To investigate whether IL-1R1 mediated signaling specifically within 5-HT neurons is required to produce MS phenotypes, IL-1R1flox/flox:ePet-1:Cre animals were generated allowing for conditional ablation of IL-1R1 expression within CNS 5-HT neurons.

Results: We found that ELS led to long-lasting elevations in mRNA levels of multiple inflammatory signaling molecules (e.g. IL-1 β, TNF- and IL-6) in both the brain and spleen, as well as elevated plasma CORT levels, in both WT and KO mice. As reported by others, we also found that ELS produced long-lasting depressive- and anxiety-like effects on behavior in adult, WT animals. Interestingly, however, IL-1R1 KO littermates did not display behavioral alterations following MS. In mice sacrificed at P15, we observed an increase in 5-HT turnover in both the hippocampus and blood of WT MS mice compared to NR littermate counterparts. IL-1R1 KO mice failed to demonstrate MS-induced increases in 5-HT turnover relative to NR IL-1R1 KO mice. IL-1Rflox/flox:ePet-1:Cre animals are viable with no gross evidence of developmental perturbations and are now being evaluated for biochemical and behavioral changes post MS.

Conclusions: Previous studies from our lab have shown that IL-1β signaling can modulate SERT activity in adult WT but not IL-1R KO mice, and that SSRIs can reverse MS-induced alterations in behavior (Yoo et al, 2013). Additionally, we recently reported that raphe-specific elimination of the IL-1β signaling enzyme p38 MAPKα prevents the induction of anxiety and depressive behavior following peripheral LPS treatments (Baganz et al, 2015). Here we provide evidence that MS leads to lifelong alterations in cytokines and CORT. In addition, MS produces short and long-term changes in 5-HT turnover and behavior in a manner that is dependent on expression of IL-1R. These findings are consistent with clinical reports showing elevated plasma 5-HT turnover in non-medicated patients diagnosed with major depression (Barton et al, 2008). To our knowledge, these studies are the first to show ELS-induced increases in adult cytokine levels and altered 5-HT turnover and suggest that IL-1R signaling pathways may be required for the effects of ELS on behavior. Ongoing studies using IL-1Rflox/flox:ePet-1:Cre transgenic to afford excision of IL-1R specifically in 5-HT neurons are being used to examine whether serotonergic IL-1R signaling is required for the biochemical and behavioral developments induced by ELS.

Keywords: Serotonin, Early-life Stress, Depression, Cytokine.
Disclosure: Nothing to disclose.

M58. Rapastinel (Glyx-13), a Rapid Acting Antidepressant, Exhibits Co-Agonist Properties at NMDA Receptors Independent of the Glycine Co-Agonist Binding Site

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Background: Multiple lines of investigation suggest that modulation of the glutamatergic system may produce a rapid and sustained clinically relevant antidepressant effect, but drugs like ketamine, which induce side effects including dissociation and nausea, may limit clinical use. Rapastinel (formerly GLYX-13), a putative NMDA receptor (NMDAR) modulator with glycine-like partial agonist properties, promises a clinical profile of rapid antidepressant activity with a better side effect profile compared with ketamine. Rapastinel is currently in late-stage clinical development as an adjunctive therapy for Major Depressive Disorder. At a molecular level, Rapastinel enhances NMDAR function, as evidenced by potentiation of [3H]MK-801 binding (Moskal et al, 2005) and NMDAR-mediated synaptic currents, and increases in the magnitude of LTP in hippocampal slices (Zhang et al, 2008). Paradoxically, a single infusion of the non-competitive NMDA receptor antagonist ketamine also rapidly alleviates depression symptoms in treatment-resistant depressed patients, and these effects may be sustained for more than a week. However, ketamine is a drug of abuse and ketamine blockade of NMDAR induces significant CNS adverse effects including psychotomimetic effects. Given Rapastinel's unique NMDAR-dependent mechanism of action, we further evaluated Rapastinel's mechanism of action using ligand binding to various targets, functional assays including enhancement of [3H]MK-801 binding in HEK cells expressing human NMDAR subunits and in an isolated neuron cellular assay for calcium imaging.

Methods: Radioligand binding/displacement from 16 different NMDA and non-NMDA receptor binding sites by Rapastinel, (R, S) ketamine, and specific ketamine enantiomers was assessed using a customized Cerep panel. Functional glycine site co-agonism was measured using [3H]MK-801 potentiation assays in membrane extracts prepared from human NR2A-D subtype-expressing HEK cells. For calcium imaging studies, rat brain cortical cells (E18, 19) were grown on 12 mm poly-D-lysine coated glass coverslips and loaded with calcium indicator Fluo-4 AM at 10-21 days post-seeding and mounted on the stage of an Olympus BX61WI upright microscope equipped with confocal laser scanning system. All test articles were dissolved in Mg2+-free extracellular medium (containing TTX and NBQX) and applied to the cells using a customized rapid drug application system that exhibits a 90% solution exchange time in less than a second. The change in fluorescence by 10 μM NMDA or NMDA plus Rapastinel was compared with 10 μM NMDA + 3 μM D-serine which produced maximum signal under the current experimental conditions. The effect of Rapastinel was normalized to the signal induced by NMDA "alone".
Results: Rapastinel (30 μM) did not exhibit appreciable affinity for any of the NMDA (including the PCP binding site within the NMDAR channel or the glycine site) or non-NMDA binding sites. By contrast, ketamine (and its enantiomers) exhibited significant affinity for the NMDAR/PCP site and opioid receptors (μ and κ). Rapastinel potentiated [3H]MK-801 binding in HEK cells expressing recombinant human NR1 and NR2B receptors as well as in HEK cells expressing NR1-specific mutations that ablate the glycine co-agonist site. In cultured rat cortical neurons in vitro, NMDA (10 μM) induced a small but significant increase in intracellular calcium in the absence of exogenously added D-serine or glycine. The NMDA induced signal was antagonized by the NMDAR antagonist, D-AP5 (50 μM). The magnitude of NMDA-induced intracellular calcium signal was potentiated by D-serine (50-3000 nM in a concentration-dependent manner. The basal and exogenous D-serine potentiated NMDA-induced calcium response were antagonized by NMDAR glycine site antagonist, 7-CK (7-chlorokynurenic acid, 300 μM). Rapastinel (10-300 nM) alone did not increase intracellular calcium. However, co-application of Rapastinel with 10 μM NMDA produced ~30% potentiation of the NMDA-induced calcium signal. This effect of Rapastinel was sensitive to blockade by the competitive NMDAR antagonist AP5 but not 7-CK.
Conclusions: Rapastinel significantly differs from ketamine and its enantiomers in terms of its affinity for NMDA and non-NMDA binding sites. Rapastinel acts directly on NMDA receptors to activate a high affinity/low efficacy co-agonist effect when co-applied with NMDA. Rapastinel maintains activity if the glycine co-agonist site is pharmacologically or genetically ablated, suggesting Rapastinel functions as a NMDAR co-agonist independent of the glycine co-agonist site. This novel mechanism of action may provide significant advantage over NMDAR antagonists like ketamine for the treatment of depression.
Keywords: NMDA Receptor, Calcium Imaging, Mechanism of Action.
Disclosure: Allergan, Inc.: Employee, Self.

M59. White Matter Abnormalities Associated With the Development of Substance Use in Bipolar Disorder

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Background: Individuals with Bipolar Disorder (BD) show high rates of comorbid substance use disorders (SUDs) yet it is unclear how this comorbidity develops. There has also been limited study of gender-specific mechanisms that may contribute to the development of this comorbidity although gender-specific mechanisms contributing to the development of SUDs and of mood disorders are well established. In this preliminary study we investigate white matter differences associated with development of substance use problems in BD during adolescence, a critical period in the development of BD and of SUDs, and explore if associations differ for females and males.
Methods: Adolescents with BD and minimal alcohol/substance exposure ($n = 27$, 44% female, meanage+stdev = 16.1+2.2 years) completed diffusion tensor imaging. Subjects completed the CRAFFT interview, on average six years later, to assess alcohol and substance use problems. Subjects were categorized into two groups: individuals with total CRAFFT scores meeting threshold for alcohol/substance problems and individuals not meeting threshold. Group differences in fractional anisotropy (FA) were assessed and gender-related associations in FA explored.
Results: For the overall group, decreased right frontal white matter FA was associated with higher CRAFFT scores at follow-up ($p < 0.005$, clusters > 20 voxels) and associated with baseline depression, with results driven by females.
Conclusions: These preliminary results suggest decreased frontal white matter FA is associated with future substance use in female adolescents with BD and suggest sexual dimorphism in white matter abnormalities associated with substance use development.
Keywords: Mood Disorders, Adolescence, Diffusion Tensor Imaging, Substance-related Disorders, Sexual Dimorphism.
Disclosure: Nothing to disclose.

M60. Sleep Quality and Depressive Symptoms are Differentially Associated With Cognitive Changes Among HIV+ and HIV- Adults

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Background: Sleep quality has been associated with health, stress response, satisfaction with life, and feelings of tension, depression, anger, fatigue, and confusion1,2, 3. Moreover, depression has been associated with greater self-reported sleep problems4,5,6. Poor sleep health may also signal cognitive decline7,8,9 and is a common problem among individuals with HIV-infection10. Given the well-documented association between HIV-infection and cognitive outcomes11,12, it is important to investigate sleep

quality and depression are associated with cognitive outcomes in this population. This study examined how changes in depression symptoms and sleep quality are associated with cognitive changes over the course of 18-24 months, and if this relationship is different in the context of HIV infection.
Methods: Participants consisted of 39 HIV+ and 30 HIV- adults recruited from local HIV clinics, participant social networks (e.g., friends), and community advertisements in the Greater Los Angeles area.
Measures: Sleep quality: Subjective sleep quality was assessed at follow-up visit (18-24 months from baseline) using the 6-item Regularity, Satisfaction, Alertness, Timing, Efficiency, and Duration (R U SATED?) scale of sleep quality12. Each item is scored on a scale from 0-2, with higher scores reflecting good sleep health. Depression: Depressive symptom severity was measured using the Beck Depression Inventory-II. The Cognitive-Affective subscale was calculated using the first 13-items and the Somatic-Performance subscale was calculated using items 14-21, with item 16 (sleep item) removed. Reliable change: Depression & Cognition: The cut-off for reliable change ($>$ 4 BDI points) was determined by calculating 1.96 times the standard error of the difference between scores of a given measure administered on two occasions. We categorized participants as experiencing a reliable worsening of depression ($\geq$ 4 BDI point increase), no reliable change (less than 4 point change in either direction), and reliable improvement in depression ($\geq$ 4 BDI point decrease). Reliable change indices were performed across all cognitive domains to correct for practice errors (Heaton et al, 2001).
Results: Depression: HIV+ participants had significantly greater total BDI-II scores than HIV- participants, $F = 6.49$, $p = 0.01$. Similar group differences were found in the Cognitive-Affective, $F = 3.89$, $p = .05$ and Somatic-Performance subscales, $F = 6.35$, $p = .01$. There were no significant HIV group differences in reliable change scores for the Cognitive-Affective or Somatic-Performance subscales of the BDI-II or cognitive performance. Sleep quality: While HIV+ participants reported lower scores on subjective sleep quality ($M = 7.15$; $SD = 2.80$) than HIV- participants ($M = 8.06$; $SD = 2.43$), this difference did not reach statistical significance ($p = .12$). Sleep quality, depression, and cognition: For the entire sample, there was a significant association between sleep quality scores and changes in somatic depressive symptoms, $F = 3.079$, $p = .05$, but not cognitive affective symptoms ($p > .10$). Specifically, individuals who reported better sleep quality at follow-up showed no change depressive symptoms ($M = 7.94$; $SE = .35$) versus those who reported a worsening of symptoms ($M = 5.93$; $SD = .83$). There was a significant association between subjective sleep quality and global cognitive change, $r = .304$, $p = .013$. Regression analyses: Linear regression was used to determine the independent associations of sleep quality and somatic depressive symptoms on cognitive change scores stratified by HIV group. The overall model was significant ($p = .010$) for the HIV+ group, but not in the HIV- group. Examination of standardized beta coefficients revealed that sleep quality ($B = .229$) and changes in somatic symptoms ($B = -.226$) each contributed to cognitive change outcomes. For the HIV- group, examination of standardized beta coefficients revealed that sleep quality ($B = .229$) was the only factor significantly associated with global cognitive change.
Conclusions: While the results are consistent with previous reports of relationships between subjective sleep quality, cognition, and symptoms of depression, they add to the extant literature by identifying a subset of depressive symptoms (i.e., somatic) that, when increased, are associated with cognitive changes and sleep quality. Our results suggest that changes in depressive symptoms and sleep quality are associated with cognitive changes for HIV+ individuals, whereas only poor sleep quality was associated with cognitive change for HIV- participants. Therefore, clinicians and other healthcare providers working with HIV+ individuals should be aware of how poor sleep and changes in somatic symptoms may signal cognitive decline.
Keywords: Sleep Disturbance, HIV, Depression, Cognitive Functioning.
Disclosure: Nothing to disclose.

M61. The Neural Basis of Cognitive and Emotional Processing in Persistently Depressed Patients Treated With Desvenlafaxine

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Background: Numerous investigations have reported altered cognitive and abnormal emotional processing in major depressive disorder (MDD). However, it is unclear whether similar abnormalities are present in patients with dysthymic disorder (DD), a common type of persistent depressive disorder that is associated with significant disability. Moreover, the effects of antidepressant medications on the neural basis of cognitive and emotional processing in DD patients are poorly understood. Our objective was to map the neural basis subserving cognitive and emotional processing in DD patients vs. healthy control (HC) subjects, and to identify brain activation correlates of antidepressant treatment and symptomatic response.
Methods: Clinical trial: In a research clinic (Depression Evaluation Service, NY State Psychiatric Institute), we enrolled adult outpatients with SCID diagnosis of dysthymic disorder (DD) (DSM-5 Persistent Depressive Disorder without current major depression) in a double-blind placebo controlled clinical trial. We also matched 25 DD patients with 11 HC subjects by age and sex. At baseline, we obtained functional magnetic resonance imaging (fMRI) scans of two groups–DD patients and HC subjects. DD patients underwent a 12-week prospective, double-blind, placebo-controlled trial of the serotonin-norepinephrine reuptake inhibitor medication desvenlafaxine, with fMRI scans repeated at week 12. Scans included an event-related fMRI paradigm based on the circumplex model of affect, which interprets all emotions as linear combinations of two independent neurophysiological dimensions, valence and arousal. Subjects were presented emotionally evocative sentences, and they then rated arousal and valence according to the emotional experiences induced by these sentences. Lastly, we assessed the associations of the neural activity with subject rating of arousal and valence.

Main Outcome Measures: We used fMRI to map the neural bases that subserve cognitive and emotional processing in DD patients compared with HC subjects. An analysis of variance identified brain regions for which there were significant treatment X time interactions (i.e., attributable to antidepressant treatment, not placebo). We calculated Pearson correlations between brain activity and depressive symptoms in DD patients who underwent a 12-week prospective, double-blind, placebo-controlled trial, using voxelwise analyses.

Results: Compared with HC subjects, DD patients showed hyperactivity in the anterior insula (AI) and the default mode network (DMN) for the arousal contrast, and hypoactivity in cortico-striatal circuitry for the valence contrast. Following a 12 week clinical trial, activity for the arousal contrast was reduced within the precuneus, caudate, and putamen in the desvenlafaxine relative to the placebo group. In contrast, activity for the valence contrast was increased in the dorsolateral prefrontal cortex (DLPFC) in drug vs. placebo-treated patients. Activity in the inferior frontal gyrus (IFG) showed a significant correlation with the first age at onset of depressive illness (1stAge). Dysfunction of the precuneus at baseline was normalized by antidepressant treatment, but not by placebo, on the arousal contrast, but not the valence contrast.

Conclusions: Our findings demonstrate the involvement of the AI and DMN, striatal system, DLPFC, and IFG underlying DD. They suggest that some regions may be involved in the pathophysiology of both acute and chronic depressive disorders while others may be more specific to chronic depressive disorders. A significant association between activity in the IFG and the 1stAge suggests that dysfunction of the IFG may be an important feature in the pathophysiology of chronic depression. Converging evidence across the baseline comparisons, treatment X time interactions, and correlation analyses imply that the precuneus may act as a crucial action site of antidepressant medications.

Keywords: Depression, Functional MRI (fMRI), Clinical Trial, Psychopharmacology.

Disclosure: Pfizer, Inc.: Research Grant Support, Self.

M62. Perinatal Exposure to the SSRI Paroxetine Increases Offspring's Depression-Like Behavior Through Changes in Hippocampal DNA Methylation

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Background: Selective serotonin reuptake inhibitor (SSRI) antidepressants are the mainstay treatment for the 10-20% of pregnant and postpartum women who suffer major depression, which, in turn, exposes tens of thousands of children annually to serotonergic agents during crucial developmental periods. SSRIs are generally considered safe, with minimal risk for pregnancy complications or teratogenic risk, although accumulating evidence suggests that in utero antidepressant exposure perturbs neurodevelopment and may elicit detrimental effects on offspring's' neuropsychological health. These findings in humans are consistent with data in rodents showing that manipulating serotonin in the developing brain negatively impacts lifelong emotional behavior. For example, neonatal SSRI exposure produces disturbances within multiple behavioral domains that are typically associated with mood disorders, including increased helplessness in the Forced Swim Test (FST), enhanced anxiety-like behavior, anhedonia, perturbed sleep, and diminished sexual performance. Perinatal SSRI exposure also leads to abnormal sensory perception and sociability, suggesting that perturbing early-life serotonin transmission is relevant to neurodevelopmental disorders like autism. Interestingly, both human and rodent studies suggest that certain individuals are more vulnerable to perinatal SSRI exposure than others, but the mechanisms driving this differential susceptibility are unknown, representing a critical barrier in the field. Our research group recently showed that Sprague-Dawley rats selectively bred for high versus low novelty seeking traits exhibit differential susceptibility to perinatal SSRI exposure, with low novelty responding (LR) rats displaying increased FST immobility in response to early life SSRI exposure while high novelty responder (HR) rats were unaffected (Glover et al, Neuroscience 2015).

We also conducted a transcriptome profiling study showing marked SSRI-induced gene expression changes in the early postnatal hippocampus, including decreased expression of DNA methylation-related genes Dnmt3a and Mecp2. Our new experiments are testing the working hypothesis that the adverse behavioral consequences of perinatal SSRI exposure are mediated by perturbed DNA methylation in the early postnatal hippocampus.

Methods: We hypothesize that perinatal SSRI exposure downregulates DNA methylation in the early postnatal hippocampus and that this may provide a molecular switch that triggers adverse downstream effects on neurodevelopment and emotional behavior. To test this, the present study examined global DNA methylation (5-methylcytosine) levels in brains of neonatal rats that were perinatally exposed to the SSRI paroxetine. Because we previously showed that LR rats are susceptible to behavioral effects of perinatal SSRI exposure while HRs are resistant, we predicted and indeed found that SSRI exposure selectively affected DNA methylation in brains of LR offspring. Using next-generation sequencing, we interrogated gene-specific methylation changes in the hippocampus of perinatal SSRI-exposed LR offspring. Our final experiment tested the hypothesis that siRNA-mediated suppression of Dnmt3a expression in the hippocampus of early postnatal rats would elicit adverse behavioral consequences (e.g., enhanced FST immobility) akin to what occurs following perinatal SSRI exposure.

Results: Perinatal SSRI exposure led to decreased hippocampal DNA methylation levels specifically in LR offspring relative to vehicle-exposed LR controls; notably no changes were observed in other limbic brain regions. HR offspring were not affected by SSRI exposure, with both SSRI- and vehicle-exposed HR groups showing similar DNA methylation levels. Next generation sequencing analyses identified numerous gene-specific methylation differences in the hippocampus of perinatal SSRI-exposed rats, with a preponderance of hypomethylated genomic regions in the Cornu Ammonis (CA) and dentate gyrus subregions. Results of our siRNA study showed that transient knock-down of Dnmt3a in the early postnatal hippocampus lead to greater depression-like behavior (FST immobility) in adult offspring

with no effect on anxiety-like behavior. These results were highly reminiscent of the behavioral consequences of perinatal SSRI exposure.
Conclusions: This study found that perinatal SSRI exposure induced DNA methylation changes in the early postnatal hippocampus, which may drive adverse behavioral consequences such as increased adult depression-like behavior. These SSRI-induced methylation changes were associated with reduced neonatal hippocampal expression of the DNA methylating enzyme, Dnmt3a, and we found that siRNA-mediated Dnmt3a knockdown in the neonatal hippocampus recapitulated the behavioral effects of early-life SSRI exposure in both male and female rats. Our results may have important implications on the use of SSRIs during pregnancy and on the etiology of depression in general, as early-life changes in epigenetic mechanisms disrupt normal hippocampal development, leading to an increased risk for depression in adulthood.
Keywords: SSRI, DNA Methylation, Neurodevelopmental Disorders, Hippocampus, Depression.
Disclosure: Nothing to disclose.

M63. The Photic Regulation of Arousal and Mood (PRAM) Pathway: Chemogenetic Manipulation of a Retina-Brain Circuit Involved in Affective Disorders

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Background: Research indicates a profound effect of light on mood. A specific type of depression, seasonal affective disorder (SAD), is associated with decreased light availability. In rats, short day-length lighting schedules induce depression- and anxiety-like behavior, and chronic light-deprivation also leads to a depressive phenotype. Our lab has previously shown that this light-deprivation induced depressive phenotype is due to a locus coeruleus-noradrenaline (LC-NA)-dependent mechanism (Gonzalez and Aston-Jones, PNAS, 2008), where light-deprivation induces apoptosis in LC monoamine cells, and loss of noradrenergic LC cortical fibers in frontal cortex (found also in other animal models of depression). To determine the photic circuit underlying this phenotype, we examined the role of retinal ganglion cells (RGCs) on mood and LC-NA cell apoptosis.
Methods: Experiment 1. Male Sprague-Dawley rats received bilateral intraocular injections of either excitatory designer receptor exclusively activated by designer drugs (DREADDs: AAV2-hSyn-HA-hM3D(Gq)-mCitrine; $n = 11$) or control virus (AAV2-hSyn-EGFP; $n = 11$). Following virus expression (4 weeks), all rats were placed in continuous darkness for 6 weeks, and were concurrently subjected to daily intraperitoneal (i.p) injections of clozapine-N-oxide (CNO), the DREADD-activating ligand. Rats were then subjected to assays of mood (saccharin preference test, elevated plus maze and forced swim test) and vision (electroretinagram: ERG) before being anesthetized and perfused. Brains were sectioned and LC tissue was stained for both Poly ADP ribose polymerase (PARP) and tyrosine hydroxilase (TH, a marker of NA neurons).
Experiment 2. To determine the retinal cell-type responsible for dark-induced depression-like behavior, intrinsically photosensitive retinal ganglion cells (ipRGS) were bilaterally ablated using a targeted saporin toxin that selectively recognizes and eliminates melanopsin-expressing cells (Mel-SAP; $n = 7$). A second group of rats received bilateral intraocular injections of vehicle (0.01 M PBS; $n = 5$). Ten weeks following intraocular injections, rats were subjected to identical behavioral assays of mood, and immunohistochemical analysis as rats in Experiment 1.
Results: Experiment 1. ERG analysis showed that CNO (i.p.) increased retinal activity in animals with excitatory DREADDs expressed in retina. Constant darkness induced a depression-like phenotype in control animals, which was reduced in DREADD animals given CNO.
Experiment 2. Mel-SAP induced a depression-like phenotype as measured by a reduced preference for saccharin and increased immobility during the forced swim test. Mel-SAP had a slight anxiolytic effect on behavior, as measured by the elevated plus maze. Mel-SAP was also associated with increased apoptosis in LC-NA cells as seen with increased PARP staining.
Conclusions: A loss of ipRGC outflow may induce neural damage in LC-NA neurons, and this damage is associated with a depressive behavioral phenotype. Recovery from darkness-induced depression by DREADD activation of RGCs has implications for therapy to treat at least some types of depression.
Keywords: Depression, Anxiety, Locus Coeruelus, Retina, Light Therapy.
Disclosure: Nothing to disclose.

M64. Association Between Molecular Markers of Neuroendocrine Function and Cellular Metabolism With Early Life Stress and Psychopathology

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Background: Promoter methylation of the glucocorticoid receptor (GR) gene (NR3C1) is a proposed mechanism by which early stress may impact neuroendocrine function. Mitochondria are key to cellular stress responses and recent evidence shows that mitochondrial DNA copy number (mtDNAcn) is increased in adults with a history of early stress or psychopathology. No prior work has examined the role of NR3C1 methylation in this association.
Methods: Adult participants ($n = 395$) without lifetime bipolar, obsessive-compulsive, or psychotic disorders completed diagnostic interviews and questionnaires to characterize early stress and lifetime psychiatric symptoms. Medical conditions, current substance abuse, and prescription medications other than oral contraceptives were exclusionary. Individuals were categorized according to presence or absence of early stress (ncase = 213; ncontrol = 182) and threshold psychiatric disorders (ncase = 170; ncontrol = 225). Whole blood mtDNAcn was measured using qPCR; pyrosequencing detected NR3C1 methylation. Age and

telomere length were included as covariates given prior literature regarding their effects on mtDNAcn. Multiple regression and bootstrapping procedures tested NR3C1 as a mediator of effects of psychopathology/early stress on mtDNAcn.
Results: NR3C1 methylation was found to be a mediator of the link between early stress and mtDNAcn (95% CI: .0042 to .026) and a partial mediator of the link between psychopathology and mtDNAcn (95% CI: .0046 to .026). We found a significant positive association between early stress and mtDNAcn ($p = .05$) and between psychopathology and mtDNAcn ($p = .002$). When including NR3C1 methylation in the model with conditional testing, early stress was no longer significantly associated with mtDNAcn ($p = .18$), and the relationship between psychopathology and mtDNAcn became less robust ($p = .013$).
Conclusions: This is the first study examining associations between molecular markers of neuroendocrine function and mtDNAcn. GR signaling may be part of a mechanism by which mtDNAcn is altered with early stress and psychopathology.
Keywords: Early Life Stress, Epigenetics, Mitochondria.
Disclosure: Nothing to disclose.

M65. Differential Regulation of NPY and CGRP in CSF in Patients With Major Depressive Disorder and Parkinson´s Disease Patients With Co-Morbid Depression

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Background: Depression is common at all stages of Parkinson's disease (PD), ranging between 10 and 70% mainly depending on the criteria used for defining depression (Aarsland et al, 2010). There is a poor understanding of the pathophysiology underlying depression in PD, resulting in a lack of consensus on the therapeutic antidepressant strategies. Hypofunctional dopamine neurotransmission does not only cause movement dysfunctions in PD, but also anhedonia and loss of motivation which characterize depression in PD. However, depression is also seen in PD patients adequately treated with dopamine replacement, indicating that additional mechanisms are involved. Biological research in major depressive disorder (MDD) has provided a wealth of evidence that in addition to monoamine deficiency, stress hormones, immune mediators, neurotrophic factors and neuropeptides underlie different aspects of the depressive symptomatology (Krishnan and Nestler, 2010). In particular, reduced expression of neuropeptide Y (NPY) and, in part, altered calcitonin gene-related peptide (CGRP) play a role (Heilig et al,2004; Husum, Mathé 2002; Husum et al,2002 and 2006; Nikisch et al,2012; Sandberg et al,2014; Soleimani et al,2014; Wu et al,2011; Wörtwein et al,2006). To increase our understanding of the biochemical basis of depression in PD patients, we examined the levels of the NPY and CGRP in cerebrospinal fluid (CSF) from PD patients with or without comorbid depression and compared them to the levels in patients with MDD.
Methods: Non-demented age and gender matched PD patients with ($n = 11$) or without ($n = 13$) comorbid depression and 10 patients with major depression were included in the study. The severity of the depression was scored on the Montgomery–Åsberg Depression Rating Scale (MADRS), whereas Unified Parkinson's disease rating scale (UPDRS) was used to assess PD. A standardized LP procedure was performed at the L4-5 level with the patient in a supine position. Between 12 and 15 ml CSF from the first portion was collected in order to minimize the gradient influence. All CSF samples were aliquoted and stored at -80° C until assayed. NPY and CGRP were measured by RIA (Husum, Mathé 2002; Wörtwein et al,2006; Sandberg et al, 2014). Data were analyzed with one-way ANOVAs followed by Bonferroni´s test for pairwise comparisons (GraphPAD Prism).
Results: There was no significant difference in severity of depression according to MADRS between PD patients with co-morbid depression (17.2 + 4.1) and MDD patients (20.5 + 5.5).
The levels of NPY in PD patients with or without comorbid depression and in patients with MDD were 83.1+7.9, 64.4 +6.3 and 46.8+5.3 pmol/L, respectively. These levels are comparable to previous reports (e.g. Sandberg et al, 2014). A one-way ANOVA revealed that the NPY levels differed between the groups ($F2,31 = 6,99$; $P < 0.003$) and post hoc analysis demonstrated significantly ($p < 0.001$) higher levels of NPY in depressed PD patients when compared to patients with major depression. The levels of CGRP in PD patients with or without comorbid depression and in patients with major depression were 11.7+0.5, 11.6+0.6 and 8.5+0.2 pmol/L, respectively. These levels are comparable to previous reports (e.g. Mathé et al, 2002). Similar to the NPY data, a one-way ANOVA revealed that the CGRP levels differed between the groups ($F2,32 = 9.34$; $P < 0.001$) and post hoc analysis demonstrated significantly ($p < 0.001$) higher levels of CGRP in depressed PD patients when compared to MDD patients.
Conclusions: These data show that both NPY and CGRP levels are higher in CSF in patients with PD and comorbid depression when compared to MDD patients. In comparison to MDD, PD patients with comorbid depression have less thoughts of guilt or suicidal ideation. NPY levels are reduced in brain and CSF from patients with suicidal attempts and ideation (Caberlotto and Hurd 2001; Heilig et al,2004; Sandberg et al, 2014). Our data therefore raise the possibility that the difference in NPY and CGRP levels in CSF between PD depression and MDD reflect the phenotype differences in depression between the patient groups and particularly the likelihood to attempt (or actually commit) suicide. Increased CSF levels of CGRP in depressed patients and in a rat model of depression have been reported and a negative correlation between NPY and CGRP in brain regions of relevance for mood disorders have been found in a rat model of depression (Mathé et al,2002, Husum et al,2002, Wörtwein et al 2006).
In summary, we found altered NPY and CGRP levels in CSF of PD patients with depression; the changes were different from those found in MDD patients indicating different pathophysiologies leading to depression phenotype in PD and MDD.
Keywords: Major Depression, Parkinson's Disease, Cerebrospinal Fluid, Neuropeptide Y.
Disclosure: Nothing to disclose.

M66. Efficacy and Safety of Intravenous Low-Dose Ketamine for Depression in an Academic Clinical Practice

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Background: Several recent studies have demonstrated that a single infusion of low dose ketamine, significantly reduces symptoms of depression in patients with treatment resistant depression (TRD) with a typical onset of action within 24 hours. These findings have led to great interest in the possibility that ketamine, or another drug designed to mimic ketamine's mechanism of antidepressant action, may represent an effective and rapid treatment for TRD. However, subjects in the aforementioned prospective published studies were met restrictive inclusion and exclusion criteria. For example, most of these studies did not allow, or placed severe limitations on concomitant psychotropic medications that subjects could be taking. A history of suicidal behavior, active suicidal ideation or comorbid psychiatric conditions were also typically exclusionary these studies. Several studies have shown that subjects recruited for clinical trials in depression are poorly representative of the depressed outpatient population. Indeed, one study estimated that 86% of outpatients with clinical depression would not qualify for a typical clinical trial in depression due to exclusion criteria and that those who would be excluded were on average more chronically ill, had more previous episodes of depression, and had greater psychosocial impairment and personality pathology than subjects in clinical trials for depression. Given the strong interest in ketamine's potential as a treatment for TRD, which is based on the highly positive results obtained in clinical trials conducted to date, it is important to investigate its efficacy and safety in patients who are representative of the TRD population. Based on the published studies suggesting strong efficacy and safety of ketamine for TRD, one of the authors (DF) initiated an outpatient ketamine treatment program for TRD patients several years ago at University of California at San Diego (UCSD). As a clinical program, the criteria for acceptance of patients for ketamine infusion treating was based on the prescribing psychiatrist's determination of a favorable risk vs benefit formulation, rather than the type of formal restrictive criteria employed in prospective research studies. This paper, describes the results of a retrospective analysis of the safety and efficacy outcomes of the index ketamine infusion administered to the first 50 patients in this clinical program.
Methods: The initial ketamine infusion administered to patients was 0.5 mg/kg over 40 minutes and it was administered in an outpatient infusion setting at UCSD in the absence of an anesthesiologist. Patients were not required to wash off medication prior to receiving their ketamine infusion. Furthermore, suicide risk did not make patients ineligible and in general enhanced patient's eligibility for ketamine treatment owing to its strengthening the patient's benefit to risk formulation.
A retrospective chart review of the first 50 TRD patients treated with low-dose IV ketamine at UC San Diego was conducted. Charts were included in the analysis, if the patient was at least 18 years old and containing a completed baseline, 1-hour, and 24-hour post infusion Beck Depression Inventory (BDI). Demographic and clinical variables were analyzed with descriptive statistics. The primary outcome was reduction in BDI score from baseline to 24-hours determined by a paired t-test. Secondary outcomes included reduction in BDI 1-hour post-infusion, response ($>$ 50% reduction in BDI score) and remission (BDI score $\leq$11) at 24 hours. Patients who were responders at 24 hours were also asked to complete a BDI at 7-days post infusion.
Results: Forty-one charts met the analysis inclusion criteria. Mean patient age was 48.6 $\pm$ 12.7 years, 78% white, and 36.6 % female. All patients had a diagnosis of major depressive disorder ($n=34$) or bipolar disorder ($n=7$) and had failed multiple antidepressants. Some had failed ECT (26.8%) or rTMS (24.4%). Significant psychiatric comorbidity existed in 73.1% of patients, with anxiety being most prevalent (58.5%). Concomitant medications included antidepressants (51.2%), antipsychotics (29.3%), mood stabilizers (19.5%), stimulants (26.8%), benzodiazepines (41.5%) and hypnotics (17.1%).
Average BDI score was 32.6 $\pm$ 8.4 prior to the infusion. This was higher than baseline scores reported in previous published clinical trials. BDI score was 16.8 $\pm$ 3.8 ($p<$ 0.001) and 16.1 $\pm$ 3.1 ($p<$ 0.001) at 1-hour and 24-hours post-infusion, respectively. There was a 53.7% response rate and a 41.5% remission rate at 24-hours post infusion. Seven-day post infusion BDI responses accessed from among patients who were responders at 24-hours revealed that 75% of them maintained their responder status at 7 days.
No serious adverse events occurred during infusions or in the 7-day period afterward. On average, ketamine induced a modest, transient blood pressure elevation during infusions that did not require medical intervention. Two patients required antiemetic medication for nausea and one patient required lorazepam for heightened anxiety during the infusion.
Conclusions: The naturalistic sample of TRD patients in this analysis had higher baseline symptom scores, greater suicidal risk, and more concomitant medications at the time of their ketamine treatment that typical cohorts published prospective studies. Despite this, low dose ketamine infusions demonstrated rapid efficacy and were well tolerated.
Keywords: Ketamine, Treatment Resistant Depression, Depression.
Disclosure: Nothing to disclose.

M67. Is Deep Brain Stimulation to the Medial Forebrain Bundle for Treatment-Resistant Depression Associated with Changes in Personality?

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Background: Individual reports of a change in patients´ behavior in their personal relationships during deep brain stimulation (DBS) for neurologic and psychiatric disease, have been raised the question whether DBS can induce changes in personality.

It is well known that depressive patients score differently on the Big Five dimensions of personality (extraversion, neuroticism, openness to experience, agreeableness, and conscientiousness) as compared to a healthy population. Scores in personality dimensions can differ before, during and after a depressive episode. Response to an antidepressant treatment has been shown to correlate with changes in dimensions of the Big Five personality, e.g. a reduction of neuroticism and an increase in extraversion.

In this study, Big Five personality dimensions were assessed in patients suffering from chronic, treatment-resistant depression (TRD) before (baseline) and six months after the onset of DBS (DBS condition). It was studied whether (1) the TRD sample differs from a healthy normative sample in dimensions of the NEO-FFI at baseline, (2) DBS treatment is associated with changes in personality, (3) the antidepressant response to DBS or the severity of depression during DBS (DBS condition) are related to dimensions of personality.

Methods: 21 patients (8 females, 13 males, mean age of 49 years, mean duration of current episode 10 years, ranging from 1 year to 30 years, mean age at onset 28 years, ranging from 13 to 48 years) suffering from TRD were assessed before and six months after onset of DBS to the superolateral branch of the medial forebrain bundle (slMFB). Personality was measured with the NEO-Five-Factor Inventory (NEO-FFI). Depression was assessed with the Hamilton Depression Scale (HAMD) and the Montgomery-Asberg Depression Rating Scale (MADRS). Baseline profiles have been compared to a normative healthy sample with t-test, baseline vs. DBS condition have been compared with student´s t-test, correlation analyses have been performed with pearson´s correlation coefficient $\alpha = 5\%$).

Results: No change in any personality dimension from baseline to six months follow-up (DBS condition) in the whole sample analysis was found ($p > 0.4$). All patients had a significant reduction in depression as measured by HAMD and MADRS ($p < 0.001$). Compared to the normative data, TRD Patients showed significant different scores on all five personality dimensions at baseline; higher neuroticism and agreeableness, and a reduced level of extraversion, openness to experience and conscientiousness have been found in the whole sample analysis. None of the scores in the five personality dimensions at baseline were found to predict the antidepressant outcome. The degree of antidepressant response (baseline vs. DBS condition) (Extraversion x difference MADRS: $r = -0.49$, $p < 0.05$; Extraversion x difference HAMD: $r = -0.45$, $p < 0.05$) as well as lower scores in HAMD and MADRS during DBS treatment (DBS Condition: Extraversion x HAMD $r = -0.50$, $p < 0.05$) were correlated with a higher score in extraversion, but not with other personality dimensions.

Conclusions: A significant antidepressant response after six month of DBS has been observed in the whole sample, but changes in personality related to DBS itself- as questioned in some publications- could not be objectified. As known from studies on personality in depressant patients, the severity of depression during DBS as well as the degree of antidepressant response were related to a change in dimensions of personality in the present sample. Patients who experienced an antidepressant effect from DBS, showed higher levels of extraversion after 6 months of DBS. Moreover, patients suffering from TRD differed from the healthy population in all five dimensions of personality; a typical personality profile for depression was found. Further evaluation will assess the putative protective role of a higher level of extraversion for the course of depression during the follow-up of this DBS study.

Keywords: Deep Brain Stimulation, Therapy-Resistant Depression, Big Five Personality Factors, Ethics, Medial Forebrain Bundle.

Disclosure: Nothing to disclose.

M68. Metabolic Influences on Emotion Regulation in Women

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Background: Over 70% of reproductive-age women meet the criteria for at least one component of the metabolic syndrome, a group of risk factors that include obesity, hyperglycemia, hypertension, elevated triglycerides, low HDL, and pro-inflammatory state. The metabolic syndrome and insulin resistance in particular are associated with increased risk of significant medical complications, and are also associated with a doubled risk for mood disorders. Relationships between insulin metabolism and mood regulation may be more pronounced in women than men, as reproductive and hormone status can influence susceptibility to both mood disturbances, as well as metabolic disorders, and each has higher prevalence in women than men.

The endogenous opioid system may act as an interface between metabolic and mood disorders. Insulin interacts with several neurotransmitter systems within the brain, and most hypothalamus/pituitary insulin receptors co-localize with endorphin synthesizing cells. Insulin dysregulation has been linked to behavioral changes including appetite, cognition, and mood, and is associated with decreased opioid tone in limbic mood regulation regions, consistent with our preliminary results of increased central resting μ-opioid receptor availability (binding potential, BPND) in women with insulin resistance, particularly in the nucleus accumbens and amygdala, regions where opioid neurotransmission is implicated in mood regulation.

We assessed the role of opioid neurotransmission in metabolic and emotion regulation in healthy reproductive-age women. By studying women prior to diagnosis of diabetes or depression, we hope to elucidate early factors underlying the increased risk for mood disorders related to metabolic function.

Methods: Healthy reproductive-age women (18-40 years) without diabetes or depression were recruited to participate in assessments of metabolic function (fasting glucose, insulin, and 2 hour glucose tolerance test), measures of depression, mood, and affective state (Beck Depression Inventory (BDI), Positive and Negative Affective Schedule (PANAS-X), and Profile of Mood States (POMS), and neuroimaging analyses of fMRI BOLD activation while viewing emotionally salient images, and resting PET

assessment of mu-opioid receptor availability using [11C] carfentanil tracer. Women were classified as control or insulin resistant (IR) using a homeostatic model assessment (HOMA-IR) cut-off score of 2.5. Procedures were approved by the University of Michigan Institutional Review Board and written informed consent was obtained from participants.

Results: Of the 30 women (age 19-40, mean 25) assessed in the study, 11 (36.7%) were insulin resistant. Age was similar in control and IR women (26.7 +/- 6.4 (SD) years control, 24.3 +/- 6.1 years IR). Insulin resistant women had consistently worse outcomes than controls on assessments of mood and affective state, although differences did not achieve statistical significance. IR women scored higher than controls on the BDI (2.3 +/- 2.3 control, 4.9 +/- 8.9 IR; $p = 0.236$), PANAS negative affect (11.4 +/- 1.8 control, 12.9 +/- 7.1 IR; $p = 0.407$), and POMS total negative mood score (2.4 +/- 9.1 control, 9.6 +/- 19.9 IR; $p = 0.279$), and lower on the PANAS positive affect (29.6 +/- 8.5 control, 23.1 +/- 9.4 IR; $p = 0.069$). Preliminary fMRI and PET +/–opioid analyses have been performed in 12 women (5 control and 7 IR). While viewing emotionally unpleasant images, IR women have increased regional activation in the right ventral anterior cingulate and left prefrontal cortex compared to controls, and increased resting mu-opioid receptor availability, consistent with decreased endogenous ligand binding, in the right nucleus accumbens (NAC). ROI analysis of mu-opioid receptor availabilities (BPND) in the NAC and amygdala were associated with measures of metabolic function and negative mood: insulin was positively correlated with mu-opioid BPND in NAC ($R = 0.683$, $p = 0.27$ L; $R = 0.706$, $p = 0.10$ R), and amygdala ($R = 0.618$, $p = 0.032$ L). HOMA-IR was positively associated with NAC ($R = 0.643$, $p = 0.024$ L; $R = 0.720$, $p = 0.008$ R), and amygdala (R-0.634, $p = 0.027$ L). PANAS negative affect was positively correlated with mu-opioid BPND in amygdala ($R = 0.533$, $p = 0.049$ R), POMS mood disturbance with NAC ($R = 0.509$, $p = 0.063$ L) and amygdala (R-0.481, $p = 0.082$ L), and BDI with NAC ($R = 0.495$, $p = 0.072$ L; $R = 0.515$, $p = 0.059$ R), amygdala ($R = 0.555$, $p = 0.039$ R). Activation in the amygdala while viewing emotionally unpleasant images was correlated with measures of negative mood, including PANAS negative affect ($R = 0.64$, $p = 0.03$ R), POMS mood disturbance ($R = 0.56$, $p = 0.07$ L), and BDI ($R = 0.69$, $p = 0.02$ R).

Conclusions: In our sample, IR women had more depression symptoms and worse scores on measures of mood than controls, and more activation in emotion regulation regions while viewing unpleasant images. Amygdala response to unpleasant images was associated with measures of mood. IR women also had increased NAC mu-opioid receptor availability than controls, and receptor availabilities in limbic emotion regulation regions were associated with measures of metabolic function, negative affect, and depression. In our sample of otherwise healthy women, more than 30% met criteria for previously undetected insulin resistance, illustrating the importance of considering metabolic factors in mental health outcomes.

Keywords: Metabolism, Mood, Depression, fMRI, PET.
Disclosure: Nothing to disclose.

M69. Pharmacogenetics of Escitalopram in Japanese Depressed Patients

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Background: Although several studies have tried to find the relationship between CYP2C19 genotype and steady-state plasma concentration of escitalopram, there is still controversial. We examined the impact of CYP2C19 genotype on steady-state plasma concentration of escitalopram in larger samples with Japanese depressed patients.

Methods: Subjects were 412 depressed patients with written informed consent to participate and receiving 5-20 mg once daily of escitaropram. Sample collections were conducted 12-14 h after the bedtime dosing. Plasma concentrations of escitaropram and its desmethylesciatlopram were quantitated using HPLC. CYP2C19 genotypes (CYP2C19*2 and CYP2C19*3) were identified using TaqMan assay.

Results: Although trends of gene-dose effect were observed, there was no significant difference in the steady-state plasma concentrations of escitaroplam and desmethylescitaroplam among genotype groups at each dosage. There were significant differences among genotype group in the dose-adjusted steady-state plasma concentrations and metabolite ratios in total subjects. ANCOVA including age, gender and bodyweight showed that CYP2C19 genotype were correlated with steady-state plasma concentration of escitaroplam ($p = 0.01$) and metabolite ratio ($p = 0.001$).

Conclusions: The present study demonstrates that CYP2C19 genotype has, to some extent, impact on steady-state plasma concentration of escitalopram in depressed patients.

Keywords: Escitaropram, Depression, Japanese, Pharmacogenetics, CYP2C19.
Disclosure: Japan Society for the Promotion of Research (JSPS, 15K19239, 15K19710 and 15H04754): Grant, Self; Asteras: Grant, Self; Dainippon: Grant, Self; Eli Lilly: Grant, Self; GSK: Grant, Self; Janssen-Pharma: Speaker, Self; Meiji: Speaker, Self; Mochida: Speaker, Self; MSD: Speaker, Self; Otsuka: Speaker, Self; Pfizer: Speaker, Self; Takada: Speaker, Self; Yoshitomi: Grant, Self.

M70. ECT Modulation of Nucleus Accumbens Network Dynamics in Major Depression Disorder: Biomarkers of Treatment Mechanism and Response

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Background: Major depressive disorder (MDD) is associated with dysfunction in reward system manifested psychologically by anhedonia and depressed mood. Structural and

functional abnormalities of the nucleus accumbens (NAc), one of the core nodes in reward network, have been extensively reported in neuroimaging studies of MDD. On the other hand, electroconvulsive therapy (ECT) is one of the fastest acting and most effective treatment modalities in MDD, particularly for treatment resistance depression. The main aim in this study is to explore how ECT can therapeutically modulate NAc network dynamics in subjects with depression.

Methods: Resting-state fMRI (rsfMRI) data was obtained from 42 subjects with MDD before and after right unilateral ECT treatment and also from 26 age- and sex-matched healthy controls at two time points. Right and left NAc were selected as reference seeds. Bivariate seed-to-whole-brain and whole-brain-to-seed Granger Analysis (GA) based effective connectivity (EC) analysis was applied on rsfMRI data to define brain regions receiving causal influence from NAc or have causal influence on NAc. Statistical analyses were performed to test if ECT can induce significant changes in the value of EC between NAc and interacting nodes and if those changes have any significant correlation with clinical improvement measured by Hamilton Depression Rating Scale (HAM-D) score.

Results: Our results revealed a significant ECT-induced increase in the right NAc-to-Amygdala EC in MDD group (Pre ECT: 0.09 $\pm$ 0.01 versus Post ECT: 0.23 $\pm$ 0.05; $P=$ 0.002). We also found that NAc-to-Amygdala EC change (delta EC) had a significant positive correlation with change in Hamilton Depression rating scores (delta HAMD) measured between baseline and post ECT scans ($r=$ + 0.3; $p=$ 0.02). Furthermore, our results showed a significant negative correlation between right Thalamus-to-NAc EC ($r=$ -0.31; $p=$ 0.04) and right NAc-to-Caudate EC ($r=$ -0.43 ; $p=$ 0.004) at baseline (before ECT) with change in HAMD score (delta HAMD).

Conclusions: Our results indicate that ECT can therapeutically modulate network dynamics of MDD relevant deep brain structures like nucleus accumbens. Furthermore, NAc effective connectivity with the thalamus and the caudate at baseline can be used as neuroimaging biomarkers of ECT treatment response in depression.

Keywords: ECT, Nucleus Accumbens, Resting State fMRI, Effective Connectivity.

Disclosure: Nothing to disclose.

M71. A Preliminary Report for the "REST-IT" Study: Self-Reported Eveningness and Actigraphic Delayed Sleep Timing Correlates With Suicidality in Depressed Individuals With Insomnia

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Background: Sleep disturbance has been identified as a modifiable risk factor for suicidality. However, few studies have focused on deviations in sleep timing variables, such as delayed sleep phase, when exploring this relationship. The current analyses examined relationships between self-report and actigraphic sleep timing indices and suicidality in depressed outpatients experiencing insomnia and suicidality, while controlling for depression severity.

Methods: The current sample is composed of 62 participants (mean age= 41.9 years +/- 13.2; 61% female; 72% Caucasian) from the Reducing Suicidal Ideation through Insomnia Treatment (REST-IT) study, a multisite, double-blind, randomized controlled trial examining whether hypnotic medication (zolpidem CR or placebo) along with open-label anti-depressant therapy impacts suicidality. For the current analyses, participants completed the self-reported reduced Morningness-Eveningness Questionnaire (rMEQ) at baseline, actigraphic assessment of sleep timing during the first week of treatment (average 7 days +/- 1.5), and the Beck Scale for Suicide Ideation and Hamilton Rating Scale for Depression at the end of the first week of treatment. Actigraphic assessment variables included the start and end time of the sleep period, variability in these times, sleep period duration, and acrophase (the peak of the rest-active rhythm assuming a cosinor curve). Hypnotic medication condition remained blinded. Bivariate correlations were completed between self-report and actigraphic sleep timing measures, as well as between all sleep timing measures and suicidality. Any statistically significant relationships between sleep timing measures and suicidality were further explored using multivariate regression, controlling for depressive symptom severity.

Results: Results revealed significant relationships between the rMEQ and actigraphic timing variables, including lower rMEQ scores (i.e., greater eveningness) relating to a later start ($r=$ -0.49, $p<$ .0001) and end of the sleep period ($r=$ -0.47, $p=$ 0.0002); greater variability in the start of the sleep period ($r=$ -0.29, $p=$ 0.02); and later acrophase ($r=$ -0.56, $p<$ 0.0001).

Results also revealed that lower rMEQ scores (i.e., greater eveningness), a later end time to the sleep period, and a later acrophase were related to higher levels of suicidality ($r=$ -0.30, $p=$ 0.02; $r=$ 0.32, $p=$0.01; and $r=$ 0.30, $p=$ 0.02, respectively). When controlling for depression severity, the relationship remained statistically significant between lower rMEQ scores (i.e., greater eveningness) and higher levels of suicidality ($t=$ -2.11, $p=$ 0.04), whereas the association between end time of the sleep period and acrophase with suicidality were each marginally significant ($t=$ 1.8, $p=$ 0.08; $t=$ 1.6, $p=$ 0.1 respectively).

Conclusions: At treatment start, self-report measures of eveningness and actigraphic measurement of sleep timing are significantly related in depressed outpatients with insomnia and suicidality, supporting the validity of both types of measurement in assessing sleep timing in this population. Moreover, those depressed individuals reporting greater eveningness and demonstrating more delayed timing through actigraphy reported significantly higher levels of suicidality. Only the relationship between self-reported eveningness and suicidality remained significant independent of depression severity. However, this may have been due to small sample size, as the other relationships remained at a trend level. Sleep timing measurement may be helpful in further understanding the relationship between sleep disturbance and suicidality.

Keywords: Sleep, Suicide, Depression, Insomnia, Actigraphy.

Disclosure: Merck: Grant, Consultant, Self; Jazz: Consultant, Self; Janssen: Consultant, Self.

M72. Differential Effects of the SSRIs Fluoxetine, Paroxetine, Escitalopram and Sertraline in a Rodent Model of Hypobaric Hypoxia-Related Depression

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Background: Demographic studies indicate that living at altitude (in hypobaric hypoxia) or with chronic hypoxic disorders (COPD, cardiovascular disease, smoking) increases the risk for depression and suicide. Our previous studies show that depression-like behavior (DLB) increases incrementally with altitude of housing in female rats, implying that exposure to chronic hypoxia alone could worsen depression status. We now attempt to identify whether chronic hypobaric hypoxia exposure could exacerbate treatment-resistant depression (TRD), thereby potentially increasing rates of suicidal ideation and suicide attempts. Selective serotonin reuptake inhibitors (SSRIs) are often a first-line antidepressant (AD) treatment, and SSRIs institute over 80% of the ADs prescribed in the US. However, hypobaric hypoxia is linked to brain serotonin deficits, and SSRIs have been shown to lose AD efficacy in previous animal models for low brain serotonin. We therefore examined whether housing in hypobaric hypoxia could alter the AD efficacy of SSRIs in an animal model for hypoxia-related depression.
Methods: Sprague Dawley rats were housed for a week at altitudes of sea level (SL), 4,500ft (4.5K, local conditions) or 10,000ft (10K). Rats were then treated with ADs, and tested for DLB in the modified forced swim test (FST). SSRIs tested include fluoxetine (FLU, Prozac®, 20 or 40mg/kg), paroxetine (PAR, Paxil®, 20mg/kg), escitalopram (ESC, Lexapro®, 20mg/kg) and sertraline (SER, Zoloft®, 10mg/kg). The tricyclic AD (TCA) desipramine (DES, 10mg/kg) was used as a positive control, and vehicle controls (C) received saline injections. AD doses were chosen as optimal doses shown to have AD efficacy in the rat FST in previous studies (Borsini & Meli, 1988. Psychopharmacol 94:147; Borsini 1995. Neuro Biobehav Rev 19:377.) In addition, FLU was tested at both an optimal dose of 20mg/kg and a high dose of 40mg/kg.
Behavior in the FST was scored for swimming, climbing and immobility, and latency to immobility (LTI) is measured. DLB in the FST is defined by more immobility and a shorter LTI. Effective ADs cause a 20% drop in immobility and increase in LTI (Borsini & Meli, 1988), by increasing activity. SSRIs increase swimming, while noradrenergic ADs such as DES increase climbing in the FST.
Results: (1) AD efficacy of SSRIs vs. DES at Altitude: In females, the TCA DES showed strong AD efficacy at each altitude. DES reduced immobility by 30%, and increased LTI by 50% ($n =$ 10-15). The SSRIs FLU, PAR and ESC however did not exhibit AD efficacy in females in all three altitude groups: time spent immobile and LTI did not improve with treatment ($n =$ 10-15). In preliminary studies ($n =$ 5-10), the fourth SSRI tested, SER showed AD efficacy in female rats at SL, 4.5K and 10K. SER decreased immobility by 50% and increased LTI by about 50% in all three altitude groups. (2) SSRI Effects on Active Behavior in the FST: The SSRIs FLU and ESC did not alter swimming behavior in the FST, while both PAR and SER significantly increased swimming at SL and 4.5K but not at 10K. FLU had no impact on climbing behavior, while both PAR and ESC significantly decreased climbing at all three altitudes, thereby creating a net zero effect on total activity in the FST. SER however increased swimming by 50% at all three altitudes, and showed a trend towards increasing climbing at SL. (3) Gender Effects of SSRIs vs. DES: Since our prior studies had shown significant gender-based differences in the impact of altitude on DLB in the FST, we also tested male rats for SSRI function in this model. Male rats in all three altitude groups showed no response to FLU, vs. saline controls. Studies are in progress to determine the AD efficacy of the TCA DES and the SSRI SER in male rats.
Conclusions: (1) In previous studies, we had shown that the SSRIs FLU, PAR and ESC did not exhibit AD efficacy in all three altitude groups in our model. Pilot studies imply that a fourth SSRI, SER does exhibit AD efficacy in the FST after a week of housing rats at altitudes of sea level, 4,500ft or 10,000 ft. (2) These SSRIs have been documented to vary pharmacologically in their effects on brain serotonin, dopamine and norepinephrine in different brain regions linked to depression (Preskorn 1996. Clinical Pharmacology of SSRIs). In addition, housing at altitude alters brain neurochemistry, and brain monoamines are particularly vulnerable to hypoxia (Katz 1982. Brain Res. 231:399; Ray et al, 2011. Neurochem. Intnl 58:112). Hypoxia-induced changes in brain chemistry are expected to be responsible for the altered response to SSRIs seen in our model. Studies are in progress to analyze rat brain monoamine levels in our model of hypoxia-related depression. (3) Since over 80% of AD prescriptions in the US are for SSRIs, unresolved depression in SSRI-treated MDD patients could potentially account for the higher rates of suicidal ideation and suicide attempts observed in people exposed to chronic hypoxia. These data suggest that sertraline may be the preferred SSRI for prescription to people exposed to chronic hypoxia via living at altitude or with chronic hypoxic diseases. (4) Future studies will assess the efficacy of noradrenergic ADs as well as novel non-traditional ADs in this animal model of hypoxia-related depression.
Keywords: Depression, Altitude, Hypoxia, Forced Swim Test, SSRI.
Disclosure: Nothing to disclose.

M73. Cortisol Response Predicts Magnitude of Suicide Ideation Increases to Life Events

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Background: The stress-diathesis model of suicidal behavior (SB) posits that suicide risk is determined by a trait-like predisposition in the face of a stressor. While trait

characteristics, such as impulsivity and aggression, have been identified as diatheses, biological diatheses have been harder to identify. Cortisol, which is secreted via the hypothalamic-pituitary-adrenal (HPA) axis in response to stress, is a potential biomarker given findings of HPA axis dysregulation in depressed individuals (Rao, 2008; Young et al,2000), and those at risk for SB (Mann 2003, McGirr et al, 2010). The Trier Social Stress Test (TSST) is a highly effective psychosocial stress paradigm used to measure HPA axis activation in response to induced stress (Kirschbaum et al, 1993). Ecological Momentary Assessment (EMA), real-time data collection in an individual's natural setting, allows for the identification of changes in affect and behavior that precede SB within a natural context. The purpose of the present study is to relate suicidal ideation (SI) endorsed through EMA with cortisol response to stress, as measured by TSST, to determine if there is a biological subtype of suicidal individuals who are more prone to experience SB in response to life events.

Methods: Subjects ($n = 50$) who met criteria for borderline personality disorder participated in this study after providing written informed consent. Most subjects had comorbid Major Depressive Disorder (76%, $n = 38$) and a prior suicide attempt (80%, $n = 40$). The sample was mostly female (86%, $n = 43$), Caucasian (56%, $n = 28$), single (82%, $n = 41$), and college-educated (46%, $n = 23$).

Subjects completed the TSST, a well-validated laboratory paradigm that measures salivary cortisol response to psychosocial stress. Subjects also completed EMA, where they were given a personal digital assistant that randomly prompted subjects to answer a series of questions 6 times daily for one week. The questions assessed changes in both stressors and SI that occurred since the last prompt.

Cortisol response to the TSST was defined as the area under the curve of log-transformed cortisol values (AULC), calculated from the subject's baseline cortisol level. We assessed the effect of trigger events and cortisol response on the change in SI using mixed effect regression models by including an event indicator, the cortisol response measure, and their interactions as fixed effects, in addition to subject-specific random intercepts.

Results: TSST cortisol response predicted higher increase in SI in response to a negative life event, as reported on the EMA ($t = 2.41$, $df = 870$, $p = .016$) in the joint model that included all negative stressors. As an integrity check, we also measured whether TSST cortisol response predicted higher increase in SI in response to positive stressors, and found that it did not. In the individual models for each stressor, cortisol response was found to predict higher SI in response to two particular stressors: being disappointed by someone ($t = 2.27$, $df = 870$, $p = .0237$) and being reminded of something painful from the past ($t = 3.8$, $df = 870$, $p = .0002$).

Conclusions: Our results suggest that there is a biological subtype of suicidal individuals who are more reactive to life events. Stress response, as measured by the TSST, suggests that cortisol response serves as a biomarker to distinguish a more highly reactive, and thus more acutely at-risk population for suicide. This finding is particularly interesting because the sample in this study, individuals with borderline personality disorder, represent an extreme of stress reactivity, thereby restricting the range.

Keywords: Suicide, Cortisol, Ecological Momentary Assessment.

Disclosure: Nothing to disclose.

M74. Genetics of the HPA-Axis Predict Limbic Connectivity Patterns

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Background: Dysregulation of limbic brain networks and dysregulation of the HPA-axis are both considered likely contributing factors to the development and maintenance of emotional symptoms in depression. Our group has shown that HPA-axis signaling plays are role in modulating the activity and connectivity of limbic structures. This work demonstrates how HPA-axis genetic polymorphisms that affect ligand-receptor interactions have effects on large-scale patterns of limbic connectivity.

Methods: This study uses an array of naturally occurring single nucleotide polymorphisms (SNPs) of HPA-axis genes (corticotropin releasing hormone, and corticotropin releasing hormone receptors 1 & 2, glucocorticoid receptor, mineralcorticoid receptor), age, gender, and severity of depressive symptoms to predict large scale patterns of resting state limbic functional connectivity in a mixed group of healthy participants and depressed patients. Limbic functional connectivity was assessed using a combination of 1) a novel hierarchical clustering approach to reduce non-homogenous time-courses within 2) an anatomical seed-based simple-regression approach. All predictive models were adjusted for multiple comparisons. These analyses do not use the cluster-level inference approach that has recently been shown to be particularly vulnerable to type-1 error.

Results: Depressive symptoms in combination with a variety of HPA-axis SNPs were able to predict limbic connectivity with the subgenual cingulate (Brodmann Area 25), the putamen and the thalamus. Independent of depressive symptoms different combination of HPA-axis SNPs were also able to predict limbic connectivity to a variety of other brain regions including the amygdala, caudate, middle cingulate cortex, posterior cingulate cortex, claustrum, hippocampus, hypothalamus, midbrain, pallidum, parahippocampal cortex, pons, and brainstem.

Conclusions: This study suggests that the limbic connectivity of the subgenual cingulate (BA25), putamen, and thalamus may play a critical role in depression pathophysiology. It also suggests a prominent role for neuroendocrine signaling as a potential modulator of large-scale intra-limbic communication networks that are thought to underlie the neurophysiology of emotion.

Keywords: HPA Axis, Resting State Functional Connectivity, Limbic Cortex, Brodmann Area 25, Subgenual.

Disclosure: Nothing to disclose.

M75. Adult Hippocampal Neurogenesis Promotes Stress Resilience by Inhibiting Ventral Dentate Gyrus Activity

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Background: Adult hippocampal neurogenesis is necessary to confer antidepressant effects and to prevent stress-induced behavioral abnormalities. However, it is unknown how the small population of adult-born neurons exerts such profound effects on behavior. Previous research has shown that adult-born neurons may regulate the overall excitability of the hippocampal dentate gyrus. Therefore, we investigated how neurogenesis regulates dentate gyrus function to mediate stress resilience.

Methods: We used three complementary transgenic mouse models to investigate the effects of neurogenesis on dentate gyrus physiology and stress resilience: 1) Mice with adult-inducible deletion of the pro-apoptotic gene, Bax, from neural stem cells and their progeny, which have 2 fold higher levels of neurogenesis than control mice. 2) Mice in which the activity of adult-born neurons can be silenced using the Gi-protein coupled inhibitory Designer Receptor Exclusively Activated by Designer Drugs (DREADD), hM4Di. Here we used daily cannula infusions of the DREADD-receptor ligand, clozapine-N-oxide (CNO), to activate the hM4Di receptor and inhibit adult-born neurons specifically in the ventral dentate gyrus. 3) Mice in which we can silence or excite mature granule cells in the ventral dentate gyrus, using either the Gi-protein coupled inhibitory hM4Di receptor, or a Gq-coupled, excitatory hM3Dq receptor, respectively. We then subjected all mice to 10 days of social defeat stress and examined stress susceptibility and resilience using behavioral tests of anxiety. We also examined neuronal activity in the dentate gyrus, using immunohistochemistry for the immediate early gene, c-fos, and in vitro electrophysiology.

Results: At the behavioral level, socially defeated mice spend ~40% less time interacting with a novel mouse (Ctrl: 104 ± 5 sec, defeated: 62 ± 11 sec; $n = 16$; $p < 0.01$) and ~42% less time in the center of the open field arena (Ctrl: 43 ± 2 sec, defeated: 18 ± 1 sec; $n = 14$; $p < 0.01$). Defeated mice with increased neurogenesis are resilient to social defeat stress and exhibit higher levels of social interaction time (95 ± 9 sec; $n = 14$, $p < 0.01$) and time in the center of the open field (72 ± 8 sec; $n = 14$, $p < 0.05$). In contrast, defeated mice in which adult-born neurons in the ventral dentate gyrus are silenced using DREADD receptors, are more susceptible to stress and spend less time interacting with a novel mouse (45 ± 3 sec, $n = 9$, $p < 0.05$) and less time exploring the center of the open field (7 ± 2 sec, $n = 9$, $p < 0.05$).

At the cellular level, social defeat stress increases the number of activated, c-fos+, mature granule cells in the ventral dentate gyrus (by 4.6 ± 0.5 fold, $n = 8$, $p < 0.001$). This effect is attenuated in mice with increased neurogenesis (2.7 ± 0.5 fold, $n = 9$, $p < 0.05$) and enhanced in mice in which adult-born neurons are silenced using DREADD receptors (12.5 ± 1.2 fold, $n = 8$, $p < 0.01$). Accordingly, in vitro electrophysiological recordings from ventral dentate gyrus sections of stressed mice show lower EPSP charge in mice with increased neurogenesis compared to stressed mice with normal levels of neurogenesis (-3.9 ± 0.7 fold, $n = 9$, $p < 0.05$). Silencing adult-born neurons, using DREADD receptors, causes a strong increase in charge compared to control mice in which adult-born neurons are not silenced (68 ± 23 fold, $n = 5$, $p < 0.05$).

To determine whether this neurogenesis-mediated inhibition of dentate gyrus activity is necessary and sufficient to confer stress resilience, we injected the Gi-protein coupled inhibitory DREADD receptor, hM4Di, into the ventral dentate gyrus. We then chronically silenced mature granule cells during social defeat by daily injections of the DREADD-receptor ligand, clozapine-N-oxide (CNO). Indeed, silencing mature granule cells during chronic defeat increases interaction time (VEH: 85 ± 9 sec, CNO: 125 ± 7 sec; $n = 11$; $p < 0.01$) and open field center time (VEH: 17 ± 3 sec, CNO: 28 ± 4 sec; $n = 11$; $p < 0.05$). Accordingly, stimulating mature granule neurons using the Gq-protein coupled DREADD receptor, hM3Dq, decreases social interaction time (VEH: 74 ± 6 sec, CNO: 37 ± 8 sec; $n = 8$; $p < 0.05$) and open field center time (VEH: 42 ± 5 sec, CNO: 23 ± 7 sec; $n = 8$; $p < 0.05$).

Conclusions: Our findings demonstrate that hippocampal neurogenesis inhibits the activity of mature granule cells in the ventral dentate gyrus during chronic stress. This effect of adult-born neurons on the neural circuitry is necessary and sufficient to confer stress resilience. Inhibiting dentate gyrus function, either by increasing neurogenesis or using pharmacological interventions, could be a promising new therapeutic strategy to target stress-induced psychopathology.

Keywords: Adult Hippocampal Neurogenesis, Depression, Dentate Gyrus, Anxiety, Stress Response Circuitry.

Disclosure: Nothing to disclose.

M76. SAGE-547 for the Treatment of Severe Postpartum Depression

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Background: Although postpartum depression (PPD) is known to be common in the perinatal period, affecting at least 10-20% of women, it remains understudied and underdiagnosed. Further, the failure to effectively treat PPD can have severe consequences for the mother and family. There are no pharmacologic therapies specifically approved for PPD, and current therapeutic approaches include cautious use of medications, including selective serotonin reuptake inhibitors (SSRIs) as first choice. Evidence suggests that changes in reproductive steroids (progesterone and estradiol) during pregnancy and postpartum are thought to contribute to the development of PPD.

Allopregnanolone, a major metabolite of progesterone, is a potent endogenous neuromodulator that influences multiple receptor systems, including the GABAA receptor family (Majewska et al, 1986). Allopregnanolone acts at nanomolar concentration to augment responses to GABA across a broad

range of expressed GABAARs in the brain, including both synaptic and extrasynaptic sites (Lambert et al. 2001; Belelli et al. 2002). Although allopregnanolone positively modulates most, if not all, GABAA receptor subtypes, extrasynaptic δ-subunit-containing GABAARs are particularly sensitive to modulation by neurosteroids (Belelli et al. 2002; Stell et al. 2003).

Pre-clinical and preliminary clinical studies suggest that the rapid postpartum decline in serum and brain levels of allopregnanolone may trigger PPD in some women, leading to the hypothesis that PPD could be treated with therapeutic doses of allopregnanolone equivalent to third trimester serum levels. SAGE-547 injection is a proprietary, soluble, i.v. formulation of allopregnanolone that was evaluated as a potential treatment for severe PPD in a Phase 2, randomized, placebo-controlled, double-blind trial.

Methods: This Phase 2 placebo-controlled study enrolled 21 women with severe PPD at 11 clinical trial sites. The patients were required to have had a major depressive episode that began no earlier than the third trimester and no later than the first four weeks following delivery and to be less than six months postpartum at the time of enrollment. Trial participants were also required to have a 17-item Hamilton Rating Scale for Depression (HAM-D) score of 26 or above prior to treatment. Patients were randomized one-to-one to receive either SAGE-547 injection or placebo, which were blindly administered as a continuous infusion for 60 hours. Subjects on psychotropic medications at baseline were permitted to remain on these as long as the dose remained constant during the treatment period. Acute assessment of HAM-D was assessed at 2 h, 6 h, 8 h, 12 h, 24 h, 48 h, 60 h, 72 h, 7 days, and 30 days. The primary efficacy endpoint was the change from baseline in HAM-D total score compared to placebo at 60 hours. Secondary efficacy endpoints were measured using the Montgomery–Åsberg Depression Rating Scale (MADRS). Safety assessments and adverse events were also recorded.

Results: Ten patients were randomized to SAGE-547 injection, and 11 patients were randomized to placebo. Three subjects in each group remained on their psychotropic medications that had been initiated prior to enrollment. SAGE-547 injection administration to women with severe PPD resulted in a statistically significant reduction in HAM-D total score compared to placebo at 60 hours ($p = 0.008$). Even though baseline mean HAM-D scores were high, indicating severe depression (28.1 for SAGE-547 injection; 28.8 for Placebo), patients treated with SAGE-547 injection, on average, showed a reduction in HAM-D of greater than 20 points at 60 hours, and this improvement was 12 points greater than that observed with placebo. The effect was statistically significant at 24 hours of treatment ($p = 0.006$) and was maintained through 30-day follow-up ($p = 0.01$). Remission rates (HAM-D ≤ 7) at 60 hours were 70% for SAGE-547 injection treated patients and 9% for placebo treated patients, respectively ($p = 0.008$), and remission was maintained through 30 days (70% vs.18%, respectively; $p = 0.03$). Secondary efficacy endpoints showed a reduction from baseline MADRS total scores (37.5 for SAGE-547 injection; 37.0 for placebo) of 27.9 points at 60 hours versus 12.2 for placebo. The improvement in MADRS ratings for the SAGE-547 injection treated group was 15 points greater than for the placebo treated group at 60 hours ($p = 0.01$), with this improvement in depressive symptoms maintained through 30 days ($p = 0.01$). SAGE-547 injection was generally well tolerated with no serious adverse events (AEs), deaths, or discontinuations due to AEs. Four patients reported AEs while on SAGE-547 injection, and 8 patients reported AEs with placebo.

Conclusions: SAGE-547 treatment of patients with severe PPD resulted in a marked reduction in depression scores when compared to placebo treated patients at 60 hours, achieving the primary endpoint. This improvement in mood was maintained at 30 days. Secondary measures of efficacy using the MADRS confirmed the primary efficacy endpoint of change in HAM-D total score. SAGE-547 injection was generally well tolerated, with fewer adverse events than placebo. Double-blind, placebo-controlled, multi-center, dose-ranging Phase 2 expansion studies of SAGE-547 injection in moderate and severe PPD are currently underway.

Keywords: Postpartum Depression, Neuroactive Steroid, Allopregnanolone, SAGE-547.

Disclosure: Sage Therapeutics: Employee, Self; Sage Therapeutics: Patent Holder, Self.

M77. Effect of Glucocorticoids on Tspo Expression in Nonhuman Primate Brain Evaluated Using Positron Emission Tomography

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Background: The perception that glucocorticoids have an exclusively immunosuppressive role is debated. Accumulating evidence suggests that in addition to their well-known anti-inflammatory properties, glucocorticoids may promote inflammation in the central nervous system (CNS) under specific circumstances. In rodents, glucocorticoids are capable of potentiating neuroinflammatory processes and have a permissive function in neuroinflammatory priming. Exposure to high levels of glucocorticoids induces microglia proliferation, enhances the expression of the NLRP3 inflammasome in macrophage cultures and hippocampal microglia; upregulates hippocampal TLR-2 and microglia activation markers such as IBA-1 and MHC-II. Both acute and chronic treatments with glucocorticoids have been demonstrated to prime the responsiveness of hippocampal microglial cells to a subsequent immune challenge with LPS. The priming effects of glucocorticoids on microglia have only been characterized in rodents ex-vivo and have not been tested in vivo.

Positron emission tomography (PET) with 18 kDa translocator protein (TSPO) targeting radioligands such as [18F] PBR111 is a quantitative approach that enables assessment of microglia and thus inflammation in vivo. Here we investigate the effects of glucocorticoids on microglia in vivo using [18F] PBR111 in healthy non-human primates.

Methods: Five female oophorectomized cynomolgus macaques were studied. All animals had a T1 weighted gradient

echo structural MRI. 120min dynamic [18F]PBR111 PET scans with arterial blood sampling were acquired before and after sub-chronic treatment with prednisolone. All subjects received a baseline PET scan followed by a loading prednisolone dose of 30 mg/kg; p.o. on day 1 and once daily 10 mg/kg p.o. for the ensuing 6 days. The post-treatment PET scan was performed approximately 1 hour post the last dosing on day 7. Venous blood samples were collected at baseline, day 1 and day 7 and analyzed for changes in hematological parameters and evaluated for a panel of inflammation-related biochemical markers with and without in vitro LPS stimulation. To estimate the total volume distribution (VT), the Logan graphical method with a fixed t* of 35 min was applied utilizing the metabolite corrected plasma input function with fixed blood volume (5%). The hippocampus was chosen as a priori region of interest, based on previously reported findings. The whole brain was also studied to assess whether the effects of glucocorticoids on microglia were localized or global. In addition to VT, the distribution volume ratio (DVR) using white matter as the reference region was also investigated to control for the variability associated with arterial input function. Statistical inferences on all measures were performed using two-tailed Wilcoxon sign-rank test on the paired post-treatment vs baseline data.

Results: [18F]PBR111 VT in the whole brain and hippocampus were increased after treatment with prednisolone in all five subjects ($Z = 2.02$, $p < 0.05$). The median increases were 8.3% in the hippocampus (range: 6%-71%;) and 10.7% in the whole brain (range: 2%-74%). There was a trend toward DVR increase in the hippocampus ($Z = 1.75$, $p = 0.08$) but not in the whole brain. White blood cell count was increased following prednisolone treatment in all subjects ($p < 0.05$), this increase being driven primarily by an increase in neutrophils. A change in the metabolite corrected plasma input function following prednisolone administration was observed likely driven by increased radioligand binding to white blood cells. Among the evaluable biochemical markers pretreatment of the samples with prednisolone in vitro had an immunosuppressive profile to LPS stimulation at baseline. However, following in vivo dosing with prednisolone, LPS stimulation of the day 1 and day 7 samples demonstrated elevated IL1ra, MIP1beta and MMP9 ($p < 0.05$) and a trend towards reduction in MIP1alpha ($p = 0.08$) relative to baseline LPS-stimulated samples.

Conclusions: Sub-chronic treatment with high dose of glucocorticoids in nonhuman-primates induced an increase in [18F]PBR111 brain binding, primarily in the hippocampus, interpretable as an increase in TSPO expression. A concomitant increase in systemic release of inflammatory factors was seen likely due to prednisolone-induced neutrophilia. The observed increase in TSPO binding after prednisolone administration may reflect the priming effect of glucocorticoids on microglia, and our experimental paradigm may be a useful approach to quantify microglial priming in the living brain. Further studies are required to clarify the microglial immunophenotype associated with glucocorticoid-induced increase in TSPO expression.

Keywords: TSPO, Glucocorticoids, Microglia Priming, PET Imaging, Neuroinflammation.

Disclosure: AbbVie: Employee, Qi Guo, Peer Jacobson, David Reuter, Ann Tovcimak, Ji-Quan Wang, Cecelia Schroeder, Martin Voorbach, Paul Makidon, Rajasimhan Rajagovindan, Biogen, Author is employee, John Beaver, Imanova, Author is employee, Roger Gunn, Eugenii Rabiner, AbbVie, Study conduct and financial support.

M78. Do More Recent Antidepressant Clinical Trials Have a Higher Rate of Success?

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Background: Among the 52 antidepressant trials submitted for approval of nine antidepressants by the US FDA between 1985 and 2000, it was noted that 45% of the treatment arms consisting of the investigational antidepressant that received approval were statistically superior to placebo. Based on this relatively poor performance of the approved antidepressants, several suggestions were made to improve the outcome of future antidepressant clinical trials.

First of these was to make changes in the design and conduct of the antidepressant clinical trials based on post-hoc analysis. Second, to increase reliability of depression severity ratings by having remote and independent raters or to have the ratings taped so that baseline score inflation is curtailed. Third, to revise power calculations using a lower effect size based on the data from earlier antidepressant clinical trials. This implies that the number of trial participants needs to increase. This method has proven to be useful in anti-hypertensive agent trials.

Methods: Between 2001 and 2013, seven more new antidepressants have been approved by the US FDA. We reviewed the Medical Review of Efficacy of these new antidepressants available from the FDA website evaluating the research design features, rating methods, power calculations used in the study and reporting of trials by the US FDA.

Results: Forty-one large scale multicenter, parallel design, randomized and placebo controlled antidepressant trials were submitted and reviewed by the US FDA for the approval of these seven new antidepressants. The number of treatment arms that received approval that included therapeutic doses were 54 and 32 of these (59.2%) showed statistical superiority over placebo.

A detailed review showed that the newer antidepressant trials did not incorporate any of the suggestions made regarding research design features. Also, two of the 41 antidepressant clinical trials used either taped interviews or remote raters and neither of these trials showed superiority over placebo for the antidepressant.

The major finding was that the power calculations had changed significantly. The mean number of subjects per treatment arm was 143.6 among the newer antidepressant trials compared to 66.9 in the earlier antidepressant trials. Interestingly, the mean effect size was similar among the newer (Hedges' $g = 0.31$) and the older (Hedges' $g = 0.36$) antidepressant trials.

Conclusions: These data suggest that the success rate of more recent antidepressant clinical trials (59.2%) is higher than the

success rate of earlier antidepressant clinical trials (45%). This increase in success rate may be related to the revised power calculations resulting in an increase in the number of trial participants for the antidepressant clinical trials. Specifically, the number of participants has increased by more than two-fold, although the effect size has not significantly changed.

Keywords: Clinical Trials, Antidepressants, Clinical Trial Methodology, Clinical Trial Rating Methods, Effect Size.

Disclosure: Nothing to disclose.

M79. Longitudinal Diffusion Tensor Imaging of Bipolar Disorder in Adolescence

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Background: Bipolar disorder (BD) is increasingly thought of, for at least a subset of individuals with the disorder, to be a neurodevelopmental disorder and adolescence to be a critical period of developmental differences. In adults with BD, converging evidence supports abnormalities in the structure, function and connectivity of a neural system that subserves emotional regulation, including the ventral prefrontal cortex (VPFC) and amygdala. Abnormalities in white matter connections between these regions, e.g. in the uncinate fasciculus (UF), are suggested to result in VPFC-amygdala functional uncoupling and emotional dysregulation. Differences in developmental trajectories of this system during adolescence are implicated in the disorder, and data from our group and other research groups support differences in the trajectories of the structure and function of gray matter nodes in the system. Using structural imaging techniques, we recently reported differences in longitudinal changes of white matter (WM) morphology in adolescents and young adults with BD. Longitudinal diffusion tensor imaging (DTI) was used to examine differences in WM structural integrity changes over time during adolescence in BD, and to test hypotheses that there are decreases with age and over time in UF structural integrity in adolescents with BD compared to healthy control (HC).

Methods: 64 adolescents: 27 with BD [mean age (years) at time one $\pm$ SD = 17.6 $\pm$ 2.6] and 37 HC (16.7 $\pm$ 2.1) completed two DTI scans to study fractional anisotropy (FA) changes over time in a UF region of interest, with an average time between scans of 2.5 years.

Results: There were significant group by age and group by time interactions for UF FA ($p < 0.05$). HC subjects showed significant increases over time and age ($p < 0.05$), while no significant changes over time or age were observed in the BD group.

Conclusions: These findings provide evidence to support abnormalities in the developmental trajectory of VPFC-amygdala WM connections during adolescence in BD and implicate differences in WM development in BD during adolescence as potential targets for treatment and prevention strategies.

Keywords: Neuroimaging, Bipolar Disorder, Adolescent Development, DTI, Emotion Regulation.

Disclosure: Nothing to disclose.

M80. Continuation Phase Intravenous Ketamine in Adults With Treatment-Resistant Depression

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Background: Multiple controlled trials have demonstrated the short-term, acute-phase effectiveness of single and repeated administration of subanesthetic doses of intravenous (i.v.) and intranasal ketamine, a potent non-competitive glutamatergic N-methyl-D-aspartate (NMDA) antagonist, for treating the symptoms of non-psychotic treatment-resistant unipolar and bipolar major depression. However, little is known about the antidepressive effects of repeated intravenous ketamine infusions beyond the acute phase of treatment in patients with refractory depression.

Methods: This was a single-arm, open-label study that proceeded in three phases. During the acute phase, twelve subjects with treatment-resistant, non-psychotic, SCID-confirmed diagnoses of unipolar or bipolar major depression were given repeated (up to 6) thrice-weekly acute-phase intravenous infusions of ketamine (0.5 mg/kg, administered over 100 minutes). Those who remitted (Montgomery Åsberg Depression Rating Scale [MÅDRS] total score < 9 measured 24 hours post-infusion) during the acute phase received continuation phase treatment that consisted of 4 weekly ketamine infusions at the same dose and infusion rate. This was followed by 4 weeks of post-continuation phase follow-up, during which no further ketamine infusions were administered. MÅDRS and Clinical Global Impression-severity (CGI-S) and –change (CGI-C) subscale ratings were assessed at baseline, at 24 hours following each acute phase infusion, at the last acute phase observation, and during continuation and post-continuation phase follow-up (acute phase remitters only). Positive treatment response was defined as achieving a $> 50\%$ decrease (improvement) in MÅDRS total score from baseline.

Results: All 12 enrolled subjects received at least one acute-phase ketamine infusion. Subjects were predominantly middle-aged (mean age, 45.8 $\pm$ 8 years), female ($n = 11$, 91.7%), and Caucasian ($n = 11$, 91.7%). Nine (75.0%) had confirmed diagnoses of MDD; all others had diagnoses of bipolar I ($n = 1$) or II ($n = 2$) depression. Subjects had failed to respond to 3 or more treatments during the current depressive episode, including four who responded poorly to electroconvulsive therapy. Five of the 12 enrolled subjects (41.7%) remitted and 7 (58.3%) responded to ketamine treatment during the acute phase. The majority of subjects who remitted (4/5, 80.0%) did so after the first acute infusion. The remaining subject remitted after the third acute infusion. Baseline MÅDRS total scores did not differ significantly between eventual remitters and non-remitters (29.4 $\pm$ 8.2 vs. 29.4 $\pm$ 7.7, $p =$ n.s.). There was a mean 41.5% reduction in MÅDRS total scores between baseline and the last acute phase observation in the entire sample.

Significantly greater reduction in MÅDRS total scores (baseline to last acute-phase observation) occurred in remitters than non-remitters (79.1% vs. 14.6%, $p<0.001$). Similar results were observed for MÅDRS factors and CGI-S scores. All five subjects who remitted during the acute phase experienced further depressive symptom improvement during continuation phase treatment. Although four of five subjects lost their remitter status during post-continuation phase follow-up, all were still classified as positive treatment responders at the end of the post-continuation phase. Two subjects were withdrawn during the acute phase due to adverse effects, while three elected to stop acute treatment after receiving multiple acute phase infusions owing to lack of antidepressive benefit lasting beyond 24 hours. The most commonly reported adverse effects during acute-phase treatment were dissociation ($n=9$), dizziness ($n=7$), numbness or tingling in the extremities ($n=7$), sleepiness or sedation ($n=6$), tearfulness/emotionality ($n=4$), and facial numbness ($n=3$). Adverse effects were generally mild and transient during both acute- and continuation-phase infusions. None of the subjects experienced visual or other types of hallucinations. No new adverse effects were reported during continuation phase treatment.

Conclusions: The continuation phase administration of ketamine at weekly intervals to patients with treatment-resistant depression who remitted during acute phase ketamine treatment can extend the duration of depressive symptom remission, without increases in adverse effects. The antidepressive effect of ketamine may persist for several weeks after the end of continuation phase treatment.

Keywords: Ketamine, Treatment Resistant Depression, Continuation Phase.

Disclosure: Nothing to disclose.

M81. Lurasidone for Prevention of Recurrence in Patients With Bipolar I Disorder: Comparison of Adjunctive Therapy With Lithium Vs. Valproate

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Background: Little information is available that evaluates background use of specific mood stabilizers on the recurrence prevention efficacy of atypical antipsychotic drugs [1]. This secondary analysis of a maintenance treatment study in patients with bipolar I disorder compared the recurrence prevention efficacy of 28 weeks of treatment with lurasidone when used adjunctively with lithium (Li) or with valproate (VPA).

Methods: Patients with a diagnosis of bipolar I disorder with ≥ 1 manic, mixed manic, or depressed episode in the past 2 years, and with a Young Mania Rating Scale (YMRS) or Montgomery-Åsberg Depression Rating Scale (MADRS) total score ≥ 14 (if on Li or VPA), or ≥ 18 (if not on Li or VPA) received open-label treatment with lurasidone 20-80 mg/d adjunctive with Li or VPA for up to 20 weeks during an open-label stabilization phase. Patients who achieved predefined stability criteria were randomized to 28 weeks of double-blind treatment with lurasidone, 20-80 mg/d, adjunctive with Li ($N=104$) or VPA ($N=134$), or placebo adjunctive with Li ($N=109$) or VPA ($N=141$). A Cox proportional hazards model was used to separately assess (by adjunctive mood stabilizer utilized) hazard ratios for risk of recurrence of any mood episode for all patients, and for patients with a current (index) depressive episode or manic/hypomanic/mixed manic episode. Kaplan-Meier estimates of the probability of time to recurrence were also calculated.

Results: Baseline characteristics were comparable for patients receiving adjunctive therapy with Li vs. VPA in terms of mean age (42.9 vs. 45.6 years), proportion of females (59.6% vs. 53.1%), depressive index episode (55.9% vs. 50.5%), current episode duration (26.3 vs. 35.0 weeks), and age of bipolar illness onset (26.7 vs. 30.0 years). For the total intent to treat (ITT) sample, Cox model hazard ratios assessing the reduction (vs. placebo) in risk of recurrence of any mood episode were similar for lurasidone + Li compared to lurasidone + VPA (0.74 vs. 0.67), representing non-significant risk reduction rates of 26% vs. 33%, respectively. Among patients presenting with a current depressive episode, lurasidone + VPA was associated with a significant risk reduction rate vs. placebo + VPA of 61% (hazard ratio, 0.39; Cox model $P=0.027$; log-rank test $P=0.031$), while lurasidone + Li was associated with a non-significant risk reduction vs. placebo + Li. Among patients presenting with a current episode of mania/hypomania/ mixed mania, lurasidone + Li was associated with a non-significant risk reduction vs. placebo + Li of 37% (hazard ratio, 0.63; Cox model $P=0.367$; log-rank test $P=0.237$), while lurasidone + VPA was associated with a non-significant risk reduction rate vs. placebo + VPA.

Conclusions: In this double-blind, placebo-controlled, 28-week, randomized withdrawal study of patients with bipolar I disorder, treatment with lurasidone was comparably effective in preventing recurrence of any mood disorder regardless of whether the background mood stabilizer was lithium or valproate. In the group of patients presenting with an index episode of depression, reduction in recurrence risk was greater for lurasidone + valproate (vs. lurasidone + lithium); while risk reduction was greater on lurasidone + lithium (vs. lurasidone + valproate) in patients with an index episode of mania/hypomania/mixed mania.

Keywords: Clinical trial, Bipolar Disorder, Durasidone.

Disclosure: Xhale: Consulting/Stockholder, Self; Takeda: Consulting, Self; Mitsubishi Tanabe: Consulting, Self; Taisho: Consulting, Self; Lundbeck: Consulting, Self; Prismic Pharmaceuticals, Consulting: Self; Bracket/Clintara, Consulting: Self; Total Pain Solutions: Consulting, Self; Gerson Lehrman Group Healthcare and Biomedical Council, Consulting: Self; Fortress Biotech, Consulting: Self; Sunovion, Consulting, Self; Sumitomo Dainippon: Consulting, Self; Xhale, Celgene, Seattle Genetics, Abbvie, OPKO Health, Inc., Bracket Intermediate Holding Corp., Network Life Sciences Inc.: Stockholder, Self; American Foundation for Suicide Prevention (AFSP), Brain and Behavior Research Foundation (BBRF) (formerly named National Alliance for Research on Schizophrenia and Depression [NARSAD]), Xhale, Anxiety Disorders Association of America (ADAA), Skyland Trail, Bracket (Clintara), RiverMend Health LLC, Laureate Institute for Brain Research, Inc.: Scientific Advisory Board, Self; AFSP, Gratitude America, ADAA: Board of Directors, Self.

M82. Role of Medial Prefrontal Cortical Brain-Derived Neurotrophic Factor in the Antidepressant-Like Effects of Fluoxetine and Scopolamine in Mice

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Background: Traditional antidepressants require at least 2-4 weeks of continuous treatment to elicit a therapeutic response. In contrast, scopolamine, a muscarinic acetylcholine antagonist, induces antidepressant effects within days of a single administration in humans. Brain Derived Neurotropic Factor (BDNF) is a trophic factor induced in the medial prefrontal cortex (mPFC) by almost all known antidepressants. However, one potential deviation from this is scopolamine, which has been shown in multiple studies to decrease BDNF levels. The present study aimed to examine the necessity of BDNF induction in the mPFC for antidepressant-like effect of scopolamine and the selective serotonin reuptake inhibitor (SSRI), fluoxetine.
Methods: BDNF floxed mice on a BALB/cJ background were infused bilaterally into the mPFC with AAV-cre-GFP or AAV-GFP control. Four weeks later, mice were either implanted with subcutaneous osmotic mini-pumps delivering scopolamine (0 or 20 mg/kg/day) or had access to 4 weeks of fluoxetine in the drinking water (0 or 15 mg/kg/d). At the conclusion of treatment, mice were tested in the open field test (OFT), followed immediately by the chronic forced swim test (cFST). In the OFT, horizontal activity was collected via automated MedPC software. In the cFST, behavior was scored for time immobile, swimming, or climbing by an experimenter blind to condition.
Results: Scopolamine significantly decreased immobility and increased swimming in the cFST, but there was no virus by drug interactions. Fluoxetine significantly increased climbing, and this effect was prevented in mice with BDNF knockdown in the mPFC. In the open field test, there were no effects of either drug and no drug by virus interactions.
Conclusions: These data suggest that induction of BDNF in the mPFC is not necessary for the antidepressant-like effects of scopolamine in the cFST. On the other hand, the fluoxetine-induced increase in climbing was dependent on mPFC BDNF induction. This study suggests that mPFC BDNF induction may be differentially necessary for the SSRI fluoxetine and the fast-onset antidepressant scopolamine.
Keywords: Depression, Antidepressant, Mice, BDNF.
Disclosure: Nothing to disclose.

M83. Pet Imaging of Translocator Protein (TSPO): Investigating the Link Between Inflammation and Depression

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Background: Neuroinflammation may be a predisposing factor for major depressive disorder (MDD). Translocator protein 18 kDa (TSPO) is a highly expressed protein in glial cells and, therefore, represents a potential biomarker of neuroinflammation. TSPO can be accurately quantified using positron emission tomography (PET) and [11C]PBR28, a TSPO tracer developed in our laboratory. The primary aim of our current study is to investigate differences between TSPO binding in subjects with MDD who are currently experiencing a depressive episode compared to healthy controls (HCs). The second aim is to determine if antidepressants affect TSPO binding in patients with MDD. The third aim is to investigate the relationship of peripheral and central inflammatory biomarkers to TSPO binding.
Methods: Unmedicated MDD ($n = 11$), medicated MDD ($n = 16$) and HC ($n = 20$) subjects underwent PET imaging using [11C]PBR28. We measured total distribution volume (VT, proportional to Bmax/Kd) using arterial input function and corrected for TSPO genotype. Based on previous post-mortem findings, we chose the subgenual prefrontal cortex and anterior cingulate cortex as regions of interest and compared VT values obtained in medicated and unmedicated MDD subjects and healthy controls. We also obtained peripheral blood samples and cerebrospinal fluid, for later analysis, to investigate the relationship between peripheral and central inflammatory markers and TSPO binding. Specifically, we utilized Enzyme-Linked Immunosorbent Assay to determine if peripheral levels of quinolinic acid, a potential biomarker of inflammation, correlated with changes to TSPO binding.
Results: Interim results of this ongoing study show significant differences in TSPO binding in unmedicated MDD subjects. Specifically, in the anterior cingulate cortex, VT was 31% higher in unmedicated MDD subjects compared to HCs ($p < 0.005$) and 27% higher compared to medicated MDD subjects ($p < 0.05$). In the subgenual cortex, VT was 33% higher in unmedicated subjects compared to HCs ($p < 0.005$) and 26% higher compared to medicated MDD subjects ($p < 0.05$). While quinolinic acid levels were significantly higher ($p = 0.008$) in medicated subjects compared to unmedicated subjects, no direct correlation to TSPO binding was observed.
Conclusions: These preliminary results suggest that inflammation in the anterior cingulate and subgenual cortex is associated with major depression and is likely affected by the medication status of the patient. While additional analysis of potential peripheral and central predictive inflammatory biomarkers is warranted, quinolinic acid levels in blood did not correlate with TSPO binding in brain.
Keywords: Neuroinflammation, TSPO and [11C]PBR-28 PET, Major Depressive Disorder (MDD), Biomarkers, Inflammation.
Disclosure: Nothing to disclose.

M84. Chronic Social Isolation Reduces 5-HT Neuronal Activity via Upregulated SK3 Calcium-Activated Potassium Channels

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Background: The dorsal raphe nucleus (DRN) is a critical site for modulation of emotional responses. Dysfunction of

serotonergic (5-hydroxytryptamine, 5-HT) neurons in the DRN has been implicated in the pathophysiology underlying anxiety disorders and depression, and these neurons are also the main targets of antidepressant action. Our goal is to identify how chronic social isolation stress affects the activity and modulation of 5-HT neurons and to decipher the molecular mechanisms underlying these stress-induced alterations.

Methods: Experiments are performed in Tph2-COP4*H134R/EYFP mice that express channelrhodopsin EYFP fusion protein driven by the Tph2 promoter (Zhao et al, Nature Methods 2011). At weaning, litter-mate male mice are housed individually (single-housed; socially isolated) or together in groups of 3-4 (group-housed) for a minimum of 7 weeks. To assess stress-induced anxiety- and depressive-like behaviors in adulthood, the socially-isolated mice and group-housed controls are tested in behavioral studies, including open-field, novelty suppressed feeding, and tail suspension. To investigate the activity and modulation of 5-HT neurons, we perform whole-cell patch-clamp recordings in DRN slices obtained from adult mice, including electrophysiological, optogenetic, and neuromodulatory stimulation paradigms. To probe the mechanisms underlying the observed stress-induced alterations in the activity of 5-HT neurons, we use specific blockers for channels (voltage-gated Ca2+ channels, small-conductance calcium-activated potassium (SK) channels) that contribute significantly to the excitability of these neurons. To examine the expression levels of SK channels, we perform western blot analysis in DRN from socially-isolated mice and controls. Additional experiments probe the behavioral consequences of SK channel modulation. All procedures are in accordance with animal protocols approved by the University of Toronto.

Results: Behaviorally, mice subjected to chronic social isolation spend significantly more time in the peripheral zone of the open field and show a greater latency to feed in a novelty suppressed feeding paradigm, indicating anxiety-like responses. They also show increased immobility in tail suspension test, indicative of depressive-like responses. Electrophysiologically, we find that the intrinsic excitability of 5-HT neurons to optogenetic and depolarizing stimuli is significantly reduced upon chronic social isolation, compared to those in DRN slices obtained from group-housed controls. Importantly, the firing activity of 5-HT neurons induced by their excitatory neuromodulators is also significantly decreased by chronic isolation stress. Mechanistically, these alterations in the activity of 5-HT neurons upon chronic stress are accompanied by changes in the magnitude of their afterhyperpolarization (AHP) potentials. Probing the underlying cellular mechanisms pinpoints a disturbance in the expression and function of SK channels and reveals an important role for both SK2 and SK3 channels in normal regulation of 5-HT neuronal excitability. In sum, chronic social isolation rendered 5-HT neurons insensitive to SK2 blockade, and blocking the significantly upregulated SK3 channels restored normal excitability. This change represents a shift from a set of inhibitory channels that is flexible to modulation in group-housed controls to one that is modulation-resistant in socially isolated mice. Therefore, we are interrogating the effects of SK3 channel manipulation on anxiety- and depressive-like behaviors in socially isolated mice.

Conclusions: Our experiments reveal a causal link for the first time between SK channel dysregulation and 5-HT neuron activity in a lifelong stress paradigm, suggesting these channels as targets for the development of novel therapies for mood and anxiety disorders.

Keywords: Social Isolation, Dorsal Raphe Serotonin Neurons, SK3 Calcium-Activated Potassium Channels, Optogenetics, Electrophysiology.

Disclosure: Nothing to disclose.

M85. Baseline C-Reactive Protein Levels Differentially Predict Response to Escitalopram Versus Bupropion: Clinical Application of Inflammatory Biomarkers to Personalize Treatment of Depressed Outpatients

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Background: One of the biggest challenges with treatment of depression is that there are no valid clinical or biological markers which can guide selection among antidepressant treatments. Hence, current practice reflects a trial-and-error approach where depressed patients switch treatments too early, stay on ineffective treatments too long, or simply drop out of treatment. A recent report from the Genome-based Therapeutic Drugs for Depression (GENDEP) study found that depressed outpatients with low levels (less than 1mg/L) of baseline C-reactive protein (CRP) had significantly greater improvement on escitalopram as compared to nortriptyline. As use of tricyclic antidepressants has fallen significantly in last two decades, there is a need to compare treatment outcomes between SSRIs and more commonly used non-serotonergic antidepressants such as bupropion in order to facilitate clinical application of inflammatory biomarkers in making antidepressant medication selection.

Methods: We used data from participants who provided baseline plasma specimens ($n = 166$) in the Combining Medications to Enhance Depression Outcomes (CO-MED) trial, a single blind randomized controlled trial that compared outcomes across the following three treatment arms: escitalopram plus placebo, bupropion plus escitalopram, and venlafaxine plus mirtazapine. After using log transformation for biomarkers that were not distributed normally, we evaluated if baseline levels of CRP and other acute-phase reactants (serum amyloid P component and alpha-2-macroglobulin) were associated with differential response to antidepressant medication(s) in using mixed model analyses and Quick Inventory of Depression Severity (QIDS-SR) as outcome measure. Additionally, we evaluated superiority of escitalopram plus placebo over other two treatment arms in participants with low (<1mg/L) CRP levels.

Results: We found a significant interaction with treatment arm for log of CRP ($F = 3.33$, df = 2,155, $p = 0.038$) but not for log of alpha-2-macroglobulin ($F = 0.11$, df = 2, 159, $p = 0.90$) or serum amyloid P component ($F = 0.80$, df = 2, 149, $p = 0.45$) suggesting that baseline CRP levels differentially predict treatment outcomes among the three treatment

arms. Higher levels of CRP were associated with smaller reductions in depression severity in escitalopram-plus-placebo and venlafaxine-plus-mirtazapine treatment arms. Conversely, we found an inverse effect in bupropion-plus-escitalopram where higher levels of CRP were associated with greater reductions in depression severity. We also found that among depressed participants with CRP level less than 1 mg/L, treatment with escitalopram-plus-placebo resulted in greater reduction of depression severity as compared to bupropion-plus-escitalopram (estimate [est.] = -3.04, standard error [SE] = 1.36, t = -2.23, $p = 0.027$) but not to venlafaxine-plus-mirtazapine (est. = -2.14, SE = 1.27, t = -1.68, $p = 0.095$).

Conclusions: Clinically, depressed patients with low CRP benefit from escitalopram while those with higher CRP benefit from bupropion. Routine use of CRP, an inexpensive readily available lab test, in personalizing antidepressant medication selection will improve outcomes and transform clinical practice.

Keywords: Antidepressant Treatment Practice, Biomarker, Inflammation, Bupropion, Escitalopram.

Disclosure: Nothing to disclose.

M86. Exposure to Paliperidone in Utero and via Breast Milk: Drug Clearance and Implications for an Animal Model of Human Exposure

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Background: There is increasing utilization of atypical antipsychotics as mood stabilizers and adjuncts to antidepressant therapy, contributing to an increase in fetal exposure to these medications. Previous studies from our group demonstrate the need for clinically relevant and sustained drug administration in animal studies that more closely mirror human exposures. Using a rat model, we rigorously controlled fetal exposure to paliperidone, the major active component of risperidone treatment, in utero and reported that prenatal paliperidone did not alter developmental trajectory in the offspring using behavioral assessments of development (motor and cognitive) prior to weaning and as juveniles (postnatal days 23-35). Because of potential differences in brain development between humans and rodents pre- and postnatally, it may be imperative to rigorously provide and control paliperidone exposure during the postnatal period in rodent pups. We have characterized drug exposure in the postnatal period in rat pups exposed to paliperidone in utero and via breast milk exposure postnatally.

Methods: Pregnant female rats were administered either paliperidone (0.55 mg/kg/day) or vehicle (2% acetic acid, pH 5.0) using subcutaneous osmotic minipumps beginning on gestational day 4. Following parturition, both male and female offspring (4 per litter) were transferred to foster dams. At least 7-8 independent litters from each treatment group were produced. Global transcriptome analyses (RNA-seq) were performed and genome-wide 5-hydroxymethylcytosine (5-hmC) of PND35 hippocampus from two pairs of samples from male offspring were profiled. Following parturition, pups were killed and serum paliperidone concentrations were measured (LC-MS/MS) postnatally while the pups stayed with their paliperidone-treated mothers. Separate cohorts of rats were also generated to assess serum paliperidone concentrations under the following conditions: 1) prenatal exposure only and 2) breast milk exposure only.

Results: In initial analyses, prenatal exposure to paliperidone (~80th percentile of human exposure; mean [serum] 34 ng/ml) only altered expression of a small number of genes and did not significantly change the abundance of 5-hmC in hippocampus of PND35 offspring. In rat pups kept with their paliperidone-treated mother (mean serum paliperidone = 35 ng/ml), serum paliperidone had decreased to 2-6 ng/ml within 24 hours of parturition, < 3 ng/ml by 48 hours and to ~ 1 ng/ml from PND5-10. In order to clarify the role of breast milk exposure postnatally, rats were exposed to paliperidone only in utero or only postnatally via breast milk. At PND9, and near the end of the osmotic minipump pumping lifetime, the dams' mean serum paliperidone concentrations were 23 ng/ml. In pups only exposed in utero, serum paliperidone concentrations at PND 1, 2, 3, 5, 7 and 9 averaged < 2 ng/ml. In pups only exposed via breast milk, serum paliperidone concentrations were 1.8 ng/ml ($n = 3$) at PND1 but were below the limit of detection (0.2 ng/ml) for all animals ($n = 3$-6 per group) at PND 2, 3, 5, 7, and 9.

Conclusions: A clinically relevant prenatal dosing paradigm has been developed for paliperidone (and by association, risperidone). Prenatal exposure was without effect on measures of preweaning development and on behavior at PND23-35. Hippocampal gene expression and DNA hydroxymethylation status at PND35 does not, in preliminary analyses, appear to be importantly affected. Paliperidone is rapidly cleared by the pups following parturition. Measured pup serum concentrations during the postnatal period are 10-20-fold less than that during gestation. Breast milk exposure does not lead to measurable systemic exposure as assessed by serum drug concentrations. These data suggest that clinically relevant exposure to paliperidone during the early postnatal period of pups that may coincide with human brain development during late gestation will require direct administration to pups beginning on PND1.

Keywords: Prenatal, Antipsychotic, Developmental Pharmacology.

Disclosure: Nothing to disclose.

M87. Analysis of Gene Expression in Peripheral Blood Mononuclear Cells in Naïve, Depressed-Like, and Stress-Resilient Rodents Reveals Possible Peripheral Biomarkers and Correlates With Brain Function

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Background: Depression is an interplay between inherent genetic vulnerability and environmental insults, such as stress, as well as resiliency to such insults. Identifying peripheral biomarkers associated with depression risk or

resiliency could not only help identify patients at risk to provide early treatment, but may also highlight novel molecular pathways that could lead to new preventative or maintenance medicines. In the learned helpless model, rodents are exposed to an acute, inescapable stressor, and only a fraction of the rodents develop a helpless/depressed-like state, where as others demonstrate resilience to the stress. Thus, the learned helpless model offers an opportunity to explore potential resilient and vulnerable factors. We used this model to highlight possible genetic correlates to such factors.

Methods: Adult Sprague-Dawley rats were exposed to a series of inescapable foot shocks. Over the next three days, the rats were given an active avoidance test to determine their stress vulnerability. Animals that consistently escaped 0 – 3 trials out of 30, were considered helpless-like, whereas animals that escaped 27 – 30 trials out of 30 were considered resilient. After the last test, peripheral blood mononuclear cells (PBMC) and various brain regions were collected. Naïve controls not subjected to stress were included. Gene expression was than analyzed using the RNA-Sequencing platform. Genes were analyzed by tissue and group (naïve, helpless-like, and resilient) and considered significantly changed when False Discovery Rate (FDR) was less than 0.05 and fold-change in expression level different by more than 1.5.

Results: Over 18,000 protein coding genes of known function were analyzed. Of these genes, approximately 1400 were significantly altered in PBMC after footshock stress. The majority of these genes (~700 genes) were altered only in the depressed-like group, while only ~ 280 genes were similarly altered in the resilient group, and deemed shock-sensitive genes. Interestingly, ~ 390 genes were associated with resilience. Out of these resilient genes, three genes were also altered in the depressed-like group, but in the opposite direction as the resilient group. Analysis of the genes altered after footshock stress revealed that many of the genes were associated with heat shock, protein folding, and glucocorticoid receptor (GR) function, protein kinases and cellular plasticity, cell adhesion, migration, and the extracellular matrix, membrane signaling pathways, and DNA damage.

The most significant genes altered in PBMC in the depressed-like group were for proteins associated with heat shock pathways and GR chaperone proteins, including Hsp90, Fkbp4, Dnaja1, Stip1, Hsp70, and AHA1. Interestingly, several altered genes were previously suggested to be peripheral biomarkers of mood disorders, such as Stip1 and Fkbp4, or reported to be altered in both humans and animals by stress, for example, Hsp90 and Dnaja1. In the resilient group, many of the genes altered in PBMC were involved in the cell cycle process, for example, cellular division and DNA repair. One interesting gene in the resilient group was Csgalnact1, which was downregulated. This gene was previously found to be upregulated in depressed patients and associated with sustained antidepressant response. The three genes that were altered in an opposite direction as in the depressed-like group were involved in extracellular matrix cell-cell interactions, adhesion, and cellular motility. In addition, the resilient group had less altered genes putatively regulated by heat shock factor protein 1 than the depressed-like group.

Next, a less stringent criterion was used to analyze whether any gene expression changes correlated between PBMC and brain. In the hippocampus, 108 genes were similarly altered in the depressed-like group, and 101 genes similarly altered in the resilient group, as in PBMC. Many of these genes were ribosomal proteins, and in the depressed-like group, a few were related to the Hsp70 pathway. In prefrontal cortex and cerebellum, only 11 and 37 genes, respectively, were similarly altered as in PBMC in the depressed-like group, while in the resilient group, only 17 genes were similarly altered in cerebellum or prefrontal cortex as compared to the PBMC. Cluster analysis did not find any similarities among these genes. In amygdala, 60 genes were similarly altered in the depressed-like group as in PBMC and the majority of these proteins were related to heat shock pathways, chaperone proteins, and protein misfolding, including Dnaja1, Stip1, and Fkbp4. 96 genes were similarly altered in the amygdala and PBMC in the resilient group, a few of which were mitochondrial proteins, and of interest, Cacna1c.

Conclusions: These data suggest that stress associated with long-term behavioral consequences elicits changes in gene expression in the periphery indicative of cellular stress and altered GR function, and that the amygdala is particularly vulnerable to similar changes. On the other hand, stress resilient animals appear to be less impacted by such events.

Keywords: RNA Sequencing, Stress Models, Blood, Brain.

Disclosure: Janssen Research & Development: Employee, Self.

M88. Engaging Medial Temporal Lobes With ECT Pulse Amplitude to Improve Clinical Outcomes

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Background: Independent of the antidepressant effect of electroconvulsive therapy (ECT), many patients experience transient cognitive difficulties such as attention and memory impairments. The ECT charge is measured in millicoulombs (mC) and derived from multiplying pulse train duration, pulse-pair frequency, pulse width, and pulse amplitude. Pulse amplitude determines the induced electric field strength in the brain and is presently fixed at 800 or 900 milliamperes (mA) with no clinical or scientific justification. This investigation will examine the clinical and neurocognitive impact of targeted medial temporal lobe engagement as a function of pulse amplitude, one of several variables that influence the ECT charge. We hypothesize that the optimal pulse amplitude for an individual patient will enhance neuroplasticity (clinical response) while minimizing disruption of dominant hemisphere hippocampal cognitive circuitry (resulting in cognitive stability).

Methods: Fourteen subjects received clinical (Hamilton Depression Rating Scale – 24 item (HDRS)), neuropsychological (Repeatable Battery of Neuropsychological Status (RBANS)), and imaging assessments (structure and resting state-fMRI) before and immediately after the right unilateral (0.3 milliseconds (ms) pulse width) ECT series. Pulse amplitude dose was fixed for the duration of the ECT series

at 500 mA, 700 mA, 800 mA, or 900 mA. We computed electric field modeling for the subjects treated with 500, 700 and 900 mA. We used individual T-1 weighted structural MRI and finite element method to determine the hippocampal electric field strength. The Automated Segmentation of Hippocampal Subfields measured medial temporal lobe volumes. A native space ROI-to-ROI method assessed changes in rs-fMRI connectivity. We used regression models that controlled for age and gender to assess the relationship between 1) electric filed strength and medial temporal lobe neuroplasticity and 2) medial temporal lobe neuroplasticity and clinical outcomes (response and cognitive impairment, the latter assessed with percent recall from the RBANS verbal declarative memory task).

Results: The clinical outcomes are as follows: 500 mA ($n = 3$, no responders), 700 mA ($n = 2$, responders), 800 mA ($n = 2$, responders), and 900 mA ($n = 7$, responders) with response defined as a 50% decrease in HDRS total score. Right hippocampal electric field strength predicted change in the right hippocampal volume ($t = 2.44$, $p = 0.03$, Cohen's $f2 = 0.53$). This relationship was not evident in the left hemisphere ($p > 0.10$). The right dentate gyrus had a trend toward predicting HDRS change ($t = 2.04$, $p = 0.08$). The rs-fMRI connectivity between the entorhinal cortex and all of regions within the default mode network predicted percent change in verbal memory recall. Specifically, longitudinal connectivity differences between the entorhinal cortex and inferior parietal lobe ($t = 2.41$, $p = 0.03$, Cohen's $f2 = 0.30$), isthmus of the cingulate ($t = 3.43$, $p = 0.003$, Cohen's $f2 = 0.62$), medial orbital frontal cortex ($t = 3.15$, $p = 0.005$, Cohen's $f2 = 0.52$), parahippocampal gyrus ($t = 2.76$, $p = 0.013$, Cohen's $f2 = 0.40$), precuneus ($t = 3.67$, $p = 0.002$, Cohen's $f2 = 0.92$) and rostral anterior cingulate ($t = 4.32$, $p < 0.001$, Cohen's $f2 = 0.98$) were all associated with change in percent verbal memory recall. These connectivity changes were not evident with the left hippocampus or parahippocampus, or right medial temporal lobe regions ($p > 0.10$).

Conclusions: Our data demonstrated that the 500 mA pulse amplitude dose was clinically ineffective despite seizure induction with adequate duration and morphology. Similar to ultrabrief bitemporal and right unilateral ECT at seizure threshold, our 500 mA ECT data is consistent with the view that a seizure is necessary but insufficient for clinical efficacy. Our data showed a strong relationship between pulse amplitude and calculated electric field strength. The electric field must have sufficient strength to induce hippocampal volumetric change. The link between neuroplasticity and clinical efficacy was supported with increased right dentate gyrus volume. Evidence supporting the association with electric field strength includes the relationship between increased electric field strength and disrupted functional connectivity between the entorhinal cortex and precuneus. In addition, the relationship between disrupted functional connectivity and cognitive performance appears robust and specific for the left entorhinal cortex/default mode network.

Keywords: Electroconvulsive Therapy, Major Depressive Disorder, Functional MRI (fMRI), Hippocampus.

Disclosure: Nothing to disclose.

M89. P11 Protein Levels in Peripheral Blood Mononuclear Cells From Parkinson's Disease Patients With and Without Depression Correlate With Disease Severity

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Background: Parkinson's disease (PD) is the second most common neurodegenerative disease globally. Disease diagnosis is based on motor symptoms which occur subsequent to the death of over half of nigrostriatal dopaminergic neurons. Mood and cognitive impairment associated with PD represents a major healthcare problem, with between 30 and 60% of PD patients suffering from depression (1). Depression is associated with a more rapid decline in motor and cognitive functions in PD patients (2). Inflammation plays a role in both PD and depression, with some patients showing an increase in pro-inflammatory mediators both centrally and peripherally. Peripheral immune stimulation can activate the serotonin (5-hydroxytryptamine, (5-HT)) transporter within the CNS. Moreover, 5-HT is expressed in peripheral immune cells and it has been suggested that 5-HT may mediate pro-inflammatory cytokine production in natural killer (NK) cells, dendritic cells and T helper cells. P11, a member of the S100 family of proteins, potentiates 5-HT signaling and animal studies indicate that p11 is a key regulator of depressive-like behaviors (3). Alterations of peripheral p11 in NK cells and monocytes is correlated with antidepressant response in patients with MDD (4). Several clinical trials are targeting inflammatory processes for the treatment of PD (5) as well as depression (6). Such trials would benefit from biomarkers which would reflect disease severity and signs. To further evaluate a biomarker role for p11, this project assessed p11 expression in peripheral blood mononuclear cells (PBMCs) in PD patients with and without depression.

Methods: Blood samples were obtained from 20 PD patients, 20 PD patients with depression (PD(MDD)) and 16 age and gender matched healthy controls (HCs). Patients were scored on the unified Parkinson's disease rating scale (UPDRS) and the Montgomery–Åsberg Depression Rating Scale (MADRS). PBMC composition and p11 protein levels were measured in monocyte and T cell subsets, and in NK cells by intracellular staining using a monoclonal p11 antibody and multicolor flow cytometry. Data was analyzed with Flow Jo and a hierarchical gating strategy was utilized to identify cell subtypes and subsequently quantify p11 staining. Monocytes were identified as classically activated (CD14+CD16-) and non classically activated (CD14+CD16+), T cell subsets were identified as cytotoxic T cells (CD3+CD8+), T helper cells (CD3+CD4+) and T regulatory cells (CD3+CD4+FOXP3+), and NK cells were identified as CD3-CD56+ CD16+ cells.

Results: There were no significant differences in PBMC composition between the groups. Monocytes from PD (MDD) patients expressed higher levels of p11 as compared to HCs ($p < 0.05$), while cytotoxic T cells from PD and PD (MDD) patients expressed higher levels of p11 as compared to HCs ($p < 0.05$). P11 levels in both CD14+CD16- and CD14+CD16+ monocytes were positively associated with total UPRDS scores (Pearson's correlation, $p < 0.001$ and $p < 0.01$

respectively). Similarly, cytotoxic T cells (CD8+) and NK cell p11 levels were positively associated with total UPDRS score (Pearson's correlation, $p<0.05$). p11 levels in NK cells were significantly associated with patient MADRS scores (Pearson's correlation, $p<0.05$).
Conclusions: We report here that peripheral p11 protein levels are altered differentially in PD patients with and without depression, and that peripheral p11 protein levels in distinct cell types correlate with disease severity and depression scores in PD patients. Further research is warranted to examine whether p11 could serve as an easily accessible biomarker to follow responses of therapies targeting inflammatory responses in PD, especially in the context of comorbid depression.
Keywords: P11, Depression, Parkinson's Disease, Biomarker, Inflammation.
Disclosure: Nothing to disclose.

M90. Serum Phosphatidylinositol as a Biomarker for Bipolar Disorder Liability

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Background: There is evidence that individuals with bipolar disorder (BPD) have abnormal serum phospholipid levels. However, it is unclear whether these alterations arise as a secondary consequence of illness state, or if phospholipids and illness risk have overlapping etiologies. If the latter supposition were true, then phospholipids and their underlying biochemical mechanisms might provide key insights into the pathophysiology of the illness. We aimed to rank-order phospholipid classes by their genetic overlap with BPD risk in order to establish which class might be most informative in terms of increasing our understanding of illness pathophysiology.
Methods: Analyses were conducted in a sample of 558 individuals, unselected for BPD, from extended 38 extended pedigrees (average family size = 14.79, range = 2-82). We calculated a coefficient of relatedness for all family members of 9 individuals with BPD in the sample ($N=$ 185), this coefficient was set to be zero in unrelated individuals ($N=$ 373). Then under an endophenotype ranking value (ERV) approach this scalar index was tested against thirteen serum-based phospholipid concentrations in order to rank order lipid classes by their respective overlap with BPD risk. Alternate psychiatric diagnoses as well as diabetes, hypertension, cardiovascular disorders, and associated medications, were co-varied for where necessary.
Results: The phosphatidylinositol class was significantly heritable (h2 = 0.3111, $p=$ 5.19x10-12). It was the top-ranked class, and was significantly associated with BPD risk after correction for multiple testing ($\beta=$ -1.032, $p=$ 3.28x10-03, ERV = 0.4203).
Conclusions: We identified a peripheral biomarker, serum-based phosphatidylinositol, which exhibits a significant association with BPD risk. Therefore, given that phosphatidylinositol and BPD risk share partially common etiology, it seems that this lipid class warrants further investigation, not only in terms of treatment, but also as a promising diagnostic and risk marker.
Keywords: Bipolar Disorder, Lipids, Psychiatric Genetics.
Disclosure: Nothing to disclose.

M91. Novel Plasma Sterols and Depressive Symptom Severity in a Population-Based Sample

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Background: Major depressive disorder (MDD) is a highly prevalent disease and a leading cause of disability worldwide. Development of peripheral blood biomarkers for MDD holds promise for clarifying etiology and diagnosis, as well as predicting treatment response and prognosis. Vitamin D deficiency and low plasma cholesterol have been associated with depression and suicidality, suggesting that plasma sterols may be depression biomarkers. In the current report, data are reported from an analysis of 34 sterols obtained from an epidemiologic sample of adults.
Methods: Data were analyzed from $n=3117$ adults in the Dallas Heart Study (DHS)-2, a large, ethnically diverse, well characterized, population-based cohort. The primary aim of the DHS is to examine risk factors for heart disease. However, participants were representative of Dallas County, except that African-Americans were intentionally over-sampled to examine heart disease risk in this population, and were not selected based on the presence of heart disease. Plasma concentrations of 34 sterols were measured by mass spectrometry as part of the DHS. Using machine learning algorithms, the ability of sterol levels to predict scores on the Quick Inventory of Depressive Symptomatology-Self Report (QIDS-SR), a validated, and widely used 16-item, depression symptom severity scale, was tested.
Results: A random forest model (RFM) constructed from 34 sterol concentrations explained 4.2% of the total variation in QIDS-SR scores in this population. The cholesterol precursor desmosterol had the highest importance by percent increase in mean squared error metric in this model. By comparison, an RFM constructed with three demographic variables (age, gender, ethnicity) explained only about 2.2% of the variation. Combining sterol concentrations with age, gender, and ethnicity improved variation explained to about 7.4%, suggesting that plasma sterol concentrations have predictive value over and above basic demographics. Next, a machine learning algorithm was used to select features from an unbiased pool of 43 variables (including demographics, general health indicators, and sterol concentrations) to explain variation in QIDS-SR scores. This model consisted of 19 variables, 13 of which were sterol concentrations, and explained about 15.4% of the variation in QIDS-SR scores. These 13 sterols for tested for their individual associations with depression symptoms scores. Desmosterol concentrations below the fifth percentile (1.9 ng/mL, OR 1.9, CI 1.2-2.9) and 7-dehydrocholesterol (a cholesterol and vitamin D precursor) concentrations above the 95th percentile (5.4 ng/mL, OR 2.4, CI 1.6-3.6) were significantly associated

with moderate-to-severe depressive symptoms (QIDS-SR score ≥ 10.5), while an association between higher cholestanol concentrations and depressive symptom scores did not remain statistically significant ($p < 0.05$) following strict correction for multiple comparisons.
Conclusions: This is the first study reporting an association between plasma concentrations of desmosterol or 7-dehydrocholesterol and depression symptom scores. Using machine learning methods, we showed that plasma sterol concentrations have predictive value over and above demographic and general health variables. These sterols are novel and promising leads for plasma biomarker development in depression.
Keywords: Depression, Sterols, Epidemiology, Biomarkers, Machine Learning.
Disclosure: Genentech: Honorarium, Self.

M92. Ketamine Reduces Fear Expression if Administered Prior to a Stressor but Does not Facilitate Extinction

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Background: Ketamine has been reported to be an efficacious antidepressant for major depressive disorder (MDD) and posttraumatic stress disorder (PTSD). Most recently, ketamine has also been shown to be prophylactic against stress-induced depressive-like behavior in mice. It remains unknown, however, when ketamine should be administered relative to a stressor in order to maximize its antidepressant and prophylactic effects. Moreover, it is unknown if ketamine can be prophylactic against subsequent stress-inducing episodes, affecting fear expression and enhance extinction.
Methods: We systematically tested the utility of ketamine relative to a fear experience to determine when ketamine may be administered to most effectively reduce fear or to prevent subsequent aversive episodes. Using a contextual fear conditioning (CFC) paradigm, mice were administered a single dose of saline or ketamine (30 mg kg-1) at varying time points before or after (when?) CFC, extinction, and reinstatement. Mice were also tested in the forced swim test (FST) and the open field (OF) paradigm to probe ketamine's effects on depressive-like and exploratory behavior.
Results: Mice administered prophylactic ketamine 1 week, but not 1 month, before CFC exhibited reduced freezing behavior when compared with mice administered saline. In contrast, ketamine administration following CFC or during extinction and reinstatement did not alter subsequent fear expression.
Conclusions: These data indicate that ketamine can buffer a fear response when given as a prophylactic, but not when given after a stress-inducing episode. Thus, ketamine may be most useful in the clinic if administered in a vaccine-like fashion in order to protect against heightened fear responses to aversive stimuli.
Keywords: Ketamine, Extinction, PTSD, Major Depression, Posttraumatic Stress Disorder, Fear Conditioning.
Disclosure: Nothing to disclose.

M93. Clinical Severity and use of Atypical Antipsychotics in Patients With Treatment Resistant Depression (TRD)

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Background: Major depression is a disabling disorder affecting about 8.2% of people yearly with worldwide prevalence rates ranging from 10% to 19% [Kessler et al, 2003]. According to the STAR*D study, more than 50% of patients suffer from treatment-resistant depression (TRD), since they do not respond to a first trial of an antidepressant [Trivedi et al, 2006].
Methods: Sociodemographic and psychopathological features and treatment outcomes were evaluated in 100 patients (59 females, 41 males) with TRD (two or more antidepressant trials and/or combinations failed) from the Register of the Mood Disorder Clinic at McGill University Health Center (MUHC), by chart review analysis. Participants' diagnoses were ascertained by the Structured Clinical Interview for Diagnosis (SCID) [First et al, 1994] carried out by skilled professional or by psychiatrists. All participants met DSM-V criteria for Major depressive disorder (MDD) without any hypomanic or manic symptoms. These patients underwent several antidepressant treatment strategies. Only the last trial, when the patient responded to treatment and remained stable for more than 6 weeks (mean 13.6 ± 5.96, T3), and the treating psychiatrist kept the treatment unchanged, was included in this study for statistical analysis. Clinical response was investigated prior to treatment (T0) and after treatment (T3) using the Montgomery-Asberg Depression Rating Scale (MADRS), the Hamilton Rating Scale for Depression (HAM-D17), the Quick Inventory of Depressive Symptomatology (QIDS-C16) and the Clinical Global Impression-Severity of Illness (CGI-S). Subjects were also grouped according to the Maudsley Staging Method (MSM).
Results: After several failed trials, patients had responded to one of two pharmacological interventions. Group A: antidepressant combinations ($n = 34$); Group B: augmentation strategies with antipsychotic and/or mood stabilizer ($N = 66$). At T0, Group B, compared to Group A showed increased history of substance abuse (21.21% vs 5.88%, $p = 0.05$), an increased number of failed treatments (4.3 ± 3.02 vs 3.18 ± 1.86, $p = 0.05$), and significantly higher baseline values in scales' mean scores (MADRS: 33.1 ± 1.0 SEM vs 26.6 ± 1.5, $p < 0.001$; HAMD: 24.7 ± 0.7 vs 20.3 ± 1.1, $p < 0.001$; QIDS-C16: 16.8 ± 0.5 vs 13.9 ± 0.7, $p < 0.001$; CGI-S: 5.3 ± 0.1 vs 4.5 ± 0.2, $p < 0.001$). At T3 (outcome) compared to T0, patients in both A and B groups showed a significant decrease in depressive symptoms on all scales ($P < 0.001$). Importantly, the delta change was superior in group B compared to group A (MADRS: 13.9 vs 9.0, $p = 0.007$; and HAM-D17 9.9 vs 6.4, $p = 0.005$).
Conclusions: These results suggest the importance of antipsychotic/mood stabilizer augmentation as a first-line treatment in patients with severe TRD. We have identified a subgroup of TRD patients (Group B) presenting with specific

psychopathological features (significantly higher scores on MADRS and HAMD-17 score at baseline, higher number of failed treatment trials, substance abuse) that respond significantly better to atypical antipsychotics and/or mood stabilizers. Randomized-controlled trials evaluating the independent roles of augmentation with antipsychotics or mood stabilizers are warranted in order to assess the initial pharmacological options for patients with severe TRD as well as to better characterize this subgroup of patients from a psychopathological and therapeutic point of view.

Keywords: Treatment Resistant Depression, Antidepressant Treatment Practice, Atypical Antipsychotics, Treatment Outcome, Mood Stabilizers.

Disclosure: Nothing to disclose.

M94. The Role of White Matter in Personality Traits and Affective Processing in Bipolar Disorder

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Background: Bipolar disorder (BD) is characterized by affective processing bias and variations in personality traits. It is still unknown whether these features are linked to the same structural brain alterations. The aim of this study is therefore to investigate whether changes in white matter (WM) properties accentuate specific personality traits and impair affective processing in BD.

Methods: 24 healthy controls (HC) and 38 adults with BDI (HC: 29.47 ± 2.23 years, 15 females; BDI: 32.44 ± 1.84 years, 20 females) completed clinical scales and the Big Five Inventory. They were also administered the Affective Go/No-Go (AGN) and the Rapid Visual Processing (RVP) tasks of the Cambridge Neuropsychological Test Automated Battery. Diffusion Tensor Imaging (DTI) assessed the microstructure of WM tracts.

Results: In BDI measures of WM properties were reduced across all major brain white matter tracts. As expected, individuals with BDI reported greater neuroticism, lower agreeableness and conscientiousness, and made a greater number of errors in response to affective stimuli in the AGN task compared to HC. Overall better WM integrity was associated with higher agreeableness and conscientiousness scores, and better performance in the AGN and RVP tasks. Notably, high neuroticism scores were associated with faster AGN reaction times in BDI, and reduced AGN accuracy in HC.

Conclusions: Our findings showed important links between WM and personality and affective processing in BD. Therapeutic interventions for BD using brain stimulation protocols will benefit from the use of DTI to target pathways underlying affective processing and personality.

Keywords: Bipolar Disorder, Diffusion Tensor Imaging, Personality, Affective Neuroscience.

Disclosure: Nothing to disclose.

M95. The Anterior Limb of the Internal Capsule in the rat: Organization and Homology With Primates

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Background: The prefrontal cortex (PFC) is composed of anatomically and functionally specialized sub-regions involved in reinforcement-based learning, response selection, attention and behavioral flexibility. The orbital (OFC) and anterior cingulate cortex (ACC) are associated with several psychiatric disorders, including obsessive-compulsive disorder (OCD) and major depressive disorder (MDD). The anterior limb of the internal capsule (ALIC) in primates carries the ascending and descending fibers from the ACC/OFC in a topographic fashion (Jbabdi et al, 2013; Lehman et al, 2011). Importantly, diseases linked to ACC/OFC show abnormalities in the ALIC. Furthermore, the ALIC is a target for deep brain stimulation and ablative surgery for MDD and OCD.

The rat represents an essential animal model to investigate normal physiological and behavioral function, and the mechanisms that underlie abnormal behaviors. However, little is known about the trajectories and organization of descending ACC/OFC fibers in rats. While the rat does not have an organized and compact ALIC as found in the primate, the striatum in both species is filled with small white matter (WM) fascicles that contain dense bundles of passing cortical fibers.

Methods: Following recognized subdivisions of the rodent PFC, we injected a selective anterograde tracer into the following five regions in rats: anterior cingulate (Cg), prelimbic area (PL) and infralimbic area (IL) of the ACC, and the ventral lateral orbital area (VLO) of the OFC. For each case, the injection site and fiber bundles passing from the cortex through the striatum to the thalamus and brainstem structures were charted. Each individual drawing was then combined into a single 3D model to reveal the topography of ACC/OFC descending projections.

Results: Fiber bundles leave the injection sites and enter the corpus callosum (CC). Here they split into two bundles: one passes to the contralateral cortex, the second descends toward and enters the striatum. This bundle then divides into small fascicles that are embedded within the striatum. There is no compact WM bundle connecting the CC with the internal capsule. Rather, axons from ACC/OFC structures travel within the WM fascicles embedded in the striatum. Nonetheless, ACC/OFC fibers maintain their topography as they pass through the striatum. Thus, medial PFC fibers travel medially within the striatum to VLO fibers. There is also a general dorsal-ventral topography, but with some overlap.

As fibers travel caudally within these fascicles, some exit at specific points to terminate in the striatum. Others continue to the level of the anterior commissure, where they enter the forming main body of the internal capsule. Here, fibers move ventrally as they travel to the thalamus and brainstem.

Conclusions: Experiments in rats provide essential information on the mechanisms of brain function, including WM composition and perturbations. Descending PFC fibers in primates form a well-defined ALIC, but the location and organization of these fibers in rats is unknown. We addressed this gap by analyzing descending fibers from injections in the ACC/OFC of the rat PFC. Descending fibers form WM fascicles that are embedded within the striatum. These bundles are arranged topographically according to origin and target location, and contain descending projections not only to the striatum, but also to the thalamus and brainstem. They can therefore be viewed as the ALIC homologue in the rat. Mapping these projections allows us to more precisely identify the fibers affected by experimental manipulations of the striatum. This process is essential for translating abnormalities of human WM to rodent models.

The major difference between the rat and primate ALIC is that most of the primate ALIC is well-encapsulated (although a large number of fascicles embedded within the primate striatum are part of the ALIC: Lehman et al, 2011), whereas the entire rat ALIC consists of fascicles. Nonetheless, several elements of the topography of the cortical descending fibers are maintained across species. A subset of rat IL thalamic fibers do not pass through internal capsule, but rather remain in a ventral position near the medial forebrain bundle. In the monkey, the descending fibers from area 25 also remain separate from the internal capsule, traveling instead in fascicles embedded in the ventral striatum and ventral to the anterior commissure. Furthermore, like rats, monkeys show topography within the ALIC according to cortical position.

Previously, we identified the rodent cortical homologues of different regions in the primate ACC/OFC (Heilbronner et al, 2016). Based on connectivity with conserved features of the striatum, we established that IL in rodents and area 25 in primates are clear homologues. In addition, we showed that medial versus lateral OFC are likely homologues. However, the homologues of regions in the primate dorsal ACC are less clear: primate area 32 is similar to rodent area PL, but primate area 24 appears similar to portions of rodent PL and CG.

In summary, the results presented here, when combined with our previous work (Lehman et al, 2011; Jbabdi et al, 2013; Heilbronner et al, 2016), allow us to translate both white and gray matter results from rodents to nonhuman primates, and, ultimately, to humans.

Keywords: Internal Capsule, Deep Brain Stimulation, Animal Models, Translational Neuroscience, Prefrontal Cortex.

Disclosure: Nothing to disclose.

M96. Deep Brain Stimulation of the Medial Forebrain Bundle: Distinctive Responses in Resistant Depression

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Background: Treatment resistant depression (TRD) is a serious, debilitating disorder. Deep brain stimulation (DBS) to the superolateral branch of the medial forebrain bundle (MFB) was reported by Schlaepfer et al. (2013) to lead to rapid anti-depressant effects, but has not yet been replicated.

Methods: In this interim analysis of an ongoing pilot study, we assessed the efficacy of MFB-DBS in a cohort of four TRD patients over a 52-week period using improvement on the Montgomery-Åsberg Depression Rating Scale (MADRS) as the primary outcome measure. Implanted patients entered a 4-week single-blinded sham stimulation period prior to stimulation initiation. Deterministic fiber tracking analysis was performed to compare modulated fiber tracts between patients.

Results: Upon stimulation at target intraoperatively, responders reported immediate increases in energy and motivation. There was no significant mean change in mood during sham stimulation phase. After initiating stimulation, 3 of 4 patients had a $>50\%$ decrease in MADRS scores relative to baseline at 7 days. One patient withdrew from study participation. At 52 weeks, two of 3 remaining patients continue to have $>80\%$ decrease in MADRS scores. One patient failed to respond; evaluation of modulated fiber tracts revealed reduced frontal connectivity to the target region.

Conclusions: This study of MFB-DBS shows rapid antidepressant effects within the first week of stimulation, as reported by Schlaepfer et al. (2013). The striking motivational effects observed are very promising, but we await full completion of this pilot study before drawing further conclusions about efficacy.

Keywords: DBS, Treatment Resistant Depression, Medial Forebrain Bundle.

Disclosure: Dunn Foundation: Funding, Self.

M97. Adolescent Sex Differences in Expression of GABA- And BDNF-Related Genes in the Basolateral Amygdala

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Background: Within the corticolimbic circuit, several studies suggest dysfunction in inhibitory gamma-aminobutyric acid (GABA) signaling in major depressive disorder (MDD). Somatostatin (SST), a marker of a subtype of GABA interneurons is more robustly reduced across several corticolimbic brain regions in female subjects with depression. Interestingly, the SST reduction in the basolateral amygdala (BLA) is specific to females, strongly suggesting important potential sex differences in the BLA of depressed subjects. In addition to the reduction in SST in MDD, there is also a deficit in expression of the GABA synthesizing enzymes glutamate decarboxylase 1 (GAD1, also known as GAD67) and GAD2 (also known as GAD65). Taken together, this suggests significant disruption of GABA regulation in subjects with MDD that is specific to females in the BLA. Brain-derived neurotrophic factor (BDNF), which is important for growth and survival of neurons, is also affected in MDD. Previous studies indicate a robust decrease in BDNF signaling in depressed subjects, which is found only in female depressed subjects in the BLA. TrkB, the BDNF receptor, is also reduced in subjects with MDD.

The sex-specificity of reduced BDNF in the BLA suggests that there may be an underlying difference in BDNF-related signaling in the BLA due to a sex-related factor(s).
Interestingly, the sex difference in MDD incidence emerges in adolescence. Adolescence is a sensitive developmental time-period in which there is extensive neuroanatomical, functional, and chemical brain maturation. Events during adolescence that change the timing of these developmental processes can increase risk for adult psychopathology. We hypothesize that sex differences in GABA and/or BDNF function during adolescence may influence adult sex-specific cortical synchronization, subsequently affecting psychiatric disorder vulnerability. Although researchers are attempting to determine the mechanisms underlying these sex differences, it is impossible to distinguish between the roles of genetic sex and gonadal sex in humans, since genetic sex determines gonadal sex. Therefore, we have been using the genetically-engineered Four Core Genotypes (FCG) mice, in which genetic and gonadal sex are decoupled, to examine the mechanisms underlying observed sex differences in humans. We recently showed an opposing effect of male genetic sex and male-like testosterone levels on anxiety-like behavior (higher anxiety in XY mice, lower anxiety in testosterone-treated mice). Notably, we found that adult BLA expression of GABA- and BDNF-related genes mirrored the behavioral effect, suggesting an underlying vulnerability for elevated anxiety in genetic males that is masked by circulating testosterone. Since these previous studies were conducted in adult mice, it is unclear whether our findings reflect a vulnerability created during adolescence. Here, we aimed to determine whether the GABA and BDNF systems differ during adolescence based on genetic sex, gonadal sex, and/or circulating hormones.
Methods: This study used adolescent (postnatal day 21) FCG mice of each genotype [XX gonadal females ($n=$ 21), XY gonadal females ($n=$ 21), XX gonadal males ($n=$ 26), and XY gonadal males ($n=$ 19)]. Adolescent mice were sacrificed by rapid decapitation. Brains were dissected out and immediately flash frozen on dry ice. Trunk blood was collected for testosterone hormone assays. Bilateral micropunches (1mm bore punch) of the BLA (between Bregma -0.94 mm and -1.82 mm) were obtained from approximately six 160μm thick coronal tissue sections cut on a cryostat. RNA was isolated and converted to cDNA. Using qPCR, we examined expression of 3 GABA-related genes (Sst, Gad67, Gad65) and 2 BDNF-related genes (Bdnf, Trkb). All experimental protocols in animal studies were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.
Results: We found that adolescent XY mice had lower BLA expression of the 3 GABA-related genes examined compared to XX mice [Gad67 ($p<$ 0.01), Gad65 ($p<$ 0.035), Sst ($p<$ 0.08)]. There was no effect of gonadal sex on expression of these genes ($p>$ 0.3 for all comparisons). We also found that XY mice had lower expression of Trkb compared to XX mice ($p<$ 0.05). There was no genetic sex effect on Bdnf expression and no gonadal sex effect on either Bdnf or Trkb expression ($p>$ 0.03 for all comparisons). There was no correlation of gene expression with circulating testosterone levels ($p>$ 0.05 for all comparisons).
Conclusions: Given that adult XY mice show elevated anxiety-like behavior, this adolescent difference in GABA-related genes and Trkb expression may contribute to adult mood-related behaviors. Differences in expression of these genes based on genetic sex in adolescents could permanently affect brain communication and/or synchronization. These results emphasize that adolescent amygdala development differs between genetic males and females, and deserves more attention given the long-lasting effects of adolescent GABA and BDNF development.
Keywords: Sexual Dimorphism, GABA, BDNF, Adolescence.
Disclosure: Nothing to disclose.

M98. Functional Connectivity Between Anterior Insula and Key Nodes of Default Mode, Salience, Central Executive and Reward Networks Distinguish Subtypes of Depression

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Background: Individuals with major depression exhibit considerable symptom heterogeneity with implications for effective treatment. Anxious depression, anhedonic depression, or irritable depression may represent meaningful depressive subtypes with differential underlying pathophysiology. Mood dysregulation in various forms (e.g. rumination, amotivation, affect lability) is seen across depressive subtypes and is linked to the inability to adaptively switch between networks supporting internal, self-referent processing (default mode network, DMN), salience processing (salience network, SN), goal-directed executive processing (executive control network, CEN) and reward processing (reward networks, RN). The anterior insula has been identified as an important hub implicated in adaptive switching between functional networks (Sridharan et al, 2008). In the present study, we sought to investigate whether distinct deficits in the connectivity between the anterior insula and functional networks might distinguish depressive subtypes. Specifically, using data from the large multi-site EMBARC study, we used and an a priori, hypothesis-driven ROI approach to examine whether differential functional connectivity between the anterior insula and key nodes of the DMN, SN, CEN and RN distinguish anxious depression, anhedonic depression, irritable depression and healthy controls.
Methods: Resting state fMRI data were analyzed from 92 depressed patients and 51 age and gender-matched healthy controls. Depression subtypes were identified using scores on the Mood and Anxiety Questionnaire (MASQ), Snaith-Hamilton Pleasure Scale (SHAPS), and Anger Attacks Questionnaire (AAQ). Patients >2 SD from the mean of healthy controls on each subtype-related measure were selected (anxious: $n=24$; anhedonic: $n=$ 23; irritable: $n=$ 22; >1 subtype $=$ 23) After standard fMRI data preprocessing steps, ROI-ROI analyses were conducted using

independently-defined bilateral insula seeds (k = 3; Kelly et al, 2014) and bilateral DMN, SN, CEN, nucleus accumbens and ventral tegmental area seeds (Seeley et al, 2007). Pearson correlation coefficients were computed between ROIs. After r-to-z transformation, one-way ANOVAs and independent t-tests were run in SPSS to identify significant differences in connectivity between groups.

Results: Anxious depression was characterized by increased right dorsal and bilateral ventral insula-right DLPFC (CEN) functional connectivity. Anhedonic depression was characterized by decreased bilateral anterior insula-nucleus accumbens functional connectivity and decreased right dorsal anterior insula-dACC (SN) functional connectivity. Irritable depression was characterized by decreased right dorsal anterior insula-VLPFC (SN) functional connectivity.

Conclusions: Anxious depressive, anhedonic depressive, and irritable depressive subtypes evidenced differential patterns of anterior insula-functional network connectivity. Anhedonic depression showed unique deficits in insula connectivity to the nucleus accumbens in the RN and the dorsal anterior cingulate in the SN, two key regions implicated in monitoring and processing reward and salience respectively. By contrast, anxious depression showed greater connectivity between insula and a right lateral DLPFC region of the CEN. Hyperactivation of right DLPFC has been linked to attentional biases towards negative emotional stimuli in both anxiety and depression. Finally, irritable depression evidenced unique deficits in insula-VLPFC functional connectivity, a key SN region implicated in adaptive emotion regulation. These data suggest deficits in insular-functional network connectivity may be an important factor underlying specific patterns of pathology in depression subtypes, a potential biomarker in need of further research.

Keywords: Mood Disorder Subtypes, Resting State fMRI, Functional Network Connectivity.

Disclosure: Nothing to disclose.

M99. Disrupted White Matter Microstructure Within Dorsolateral Prefrontal Cortex Contributes to Negativity Bias in Late-Life Depression

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Background: Individuals with late-life depression (LLD) often demonstrate a persistent negativity bias. Negativity bias is behaviorally expressed as sustained attention to negatively-valenced stimuli and impaired direction of attention to positively-valenced stimuli (Rozin & Royzman, 2001). In depressed patients, negativity bias contributes to maladaptive self-referential thinking and a tendency to focus on negative information or outcomes (Eaves & Rush, 1984). Previous research suggests that negativity bias is associated with poor response to antidepressant drug treatment (Pizzagalli, 2011). Moreover, negativity bias is reduced in remission of depression (Eves & Rush, 1984; Davidson et al, 2002). Our primary objective was to investigate the relationship between white matter integrity in frontal brain regions, which are particularly susceptible to aging, and the persistence of negativity bias following antidepressant treatment in older adults with depression. We used a tract-based diffusion tensor imaging (DTI) approach to assess frontal white matter microstructure in neural structures critical for affective processing: the dorsolateral prefrontal cortex (DLPFC) and the reward system.

Methods: We studied 12 older adults (aged 60-90) diagnosed with major depressive disorder by SCID-R/DSM-IV criteria. Patients received 12 weeks of escitalopram treatment at a target daily dose of 20mg. Before beginning treatment, patients had a baseline MRI scan that included a DTI sequence. White matter microstructure was quantified with the extraction of fractional anisotropy (FA) values. FA was restricted to our regions of interest using a binary masking procedure. We extracted FA from DLPFC; this mask encompassed portions of the superior, middle, and inferior frontal gyri. We also extracted FA from the reward system; this mask included ventromedial prefrontal cortex, ventral striatum, nucleus accumbens, putamen, parahippocampal gyri, and amygdala. To assess negativity bias, patients completed a Trait Adjective Task outside of the scanner at baseline and follow-up (after 12 weeks of escitalopram treatment). Patients viewed positive and negative personality trait words on a laptop and decided if each word described themselves or not. The number of endorsed traits and rejected traits were analyzed as a function of their positive or negative valence.

Results: Lower baseline white matter FA within right DLPFC predicted greater negativity bias at follow-up. Patients with lower FA within right DLPFC at baseline endorsed fewer positive traits at follow-up ($r(10) = 0.62$, $p < 0.05$) and rejected fewer negative traits at follow-up ($r(10) = 0.53$, $p < 0.05$). In contrast, FA within left DLPFC at baseline was not associated with endorsed positive traits ($r(10) = 0.32$, $p = 0.16$) or rejected negative traits ($r(10) = 0.38$, $p = 0.11$) at follow-up. Baseline white matter FA in the right and left reward system was not associated with persistent negativity bias at follow-up. There was no significant correlation between baseline reward system FA and follow-up endorsed positive traits (right: $r(10) = -0.03$, $p = 0.46$; left: $r(10) = 0.07$, $p = 0.41$) or rejected negative traits (right: $r(10) = 0.24$, $p = 0.23$; left: $r(10) = -0.25$, $p = 0.22$).

Conclusions: Disrupted frontal white matter microstructure within the right DLPFC is associated with the persistence of negativity bias in individuals with LLD. These results suggest that abnormal connectivity within right DLPFC may play a role in disrupted affective processing and negative self-referential thinking following antidepressant treatment. The laterality of this finding to the right hemisphere is consistent with findings of hyperactivity of the right DLPFC in major depressive disorder (Grimm et al, 2007). Thus, abnormal right DLPFC activation may underlie directed attention to negatively-valenced stimuli in depressed patients. We did not find an association between negativity bias and white matter microstructure within the reward system, which is critical for processing rewarding, positively-valenced stimuli. This finding suggests that persistent negativity bias in LLD may be the result of sustained, DLPFC-driven attention to negative information, as opposed reward system dysfunction and impaired processing of positive information. Overall, the right DLPFC plays a key role in the direction of selective

attention. Therefore, disrupted white matter integrity within this region may contribute to abnormal processing of negatively-valenced information in older adults with depression.
Keywords: Late-life Depression, Diffusion Tensor Imaging, Antidepressant Response.
Disclosure: Nothing to disclose.

M100. C-Reactive Protein Associations With Brain Structure and Function: Neural Markers of Pro-Inflammatory Risk for Depression Following Trauma Exposure

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Background: Elevated levels of inflammatory mediators, including C-reactive protein (CRP), have been linked to depression. These inflammatory mediators may enhance psychopathology risk via effects on brain structure and function. The amygdala and ventromedial prefrontal cortex (vmPFC), brain regions involved with emotional response and regulation, may be most susceptible to these effects. Thus, we examined associations among plasma CRP, BOLD response and white matter connectivity between the amygdala and vmPFC in a sample of traumatized women.
Methods: Clinical interview, serum CRP, fMRI and diffusion tensor imaging (DTI) data were collected from 14 women recruited from primary care clinics of an inner-city hospital in Atlanta, Georgia. Participants received MRI during performance of the affective number stroop, an attentional control task that included trauma-relevant, positive and neutral distractor images. Probabilistic tractography was used to examine structural integrity of white matter connections between the amygdala and vmPFC. Mean fractional anisotropy (FA) values were entered into statistical models.
Results: Serum CRP was positively correlated with BOLD activation in the amygdala ($r2 = .69$, $p < .01$) and ventromedial prefrontal cortex ($r2 = .63$, $p < .01$) during trauma-relevant distractor trials. A marginally greater right amygdala response was observed in participants with major depressive disorder histories ($p = .05$). Although no association was observed between CRP level and amygdala-vmPFC structural connectivity, a non-significant negative association was observed between right amygdala response and integrity of white matter tracts connecting the amygdala and vmPFC ($p = .14$).
Conclusions: These preliminary findings indicate that higher CRP level (and depressive history) is associated with greater amygdala and vmPFC response to trauma-relevant cues. This pattern of response was, in turn, linked to poorer structural integrity of the amygdala-vmPFC pathway. Altered structure and function of the amgydala-vmPFC pathway will be discussed as a biological marker of pro-inflammatory risk for depression following trauma exposure.
Keywords: Functional MRI (fMRI), c-reactive Protein, Inflammation, Diffusion Tensor Imaging, Depression
Disclosure: Nothing to disclose.

M101. Comparative Effects of Immune-Targeted Therapies on Depressed Mood and Anhedonia Across 18 Placebo-Controlled Immunology and Oncology Clinical Trials

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Background: Inflammatory and immune biomarker abnormalities have been reported in primary mood disorders, and the prevalence of major depressive episodes increases in some medical conditions that involve activation of pro-inflammatory signaling pathways. Moreover, while preliminary data suggest that anti-inflammatory or immune modulating drugs may alleviate depressive symptoms, it remains unclear which molecular targets may hold the greatest promise for yielding novel antidepressant treatments. A variety of immune system-targeting compounds have been tested in clinical trials for inflammatory disorders and cancers. Here we perform a meta-analysis of 18 placebo-controlled, randomized, double-blind clinical trials conducted by Janssen and GlaxoSmithKline for immunology or oncology indications ($N = 10{,}745$) to explore whether improvement in depressive symptoms occurred with immune modulating therapies, and if so, whether specific treatment mechanisms afforded greater antidepressant efficacy than others. To the extent possible we attempted to distinguish antidepressant effects from improvement in the peripheral signs and symptoms of the primary disorder.
Methods: We assessed data collected on 9 compounds and 7 molecular targets (IL-6, TNF-a, IL12/23, CD20, COX2, BLgS and p38/MAPK14) in patients being treated for 9 diseases: rheumatoid arthritis, ulcerative colitis, psoriasis, ankylosing spondylitis, asthma, osteoarthritis of the knee, neuropathic pain, systemic lupus erythematosus, and multicentric Castleman's disease. Two core depressive symptoms (depressed mood, anhedonia) were measured on the SF-36 Health Survey, version 2.0. Patients were designated as having high-depressive symptoms at baseline (HDS, $N = 1{,}921$ patients), meaning one of the depressive symptoms was present at least 'most of the time' and the other at least 'some of the time' for four weeks. One study rated the severity of depressive symptoms using the Hospital Anxiety and Depression scale, in which case a cutoff of HADS-D $>= 11$ was used. The treated arm was defined as receiving any active dose; the placebo arm was defined as in each original study, and was typically combined with standard of care. Changes in depressive symptoms from baseline to post-treatment (4-16 weeks, depending on study) were evaluated directly and with adjustment for symptom severity of the primary disease using a mixed-effects model with repeated measures. Fixed-effects included treatment group, visit, and interaction between treatment group and visit. Analysis was also performed within patients designated as non-responders to the primary disease. Treatment effects were assessed across all studies, and grouped by drug mechanism, using the standardized mean difference (SMD, or Cohen's d), to compare the change in depressive symptom improvement in

the treatment vs. placebo arm using the R package metafor. For a subset of studies, baseline CRP levels were available for correlation with percentage of patients with HDS, estimation of mean differences between HDS and LDS groups, and correlation with improvement in depressive symptoms.

Results: The fraction of patients with high depressive symptoms varied across trials, and was significantly higher in the rheumatoid arthritis studies compared with the aggregate of all non-RA studies ($p = 0.004$, 2-sided t-test). Average baseline CRP was significantly correlated with the percentage of patients with HDS at baseline (Pearson correlation coefficient = 0.57, $p = 0.04$). The overall pooled effect, taking together all 18 studies, showed greater improvement in the treated arm than the placebo arm (SMD = 0.29, 95% CI = [0.12-0.46]; z = 3.35, $p < 0.001$). The significance of the overall treatment effect on mood symptoms was maintained after adjusting for changes in primary disease severity, and also in the primary disease non-responder subgroup considered alone. Comparing effect size across drug mechanism shows some variability, with COX-2 inhibitors numerically, though not significantly, favoring placebo (SMD = -0.15 [-0.37-0.07]; z = 1.3; $p = 0.17$); p38/MAPK14 antagonist having no effect (SMD = 0.05 [-0.54-0.65]; z = 0.18; $p = 0.86$), and IL-6 antagonists (SMD = 0.80 [0.20-1.41]; z = 2.6; $p = < 0.01$) and IL12/23 antagonist (SMD = 0.74 [0.52-0.96]; z = 6.5; $p < 0.0001$) showing the largest effect sizes. After adjusting for primary disease symptom severity the effects were smaller, but trends were preserved with the largest effect sizes for the IL-6 antagonists (SMD = 0.56 [-0.04-1.16]; z = 1.8; $p = 0.07$) and the IL12/23 antagonist (SMD = 0.57 [0.35-0.79]; z = 5.1; $p < 0.0001$). Within primary disease non-responders, not adjusting for symptom severity, similar trends were observed although significance was reduced as sample size was smaller (IL-6: SMD = 0.88 [0.16-1.59]; z = 2.4, $p = 0.017$; and IL12/23: SMD = 0.53 [-0.19-1.25], Z = 1.44, $p = 0.15$).

Conclusions: These results demonstrate that some anti-inflammatory or immune modulating drugs have statistically significant, clinically moderate anti-depressant effects in patients with inflammatory disorders and high depressive symptoms at baseline. These effects were not simply attributable to improved physical symptom severity scores and appeared stronger for anti-IL6 and anti-IL12/23 mechanisms of action.

Keywords: Major Depressive Disorder (MDD), Immune Mechanisms, Meta-Analysis.

Disclosure: Janssen Research & Development, LLC: Employer and Financial Holdings, Self.

M102. Inflammasome Activation in the Brain: Impact on Depressive-Like Behaviour

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Background: Inflammasome activation causes the maturation of caspase-1 (casp1, aka interleukin converting enzyme), an enzyme that plays a role in a number of physiological and pathophysiological processes both in the CNS and periphery (i.e., immune response, microglia activation, LTP, synaptic plasticity, adipocyte differentiation, chronic inflammation). We investigated the behavioural phenotype of genetic deficiency and pharmacological inhibition of caspase 1 at baseline and after chronic stress. We also studied simultaneous gut microbiota changes.

Methods: Adult male mice (WT and casp1 knockout) were submitted to a battery of behavioural tests at baseline and after chronic restraint stress (4 h/day for 3 weeks). Forced swim test, open field test, novelty suppressed feeding, elevated plus maze, sucrose preference test and rotarod were performed. Fecal pellets were collected and used in 16S rRNA gene amplicon sequencing.

Results: Genetic caspase-1 deficiency decreased depressive- and anxiety-like behaviors, and increased locomotor activity. Pharmacological caspase-1 antagonism improved stress-induced depressive like behaviour; fecal microbiota profiling with 16S rRNA showed increased in the relative abundance of the genus Akkermansia and Blautia. We will present new metagenomics data disclosing the bacteria species involved and their network analysis.

Conclusions: The protective effect of caspase-1 inhibition in the exacerbation of post-stress depressive-like behaviour may involve the modulation of the relationship between stress and gut microbiota composition via inflammasome signalling pathways. We propose that the gut-microbiota-inflammasome-brain axis may be a viable novel therapeutic target for depression.

Keywords: Major Depression, Animal Models, Gut Microbiome, Inflammasome.

Disclosure: Nothing to disclose.

M103. Late-Life Major Depression and Neuroticism: A Preliminary Functional Connectivity Study

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Background: The role of neuroticism in late life major depressive disorder (MDD) is generally understudied. Our initial research shows that older depressed patients scoring higher on measures of neuroticism experience worse clinical outcomes than those scoring low in neuroticism, including poorer mood outcomes to acute antidepressant treatment and greater longitudinal cognitive decline. Functional magnetic resonance imaging (fMRI) may be a useful technique to understand the underlying connections between depression and neuroticism. We sought to examine neuroticism-related resting-state functional brain activity and connectivity patterns among older adults with and without MDD enrolled in the Neurobiology of Late-life Depression (NBOLD) study at UConn Health Center.

Methods: Forty-six currently depressed patients and 36 never-depressed controls enrolled in NBOLD and agreed to clinical assessment and an fMRI scan. Depressed subjects were screened using the CES-D; all had initial scores > 16. They were assessed by a study psychiatrist who administered a Montgomery-Åsberg Depression Rating Scale (MADRS) and confirmed a diagnosis of MDD during a clinical

interview. Subjects completed the DS-14, a measure of negative affectivity (NA) and social inhibition. We used NA scores as a measure of neuroticism. Control subjects scored < 6 on the CES-D and reported no depression history, as confirmed on clinical interview by a study psychiatrist. All subjects underwent a five-minute, eyes-open, resting state 3T fMRI scan at the Olin Neuropsychiatry Center at the Institute of Living of Hartford Hospital. The 5-min resting state fMRI was acquired using multi-band gradient EPI sequence (AF = 8, TR = 475ms, TE = 30ms).

We computed the voxel-wise amplitude of low-frequency fluctuations (ALFF) at the low frequency band (0.01-0.08Hz) and seed-based seed-to-voxel functional connectivity maps (http://rfmri.org/dpabi). To examine the correlations between the negative affect with resting-state activity (ALFF) and functional connectivity, we conducted whole-brain voxel-wise multivariate regression analysis on ALFF maps and ROI-based functional connectivity maps using the NA scores and depression severity (MADRS) as regressors while controlling for age and gender. Only those regions where ALFF values correlated significantly with NA scores were chosen to further examine associations between functional connectivity and negative affect. Instead of using the significant clusters as seeds, corresponding regions from Automated Anatomical Labeling (AAL) defined ROIs were identified as seeds, and functional connectivity maps from these seeds were used as input to conduct the multivariate regression analysis. For all analyses, the significance level was set at $p < 0.05$ using the false discovery rate (FDR) cluster correction for multiple comparisons.

Results: Findings for Negative affectivity using ALFF analyses:

Compared with the control group, the MDD group had decreased ALFF in default mode network (DMN) regions including the posterior cingulate (PCC), mid cingulate, and bilateral lateral parietal cortex, and increased ALFF in right insula, bilateral hippocampus, and fusiform gyrus. However, within the MDD group, greater depression severity was correlated with greater ALFF in the DMN (PCC and medial prefrontal cortex-mPFC). Higher NA was also associated with greater ALFF in default mode network (DMN) regions and executive function related regions, i.e., bilateral dorsolateral prefrontal cortex (dlPFC). Compared with the healthy control group, higher negative affect was associated with greater ALFF in the right insula, amygdala, mPFC, and ventral striatum in the MDD group. Therefore, decreased activity of the salience (insula) and emotional processing (amygdala, ventral striatum) network might be a characteristic difference in MDD patients with high neuroticism.

Findings for Negative affectivity using seed-based analyses using AAL:

Although the MDD group showed significantly reduced functional connectivity (FC) of the ventromedial prefrontal cortex (vmPFC) with PCC, amygdala, and insula compared with the control group, within the MDD group, higher negative affect was associated with greater vmPFC-PCC, vmPFC-amygdala, and vmPFC-hippocampus FC. These correlations were greater in the MDD group compared with the control group.

Conclusions: Among depressed patients, higher negative affect was associated with greater activity in the limbic (hippocampus) and emotional salience (insula) network, as well as enhanced functional connectivity of vmPFC with other regions of the default mode network and with emotion processing and salience networks. The vmPFC is a key region related to emotion regulation. Successful emotion regulation typically reveals negative correlation between vmPFC and regions in the emotion and salience networks. Increased connectivity (less negative correlation) between the vmPFC with amygdala and insula may indicate weakened function of emotion regulation-related neural circuits. Similar results have been reported in schizophrenia and anxiety disorder previously. Our study indicates that enhanced resting state activity of emotional salience network and increased vmPFC-amygdala connectivity in major depression with high neuroticism could be a marker of neuroticism that differentiates who develops depression.

Keywords: Late-Life Depression, Personality, Functional MRI (fMRI).

Disclosure: Nothing to disclose.

M104. Alterations in the Galanin System in Major Depressive Disorder: From Methylation to Peptide

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Background: Major depressive disorder (MDD) has a lifetime prevalence of about 15 % and represents a major burden to patients, families and society (Kessler et al, 2003). MDD is thought to arise from the interaction of genetic and environmental factors, with stressful life events representing an important predisposing factor (Nestler et al, 2002; Vialou et al, 2013). In spite of several therapies, many patients cannot at present be adequately treated (Trivedi et al, 2006). Neuropeptide systems have been explored as targets for the development of novel antidepressants. Extensive animal experiments suggest that galanin (GAL), a 29/30 aminoacid peptide (Tatemoto et al, 1983) acting via three G-protein coupled receptors, GAL1-3 (Habert-Ortoli et a.,1994; Lang et al, 2015), is involved in mood regulation (Fuxe et al, 1991; Lu et al, 2005; Kuteeva et al, 2008). GAL is in rat (Melander et al,1986) and human (Chan-Palay et al, 1989) co-expressed with noradrenaline (NA) in neurons in locus coeruleus (LC), and with serotonin in neurons in the rat dorsal raphe nucleus (DRN) (Melander et al, 1986). Species differences exist, e.g. human NA neurons in LC express GAL3, and not GAL1 as in rat, and human serotonin neurons in the DRN appear to lack GAL expression (Le Maitre et al, 2013).

Methods: We explored the translational potential of these results by assessing the transcript levels and DNA promoter methylation status of GAL and its three receptors in various postmortem brain regions from depressed suicide (DS) subjects and controls, using RT-RT-qPCR and bisulfite pyrosequencing techniques, respectively. We also measured GAL protein concentrations by radioimmunoassay.

Results: Gene expression levels of GAL and GAL3 were significantly increased in noradrenergic LC neurons and in the DRN/ventral periaqueductal gray of both male and

female DS subjects compared to their respective controls, in parallel with decreased methylation of these genes. In contrast, in the forebrain, GAL and GAL3 transcript levels were significantly decreased in the prefrontal cortex of male DS subjects, together with increased DNA methylation. However, no changes were found in the anterior cingulate cortex. GAL protein levels were significantly elevated only in the LC of female DS subjects.

Conclusions: Our data indicate that GAL and its receptor GAL3 are differentially regulated in brains of MDD subjects in a region- and sex-specific manner. The increased, GAL3-mediated hyperpolarisation of the noradrenergic and, presumably, serotonergic neurons of depressed patients suggest that disinhibiting the attenuated firing of these neurons by a selective GAL3 antagonist could result in increased release of noradrenaline and serotonin in forebrain areas involved in mood regulation, and thus in antidepressant activity.

Keywords: Postmortem Human Brain, Major Depressive Disorder, Neuropeptides, Transcriptome, DNA Methylation.
Disclosure: AstraZeneca: Shares, Self. Support: Swedish RC, EC (Newmood), Swedish BF, MM Wallenberg Foundation, BMS, CIHR, FRQ-S, PSG, Hungarian Acad Sci.

M105. P11 Levels in PBMC Correlate With PET Quantification of 5-HT1B Binding in MDD Patients

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Background: Mood disorders, particularly major depressive disorder (MDD), represent the 4th major cause of disability and is projected to be the 2nd by 2020. Current treatment options are not very effective: 30-40% of MDD patients fail to remit despite trying at least two different classes of antidepressant drugs. Although the pathophysiology of MDD is partly understood optimal use of the present treatment options as well as development of new treatments is hampered by the lack of peripheral biomarkers.

Accumulating evidence imply that the 5-HT1B receptor is involved in the pathophysiology of MDD. In animal studies low 5-HT1B receptor mRNA in the Dorsal Raphe Nucleus in rats has been reported to predispose to learned helplessness, and 5-HT1B receptor binding is low in the Hippocampus of maternally-separated rats. Furthermore, in a recent Positron Emission Tomography (PET) study with the 5-HT1B receptor selective radioligand [11C]AZ10419369 increased 5-HT1B receptor binding in the Nucleus Accumbens and Ventral Pallidum has been reported in non-human primates after administration of ketamine. In humans, [11C]AZ10419369 binding measured using PET was recently shown to be significantly lower in the anterior cingulum, subgenual prefrontal cortex and the hippocampus of MDD patients compared to control subjects.

P11 is an intracellular protein necessary for expression of 5-HT1B receptors, present both in the CNS and in peripheral blood mononuclear cells (PBMCs). Levels of p11 in CNS has been associated to both MDD, response to antidepressant treatment and to 5-HT1B levels. Recently, p11 levels in PBMC was associated to response to SSRI treatment of MDD patients.

Accordingly, the aim of this work was to investigate the relation between p11 levels in PBMC and 5-HT1B binding in hippocampus of MDD patients using PET, before and after treatment with cognitive behavioral treatment (CBT).

Methods: A pre/post treatment design was used. A volunteer sample of ten drug-free patients with recurrent MDD (washout period >1 month.), were recruited and examined before (MADRS mean 25.5, range 20-35) and after (7.4, 4-12; mean reduction 18.6, $p<0.001$) standardized Internet delivered cognitive behavioral treatment (CBT; 10-12 weeks). ECAT HRRT (Siemens Molecular Imaging) and the radioligand [11C]AZ10419369 (mean injected dose 385.7 ± 30.9 MBq) was used for the PET experiments. Data acquisition time was 93 minutes. Data were corrected for head motion using a frame-by first-frame realignment algorithm. Coregistration with MRI data was performed using SPM5. The main outcome measure was [11C]AZ10419369 binding (BPND) in the Hippocampus, calculated using WAPI with the cerebellum as reference region. Hippocampus and Cerebellum were defined on MRI as previously described.

Blood samples from patients were collected at time of PET and processed within 4 hours. Whole blood was diluted 1 to 1 with PBS and PBMCs were isolated by density centrifugation. Cells were stored at -80oC in 90% FCS and 10% DMSO until use. P11 levels were measured in monocyte, and T cell subsets and NK cells by intracellular staining using a monoclonal p11 antibody. Two flow cytometry panels were used, one to identify classically activated monocytes (CD14+CD16-), alternatively activated monocytes (CD14+CD16+) and NK cells (CD56+CD16+), and the second to identify T helper cells (CD4+), cytotoxic T cells (CD8+) and T regulatory cells (Foxp3+) in each sample. Stained cells were analyzed by multicolor flow cytometry carried out with a Beckman Coulter Gallios. Data was analyzed with Flow Jo using an appropriate color compensation matrix to correct for spectral overlap and autofluorescence and an isotype control antibody was used to confirm antibody specificity for p11. A hierarchical gating strategy was utilized to remove doublets and dead cells, to identify cell subtypes and subsequently quantify p11 staining within each specific cell type. Data was expressed as total p11 levels, as measured by the percentage of p11+ cells multiplied by the median fluorescence intensity of p11 staining.

Results: PET data were acquired for all 10 subjects at both time points. PBMC data were acquired for 10 patients before CBT and 7 patients at follow-up. P11 fluorescence in CD4+, CD8+ and FOXP3+ cells at baseline correlated negatively with 5-HT1B binding in hippocampus at baseline ($r = -0.78$, -0.80, -0.77 respectively, $p<0.05$). P11 fluorescence in CD4+, CD8+ and FOXP3+ cells at baseline correlated positively with change in 5-HT1B binding (%) between baseline and follow-up ($r = 0.92$, 0.88, 0.80 respectively, $p<0.05$). Finally, change in p11 fluorescence (%) between baseline and follow-up correlated positively in CD4+ and FOXP3+ cells ($r = 0.81$, $p<0.05$ and 0.89, $p<0.01$).

Conclusions: These preliminary data show that levels of p11 in PBMCs (T helper cells, cytotoxic T cells and T regulatory cells) correlate with 5-HT1B receptor binding in the Hippocampus of MDD subjects. As 5-HT1B receptor

expression in brain is dependent on p11, this suggests a possible common regulatory mechanism for central and peripheral p11. The details of this mechanism remains to be described.
Clinically, if replicated also in other cohorts, these data may pave the way for using peripheral p11 levels as a proxy for central 5-HT1B receptor levels in the Hippocampus, and possibly other brain regions, of MDD subjects. P11 may thus be useful for predicting response to treatments such as SSRI and CBT as well as for evaluating new antidepressant treatment options.
Keywords: Major Depressive Disorder (MDD), Peripheral Blood Mononuclear Cells, PET Imaging, Serotonin 1b Receptor, Biomarkers.
Disclosure: Nothing to disclose.

M106. Characterization of FKBP5 Alternative Transcripts in the Human Brain Identified by Transcriptome Sequencing, and Alterations in Bipolar Disorder

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Background: Severe psychiatric disorders, including bipolar disorder, are increasingly accepted as being induced by stress-related effects on neurobiology. A series of landmark studies have provided compelling evidence for a distinct role of FKBP5 – a key co-chaperone of the glucocorticoid receptor in response to stressors – in this process. The FKBP5 gene encodes a number of alternative transcripts, but these have not been characterized directly in the human brain despite the likelihood of tissue-specific transcriptional regulation of the FKBP5 gene. We thus aimed to identify FKBP5 alternative transcripts in human brain (anterior cingulate cortex and hippocampus) and to assess if alternative transcript levels are altered in bipolar disorder relative to matched psychiatrically healthy controls.
Methods: RNA sequencing was performed in the anterior cingulate cortex (BA24) and hippocampus of 13 healthy control and 13 bipolar disorder subjects (type I or type II), matched for age, freezer storage time, and brain pH. Sequencing was completed on the Illumina HiSeq2000 platform using 100-bp paired-end reads. Reads were aligned to the human genome reference (hg19) using TopHat, and Cufflinks was used to generate gene-level and isoform-level counts. Independent t-tests were used to assess differences in transcript expression in cases vs controls.
Results: Reads were mapped to six reported transcripts (variant 1-6) in both the anterior cingulate cortex and hippocampus in human brain. Confirmatory analyses of these variants are on-going. Analysis of the alternative transcripts revealed that, in both the anterior cingulate cortex and hippocampus, a truncated transcript of FKBP5 (variant 4) was significantly increased in bipolar disorder relative to controls (respectfully -66.39% and +35.76%). In the anterior cingulate cortex, variant (variant 1) was also increased in bipolar patients (+70.33%), while variant 2 was decreased (-36.81%); these alterations were not seen in the hippocampus. The other variants were not altered in bipolar disorder compared to controls.
Conclusions: These data characterize FKBP5 alternative transcripts in two regions of the human brain highly implicated in the etiology of stress-induced psychiatric illnesses. In addition, we show that these transcripts are differentially altered in bipolar disorder, and that such alterations may vary according to brain region (i.e. anterior cingulate cortex vs hippocampus). Further analyses are now being conducted to verify the transcripts and their functional significance relevant to glucocorticoid-mediated stress signaling. In addition, these results are being integrated with evidence from other types of tissues to determine cross-tissue relevance.
Keywords: Neurobiology, Stress, Depression, Anxiety, Adolescence, FKBP5, Glucocorticoid Receptor, Bipolar Disorder, Postmortem Brain Tissue.
Disclosure: Nothing to Disclose.

M107. Intrinsic Brain Connectivity in Youth With Depression at High Risk for Insulin Insensitivity

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Background: Depression and obesity are reaching epidemic proportions in American youth today, and when they co-occur, they may have compounding deleterious effects, including the development of impaired insulin sensitivity toward insulin resistance (a precursor to diabetes in which cells fail to respond to the normal actions of the hormone insulin). Traditionally, depression and impaired insulin sensitivity have been compartmentalized as separate emotional and physical health syndromes. However, recent evidence suggests interactions and common neurobehavioral pathways between these syndromes that can lead to worsening depressive symptoms. To date, no study has investigated the underlying mechanisms or risk factors for developing worsening of depressive symptoms in youth with depression and impaired insulin sensitivity. Compelling recent data have shown that youth with and at risk for depression, independent of changes in weight, have early disruptions of neurobiological systems critical for the response and regulation of reward, most notably in the nucleus accumbens, anterior cingulate cortex, insula, and amygdala neural reward circuit.
Methods: Resting-state functional magnetic resonance imaging data was collected from 63 adolescents, aged 12 to 17 years, comprising healthy adolescents with a familial history of depression ($n = 22$, RSK-MDD), adolescents with current symptoms of MDD ($n = 16$, MDD), and healthy control adolescents with no family history of psychopathology ($n = 25$, HC). A seed-based ROI approach was used to examine intrinsic functional connectivity with the bilateral amygdala. Depressive symptoms in the MDD group were assessed using the Children's Depression Rating Scale – Revised (CDRS-R). Blood samples from MDD participants

were processed to obtain an insulin resistance score (HOMA-IR), calculated from fasting plasma glucose and serum insulin values. Relationships between the CDRS-R and HOMA-IR scores and intrinsic connectivity estimates were assessed using the Pearson correlation coefficient.

Results: Adolescents with current MDD symptoms were distinguished from the RSK-MDD and HC groups by significantly greater negative RSFC between the amygdala and clusters in the inferotemporal cortices bilaterally. These clusters were centered on the lingual gyri but also encompassed the fusiform gyrus and lateral occipital cortices. Correlations with CDRS-R and HOMA-IR scores indicated significant associations with RSFC in these regions. Specifically, greater severity of depressive symptoms was found to be associated with stronger negative RSFC between the amygdala and inferotemporal in the left hemisphere ($r = -0.587$, $p = 0.017$). Greater insulin resistance as estimated by HOMA-IR scores was correlated with negative RSFC between the amygdala and inferotemporal cortex in both hemispheres (left: $r = -0.579$, $P = 0.038$; right: $r = -0.602$, $P = 0.029$).

Conclusions: Our study suggests that youth with but not at risk for depression, demonstrate impaired amygdala-lingual gyrus intrinsic connectivity, which correlates with more severe depressive symptoms and impaired insulin sensitivity. Impaired connectivity in these limbic and temporal regions may represent an underlying mechanism of dysfunctional approach motivation in depressed youth. Future studies tracking these associations over time will aide in developing more mechanistically informed treatments.

Keywords: Adolescent Depression, Resting State Functional Connectivity, Insulin Resistance, Amygdala-Based Networks, Risk.

Disclosure: Nothing to disclose.

M108. Parent-Child Psychotherapy Decreases Stress and Increases Maternal Brain Activity and Connectivity During Own Baby-Cry

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Background: The mother-child relationship is central to child development, providing the emotional foundation for social functioning throughout life (Leckman et al, 2004). Sensitive maternal caregiving requires attuned contingent responses for each mother to her own child's distress signals, such as cries. Maternal sensitivity thus involves complex adaptive brain systems to support the requisite thoughts and behaviors, including recognition and acknowledgment of baby-signal salience, reflective self-awareness and emotion regulation and behavioral output (Kim, Strathearn, & Swain, 2016). Certain parental brain functions malfunction with stress and mental illness (Moses-Kolko, Horner, Phillips, Hipwell, & Swain, 2014). Brain function domains both critical to parenting and impaired by mental illness include the social processes of perception and understanding of others, emotional valence systems and their respective constructs of "Perception and Understanding of Others" and "Approach Motivation" that may serve as plausible trans-diagnostic constructs to understand mental illness (Insel, 2014). However, brain-based mechanistic understanding of the adverse effects of parenting stress and benefits of therapeutic interventions on brain physiology are lacking.

Methods: We studied maternal brain responses to salient child signals as a function of Mom Power (MP), a 10-week evidence based, relationship focused psychotherapy intervention that has been established to decrease maternal distress (Muzik et al, 2015).

Twenty-nine mothers underwent two functional magnetic resonance imaging (fMRI) brain scans, including a baby-cry task designed to solicit maternal responses to child's or self's distress signals. Between scans, mothers were pseudo-randomly assigned to either MP ($n = 14$) or Control ($n = 15$) with groups balanced for depression. Pre/post intervention measures included the parental stress index (PSI). Data from s Phillips 3T scanner were analyzed with SPM 8.

Results: Compared to Control, MP decreased parenting stress and increased child-focused responses in social brain areas highlighted by the precuneus and its functional connectivity with subgenual anterior cingulate cortex (sgACC) – key components of reflective self-awareness and decision-making neurocircuitry. Furthermore, over 13 weeks, reduction in parenting stress was related to increasing child- vs. self-focused baby-cry responses in amygdala-temporal pole functional connectivity, which may mediate maternal ability to take the perspective of her child.

Conclusions: Although replication in larger samples is needed, the results of this first parental-brain intervention study demonstrate robust stress-related brain circuits for maternal care that can be modulated by psychotherapy. We thus outline objective basic neural mechanisms of change through which parent-therapy acts on the maternal brain and may relate to stress reduction. These mechanisms may be used to evaluate brain systems for attachment, optimize interventions for parent-child relational problems, and suggest personally tailored brain imaging paradigms to explore trans-diagnostic domains of brain functioning.

Keywords: Maternal Sensitivity, Non-Pharmacological Interventions, Neurobiology, Stress, Depression, Anxiety, Brain Imaging, fMRI, Baby-Cry.

Disclosure: Nothing to disclose.

M109. The Role of Calcium Signaling in Cell Models of Bipolar Disorder

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Background: Genome wide association studies have identified multiple genetic risk factors for bipolar disorder (BP), but the mechanisms by which these gene variants increase risk is currently unknown. One well-documented genetic risk factor has been identified in CACNA1C, the gene

which encodes the pore-forming subunit of the L-type voltage-gated calcium channel CaV1.2. Calcium signaling is important during neurogenesis and brain development, and plays roles in fate specification, activity-dependent gene expression, and development and maintenance of functional neuronal networks. Genetic variation within CACNA1C has been associated with BP risk, and the A allele of rs1006737 (a SNP in CACNA1C intron 3) is thought to play a role in regulating CACNA1C gene expression. However, this SNP has not been systematically studied at early developmental time points to determine how altered calcium signaling may contribute to developmental changes in the CNS in BP.
Methods: We have used mouse neurons lacking CaV1.2 to investigate how this channel mediates neuronal calcium transients observed by the fluorescent calcium indicator Fluo-4 AM. To study the risk SNP rs1006737 in bipolar disorder (BP), we have derived and characterized iPSC from fibroblasts obtained from controls (C) and patients with BP, and have differentiated them into neurons and glia. Using Fluo-4 AM, we have measured differences in spontaneous and evoked neuronal calcium responses in BP versus C neurons.
Results: While both BP and C neurons respond to depolarization, we find that BP neurons have greater calcium transients in response to certain types of stimulation than C neurons. We have also used the CRISPR/Cas9 genome editing system to edit BP cells with the rs1006737 risk genotype (AA) to the nonrisk (GG) genotype, and we are now assessing calcium signaling and differentiation potential in the corrected cells. One potential BP therapeutic, ketamine, may act by altering calcium signals in target cells, and we are using our cell models to assess how ketamine alters patterning and differentiation in BP and C neurons.
Conclusions: The overarching goal of our research is to identify novel disease phenotypes and mechanisms involved in bipolar disorder, with the ultimate aim of improving treatment.
Keywords: Calcium Imaging, Bipolar Disorder, Induced Pluripotent Stem Cells (iPSCs), Ketamine, CACNA1C.
Disclosure: Nothing to disclose.

M110. Elevated Physiological Fluctuations in White Matter in Adolescent Bipolar Disorder

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Background: Physiological fluctuations in white matter (PFwm) during resting state fMRI (rs-fMRI) are considered nuisance variables in traditional functional connectivity studies. PFwm, however, includes variance from cardiac pulsation, vasomotion, hyperintensities, and cerebrovascular dysfunction and are associated with cognitive impairment in aging. Based on the elevated vascular risk in bipolar disorder (BD), we hypothesized PFwm would be elevated in adolescent BD, years before later stages of cerebrovascular disease.
Methods: BD ($n = 32$) and healthy control (HC; $n = 32$) adolescents (13-19 years) matched for age, IQ, and sex were included. Image preprocessing was completed in CONN toolbox (SPM12). PFwm is the temporal variance from white matter voxels in rs-fMRI data (group average white matter mask ($p > .5$) of 20, 2 mm slices in MNI space, Zrange 8-48mm) after data scrubbing and removing global signals. Group differences in the PFwm maps were tested using Flame OLS (FSL4.1.9), thresholded at $p < .05$ cluster corrected at $k > 256$ for family-wise error (defined by AFNI-3DClustSim, 10000 Monte Carlo simulations).
Results: PFwm was elevated in the BD group in 3 clusters including splenium extending to left internal capsule and thalamic radiation flaring from ventricular horn (peak = -4, -32, 14), anterior dorsal body of corpus callosum (peak = -4, 2, 22), and right internal capsule (peak = 24, -14, 10).
Conclusions: Increased rs-fMRI PFwm in adolescent BD may indicate cerebrovascular susceptibility years before visible signs of pathology, such as white matter hyperintensities. Indeed, the most extensive cluster extending from the left ventricular horn is a common locus for later life small vessel disease in white matter.
Keywords: Bipolar Disorder, White Matter, fMRI Resting State, Cardiovascular, Adolescent.
Disclosure: Nothing to disclose.

M111. Development and Preclinical Evaluation of [18F] JNJ-64413739 as a PET Radioligand for P2X7 Receptors

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Background: The P2X7 receptor is an adenosine triphosphate (ATP)-gated ion-channel, which is abundantly expressed in glial cells within the central nervous system. P2X7R activation leads to the release of the pro-inflammatory cytokine IL-1b in the brain and as such the P2X7 receptor may play a role in neuroinflammation. Thus, P2X7R PET ligands hold promise as surrogate central biomarkers of neuroinflammation.
Herein we describe the in vitro and in vivo evaluation of [18F]JNJ-64413739, a 18F-labelled PET ligand for imaging the P2X7 receptor.
Methods: The P2X7R affinity and specificity, pharmacokinetics, metabolic stability, BBB permeability, and off-target binding of JNJ-64413739 were evaluated in a series of in vitro, ex vivo, and in vivo assays. The labeled radiotracer [18F]JNJ-64413739 was synthesized via a one-step radiofluorination by nucleophilic aromatic substitution. The specific binding of [18F]JNJ-64413739 was further evaluated through a series of in vitro autoradiography (ARG) experiments with rodent brain tissue sections. The tracer was also studied in rhesus macaques and the PET imaging data was analyzed with arterial plasma input function based Logan graphical analysis (LGA).
Results: The potency of JNJ-64413739 is 1.9 nM and 1.0 nM at the recombinant rat and human P2X7 receptor (IC50s, FLIPR), respectively, and the binding affinity is 2.7 nM (Ki, rat cortex binding assay) and 15 nM (Ki, human P2X7r). In vivo rat pharmacokinetics (IV dosing) studies revealed a

brain/plasma ratio of 0.91 at 30 min post injection. Blocking experiment in rats by microdosing of JNJ-64413739 demonstrated decreased brain retention by 42% after pre-treatment by a P2X7R antagonist JNJ-54175446. In a similar microdosing experiment, the brain uptake of JNJ-64413739 is 38% lower in P2X7R knock-out (KO) mice compared to P2X7R wild-type (WT) mice. In in vitro ARG blocking experiments with [18F]JNJ-64413739, the tracer's binding to rat brain tissue sections was reduced in a dose-dependent manner by two P2X7R antagonists, A740003 and JNJ-54173717. Using a humanized P2X7R rat model, an ARG experiment with rat brain sections showed a much higher tracer binding in the hP2X7R over-expressed right striatum. In non-human primate PET imaging studies, dose-dependent receptor occupancy (RO) of JNJ-54175446 was observed in two rhesus monkeys. At a 0.1 mg/kg dose (IV) of JNJ-54175446, the RO was calculated to be 17% by LGA, while a dose of 2.5 mg/kg yielded a RO of 60%.

Conclusions: The preclinical evaluation of JNJ-64413739 indicated that it is a potent and selective ligand for rat and human P2X7R. In vivo rodent studies demonstrated that the compound readily enters the brain and binds to P2X7R specifically. In vitro ARG experiments with hP2X7R rats and WT/KO mice brain sections suggested that [18F]JNJ--64413739 engaged the P2X7 receptor. The specific binding of the tracer to P2X7R was further demonstrated by ARG blocking experiments with two known P2X7 antagonists. Reproducible and dose-dependent RO of JNJ-54175446 was obtained in rhesus monkeys in the PET imaging studies with [18F]JNJ-64413739. This PET tracer exhibits in vitro and in vivo characteristics suitable for imaging the P2X7 receptor and warrants further studies in human.

Keywords: Neuroinflammation, P2X7, PET Imaging, Radiotracer.

Disclosure: Janssen: Primary Employer, Self.

M112. Sex-Dependent Role of Melanin Concentrating Hormone Receptors in Schizophrenia

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Background: Neuropsychiatric disorders including depression, anxiety, and schizophrenia are known to possess sexually dimorphic patterns. Sex differences have been established in the prevalence, age-of-onset, clinical manifestations, severity, and response to medication for these disorders. Biological factors such as genetic, hormonal, anatomical and physiological may form the basis for sex-specific differences in neuropsychiatric disorders. The hypothalamus contains sexually dimorphic neuronal populations and neural circuits that play an essential role in regulating reproductive behaviors as well as physiological responses to environmental stimuli. Existing evidence from preclinical anatomical, physiological and pharmacological studies, in addition to human genetic studies, supports the dimorphic role of melanin concentrating hormone (MCH) in regulating feeding behavior. The genetic association of melanin concentrating hormone receptor 1 (MCHR1) with schizophrenia was found to be sex-specific, predominantly seen in men. In light of the results of human genetic association studies, together with findings of animal studies, we hypothesize a sexually dimorphism of MCH system in its modulation of mood and cognition, and in the way MCHR1 gene affects the risk of psychiatric disorders.

Methods: Male and female WT and MCHR1KO mice were 10-12 weeks age, were used for the behavioral experiments. Animals were subjected to a series of behavioral assays: locomotor activity and stereotypic behavior, social interaction, marble burying, novel object recognition and location-dependent object recognition assays, prepulse inhibition (PPI) assay, forced swim test, and contextual fear conditioning assay.

Results: The MCHR1 KO male mice exhibited approximately a two-fold increase in their locomotor activities ($P<$ 0.05). They also displayed a greater level of stereotypic behaviors ($P<$ 0.01). MCHR1 KO female mice displayed an increase in distance travelled in locomotor activity ($P<$ 0.01) and stereotypic behavior ($P<$ 0.05).

In the social interaction assay, both MCHR1 KO and WT corresponding male mice displayed more interaction with the unfamiliar mice than the empty cup ($P<$ 0.001), indicating normal sociability in MCHR1 KO male mice. However, female MCHR1 KO mice displayed similar interaction with the unfamiliar mice and the empty cup ($P>$ 0.05). In the forced swim assay, the MCHR1 KO male and female mice displayed similar level of immobility time with the corresponding WT groups ($P>$ 0.05), suggesting unaffected motivated affection.

We studied the effect of MCHR1 genetic ablation on sensorimotor gating behavior in male and female mice. The MCHR1 KO male but not female mice exhibited significant decrease in PPI ratios compared with the WT males ($P<$ 0.05, for male, $P>$ 0.05 for female), suggesting an impairment in sensorimotor gating function in male MCHR1 KO mice.

In the marble burying assay, the male MCHR1 KO mice buried higher number of marbles than the WT mice ($P<$ 0.001), indicating an increase in repetitive behavior. Female MCHR1 KO mice, however, buried a number of marbles that is comparable to the WT mice.

In the novel object recognition assay, male and female MCHR1 KO mice exhibited a significant decrease in discrimination index when compared to the corresponding WT group ($P<$ 0.001). However, in the novel location recognition assay which tests spatial memory; the female, but not the male, MCHR1 KO group displayed deficit in discriminating the objects in the old and new location as does the WT group, and displays a decrease of discrimination index (male: $P>$ 0.05, female: $P<$ 0.001). In the contextual fear conditioning assay, all groups barely exhibit freezing behavior (no difference between treatments, $P>$ 0.05) before the foot-shock stimulus during the training session. All groups exhibited comparable level of increase in the freezing behavior percentage immediately after the foot-shock stimulus ($P>$ 0.05). In the retention session, however, the male and female MCHR1 KO mice exhibited a significant decrease in the freezing behavior percentage compared to the corresponding WT groups (male, $P<$ 0.05, female, $P<$ 0.01).

Conclusions: Together these results suggest that MCHR1 genetic ablation leads to social impairment and cognitive

deficits and that some of these deficits are sex-specific whereas others are sex-independent.
Keywords: Schizophrenia-Like Behavior, Sexual Dimorphism, KO Mice.
Disclosure: Nothing to disclose.

M113. Evaluating Changes in Factors Associated With Suicidal Thinking Using the Suicide Ideation and Behavior Assessment Tool (SIBAT)

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Background: Suicidal ideation, as characterized by patients on self-report measures, has been shown to be composed of multiple factors. These factors have been explored in different populations with a variety of instruments of varying lengths. However, the manner in which patients change along these dimensions has yet to be systematically examined. A promising new instrument, the Suicide Ideation and Behavior Assessment Tool (SIBAT), combines patient report, patient performance on an implicit association task, and clinician judgment in a flexible, module-based format. Our recent clinical trial of intranasal esketamine in patients with major depressive disorder allowed us to examine how patients' characterization of their thinking on these factors changes as a function of treatment.
Methods: These analyses will be completed on results of a phase 2 study (NCT02133001) in patients with major depressive disorder assessed to be at imminent risk for suicide. A factor analysis of baseline data will be performed on the "My Thinking" module of the SIBAT. This module assesses current suicidal thinking. A 7-factor solution was considered conceptually optimal. Subscales based on these factors were derived and named as follows: 1) Suicidality, 2) Hopelessness, 3) Protective Beliefs, 4) Worry and Guilt, 5) Orientation to Others, 6) Psychosis, and 7) Greater Purpose. Patients treated with esketamine plus standard of care (SoC) or placebo plus SoC were reassessed at 4 and 24 hours following treatment initiation with blinded assessments on the SIBAT. Total scores for each of the 7 component subscales will be calculated for each subject. We will then examine the change from baseline to 4 hours and 24 hours, respectively, in these scores using an ANCOVA model with treatment as a factor and baseline score as a covariate. To illustrate the data, t-scores will be calculated for each subscale based on the baseline sample.
Results: Sixty-six individuals (55% between the ages of 18-34; 53% white) were randomized to receive esketamine plus SoC ($n = 35$) and placebo plus SoC ($n = 31$). Results will characterize the presence and magnitude of changes seen across the identified subscales of the suicide thinking module from baseline to 4 hours and 24 hours posttreatment.
Conclusions: Examination of the manner in which suicidal ideation changes over the course of treatment can provide useful insights into the extent and topography of response. In this study, randomized treatment with either SoC or SoC + intranasal esketamine allows comparison of standard and experimental treatment approaches to effects on suicidal thinking.
Keywords: Suicidal Ideation, Esketamine, SIBAT.
Disclosure: Janssen Scientific Affairs, LLC: Employee, Self; Johnson & Johnson: Stockholder, Self.

M114. Effects of Ketamine on Brain Activation During an Emotional Attention Bias Task in Depression

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Background: Current pharmacological treatments for patients with major depressive disorder (MDD) are generally only effective after several weeks of use, or may not be effective at all for some patients. There is tremendous need for more rapidly acting medications, which could greatly improve treatment for patients with MDD. Ketamine, a glutamatergic modulator, has been shown to have rapid antidepressant effects, including in treatment-resistant individuals. Little is known about the mechanism of action, but interrogation into the relations between ketamine effects and brain function during behaviors affected by illness may provide insights into the underlying antidepressant mechanism. One such function in MDD is altered emotional processing, often resulting in an attentional bias toward negative stimuli. Tasks requiring emotional processing show differences in brain activation during positive and negative stimuli that differ between MDD patients and healthy volunteers in brain regions implicated in MDD, including the anterior cingulate cortex. Moreover, ketamine alters the processing of affective stimuli in prefrontal cortex in healthy volunteers. The purpose of the current study is to assess the effects of ketamine on the processing of emotional stimuli during an attentional dot probe task in patients with MDD.
Methods: Blood oxygen level dependent (BOLD) signal was measured using functional magnetic resonance imaging (fMRI) using a 3T GE HDx scanner as participants performed a dot probe task with emotional faces, as part of a large double-blind placebo-controlled crossover study. Thirty unmedicated treatment-resistant patients with MDD and 17 healthy volunteers, between ages 18 and 65, participated in the study. Participants received ketamine (0.5 mg/kg) and placebo infusions, two weeks apart. The fMRI scans were done at baseline and at two days after each infusion. During the event-related dot probe task, two faces appeared side-by-side for 500 ms, one neutral and the other either angry, happy, or neutral. This was followed by a dot that appeared on one side for 200 ms, and the participant was instructed to respond by pressing a button to indicate whether the dot was on the left or right side. Trials in which the dot was on the same side as the emotional face were considered congruent. Data were processed using AFNI, which included despiking, blurring to 6 mm, motion correction, and alignment to the MNI 152 standard template. Individual statistical maps were generated using regressors for each type of stimulus event. A whole brain analysis was conducted using a linear mixed effect model at the group level, with four factors: diagnostic group, drug, emotion of

stimuli, and congruency of stimuli. Group statistical maps were thresholded at $p<0.05$ (family wise error corrected, initial threshold $p<0.01$) to create a mask of significant regions. Average beta values from these regions were extracted from each individual and used to investigate interaction effects. Reaction time data were also analyzed in a mixed model with the same factors. Attention bias scores were calculated as the difference in reaction time between incongruent and congruent trials for each emotion, and were then analyzed in a mixed model with group, drug, and emotion as factors.

Results: A significant interaction between group, drug, and emotion was observed in bilateral anterior cingulate activation that extended to areas of the medial frontal and superior frontal gyri. Values extracted from this region indicated that higher levels of activation to angry faces in MDD patients during the placebo condition decreased following ketamine. In contrast, no difference in attention bias scores between groups, emotions, or treatment conditions was observed. Reaction time analysis showed a main effect of group, such that patients with MDD were slower overall than healthy volunteers, although no significant difference between the placebo and ketamine conditions was observed.

Conclusions: Our findings suggest that a negative processing bias evident under placebo conditions in prefrontal cortical areas is absent following ketamine in patients with MDD, as compared to healthy volunteers. This shows that ketamine normalized the emotional processing bias during the negative emotional stimuli that characterized MDD. These findings may indicate that the antidepressant effects of ketamine may be related to the process of normalization of function, leading to the correction of a neurally based bias towards the processing of negative information.

Keywords: Major Depressive Disorder, Ketamine, Functional MRI (fMRI), Emotion Processing.

Disclosure: Nothing to Disclose.

M115. Additional Evidence for Sustained Antidepressant Effects of R-Ketamine in Rodent Models: Comparison With S-Ketamine

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Background: Rapid-acting and long-lasting antidepressant effects of ketamine have recently gained much attention. Ketamine is a racemic mixture containing equal parts of R-ketamine and S-ketamine. Although S-ketamine has been regarded as an active isomer based on its higher affinity for the NMDA receptor and anesthetic potency, recent evidence has suggested that R-ketamine exhibits more potent and longer-lasting antidepressant effects than S-ketamine in rodents. However, antidepressant potential of R-ketamine remains to be fully investigated in several animal models including the one in which conventional antidepressants are not effective. Therefore, we first compared sustained antidepressant effects of R-ketamine with those of S-ketamine in behavioral despair models. Then, antidepressant effects of R-ketamine and S-ketamine in a model refractory to current medications were investigated.

Methods: Antidepressant effects of R-ketamine and S-ketamine were evaluated in a forced swimming test and a tail suspension test using mice. As a treatment refractory model, a repeated corticosterone treatment model of rat, which has been reported to be insensitive to conventional antidepressants, was used. Both R-ketamine and S-ketamine were administered intraperitoneally, and behavioral tests were conducted at 30 min, 24 h or 48 h after the treatment. Levels of R-ketamine and S-ketamine in plasma, brain and cerebrospinal fluid (CSF) after intraperitoneal administration in mice and rats were measured by liquid chromatography tandem mass spectrometry.

Results: Both R-ketamine and S-ketamine exhibited antidepressant effects at 30 min after administration (acute effects) in the forced swimming test and tail suspension test in mice. Acute effects of S-ketamine were more potent than R-ketamine, however, these effects may be ascribed to increased locomotor activity, because S-ketamine transiently increased locomotor activity more pronouncedly than R-ketamine in mice. At 24 h after administration, both R-ketamine and S-ketamine showed antidepressant effects with practically the same potency in both forced swimming test and tail suspension test. In contrast, at 48 h after administration, antidepressant effect of S-ketamine was no longer observed, while R-ketamine still exerted a significant antidepressant effect in the mouse tail suspension test. Moreover, R-ketamine significantly reversed increased immobility time induced by repeated treatment with corticosterone in rats at 24 h after administration, as assessed by the forced swimming test, while S-ketamine did not affect the increased immobility time. Both R-ketamine and S-ketamine showed the same exposure levels in plasma in mice and rats, which were rapidly eliminated from plasma ($<$4-8 h). In addition, levels of R-ketamine and S-ketamine in brain and CSF in mice and rats were practically the same, although brain and CSF levels of R-ketamine were slightly higher than those of S-ketamine in mice.

Conclusions: The present results confirmed the previous findings that R-ketamine exerted more sustained antidepressant effects than S-ketamine in animal models of depression. Moreover, we first demonstrated that R-ketamine showed a sustained antidepressant effect even in a model which is refractory to currently prescribed antidepressants, while S-ketamine was without effect in this model, indicating potential of R-ketamine for the treatment of treatment-resistant depression. Pharmacokinetic studies revealed that exposure levels of both R-ketamine and S-ketamine were practically the same and both compounds were rapidly eliminated from plasma, suggesting that differences in antidepressant efficacy between R-ketamine and S-ketamine may not be attributable to differences in exposure levels, and that sustained effects may not be due to sustained exposure. Therefore, R-ketamine may be an active isomer of racemic ketamine, which is responsible for potent and long-lasting antidepressant effects, although human studies are needed to fully address this issue.

Keywords: R-(-)-ketamine, Esketamine, Antidepressant, Treatment Resistant Depression, Ketamine.

Disclosure: Taisho Pharmaceutical Company: Primary Employer, Self.

M116. A Systematic Approach to Determining the Effects of Lithium on Neurocognitive Performance in Patients With Bipolar Disorder

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Background: Neurocognitive impairment is common in bipolar disorder (BD). Deficits stem from both abnormal development/genetic predisposition and from neurodegenerative processes related to the expression of the disease and/or to its treatment. Potential adverse effects of psychotropic medications have long been described but have not been systematically characterized, which is particularly relevant as this is one of the modifiable aspects contributing to cognitive difficulties. Lithium remains a first-line agent; however, patients frequently complain of neurocognitive dulling, which can result in non-compliance and ultimately mood de-stabilization. Nonetheless, there is a surprising dearth of controlled data evaluating the objective cognitive side effects of lithium treatment in BD.
Methods: This study is an 11-site, prospective, non-randomized, open trial of lithium designed to collect a very large cohort of patients with bipolar I disorder to be treated with lithium monotherapy in an effort to promote relapse prevention. Several biological and clinical predictors of treatment response are included in the design, with a primary focus on pharmacogenetics. As a secondary aim, we were able to test the effects of lithium treatment on neurocognitive functioning (i.e. processing speed, verbal memory, executive functions and general cognition) in the largest single cohort reported to date. We first assessed the cognitive effects of lithium treatment at study entry in 264 BD subjects and then followed a subset of these patients ($n = 70$) who achieved mood stabilization after being optimized on lithium monotherapy, allowing for longitudinal analyses of this cohort.
Results: Baseline cross-sectional analyses comparing 163 patients currently taking lithium with 101 patients not taking lithium did not reveal any significant group differences on neurocognitive functioning – even after controlling for demographics and current mood symptoms. Patients who were taking lithium monotherapy at baseline fared better than those on polypharmacy. Longitudinal analyses revealed significant neurocognitive improvement in global cognition (composite score) ($F(1, 63) = 5.66$; partial Eta square = 0.08; $p = 0.020$) and in verbal memory ($F(1, 65) = 16.91$; partial Eta square = 0. 21; $p < 0.001$). Processing speed also improved over time at a trend level of significance ($F(1, 65) = 3.5$; partial Eta square = 0.05; $p = 0.066$), while executive functions only improved slightly over time ($F(1, 63) = 1.47$; partial Eta square = 0.02; $p = 0.230$). None of the cognitive measures evidenced decline over time with lithium treatment. Positive correlations emerged between daily dose of lithium and performance on verbal memory ($r = 0.302$; $p = 0.018$) and on global cognition ($r = 0.266$; $p = 0.041$), suggesting that within therapeutic range higher doses were associated with better performance.
Conclusions: Although anecdotal evidence and patient self-report often indicate a deleterious effect of lithium on cognitive functioning, our data are consistent with many of the known intracellular/molecular effects of lithium which suggest beneficial effects on brain structure/function. Both cross-sectional and longitudinal data suggest that lithium may be pro-cognitive in patients with BD. At the very least, it does not seem to significantly impair cognition when used therapeutically.
Keywords: Lithium, Cognition, Bipolar Disorder.
Disclosure: Takeda/Lundbeck Alliance: Advisory Board, Self; Sumitomo Dainippon Pharma: Advisory Board, Self; NeuralStem: Advisory Board, Self; Sunovion: Speaker, Self.

M117. Gene Expression Differences Associated With Major Depressive Disorder in the Human Prefrontal Cortex

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Background: Major depressive disorder (MDD) is one of the most common medical disorders in the world. (ref 1) Despite its pervasiveness, its pathophysiology is unknown and current treatment regimens are often ineffective. Research assessments of structural and functional abnormalities in the brains of MDD patients have consistently implicated the dorsolateral prefrontal cortex (DLPFC) (ref 2), however the underlying biological mechanisms driving these abnormalities remain unclear. The targeted examination of gene expression changes in the DLPFC of patients with MDD holds the potential to elucidate the pathophysiology of MDD and to lead to the development of novel therapeutics. In this study we used next-generation sequencing (NGS) to examine gene expression differences in postmortem human DLPFC samples from MDD patients compared to normal controls.
Methods: As part of Phase I of the BrainSeq Consortium (ref 3) we conducted NGS RNA sequencing to quantify gene expression in postmortem human DLPFC samples from 370 subjects (146 MDD; 224 controls; age range: 17-85 years; mean age: 44.014.7 Std. Dev.). Gene expression levels were quantified on an Illumina HiSeq 2000 (Illumina; San Diego, CA USA) following a Poly-A library protocol with 100 base pair paired-end reads and a target depth of 80-100 million reads. Reads were aligned to the hg19 transcriptome using TopHat2 (ref 4). Gene counts were estimated using htseq (ref 5) with a strict intersection model. DESeq2 (ref 6) was used to model differential gene expression with covariates for age of death, sex, sample RNA integrity, post mortem interval, four multidimensional components of ancestry, and 26 components from surrogate variable analysis (SVA). As a secondary analysis, we examined a subset of 47 MDD patients that were unmedicated at time of death using the same DESeq2 modeling framework mentioned previously to identify differentially expressed genes compared to the full set of 224 healthy controls. Genes were considered differentially expressed using a False Discovery Rate threshold of $q < 0.05$ (ref 7). We performed gene-set enrichment analysis (GSEA) using the RNA-Enrich (ref 8) tool, to identify dysregulated pathways associated with MDD.

Results: We found significant evidence for 133 differentially expressed genes (at FDR q< 0.05) in the full set of MDD patients compared to controls (76 up-regulated and 57 down-regulated). The most significant finding was a small non-coding RNA, SCARNA5 (P= 7.97x10-24; log2 fold change (LFC)= -0.423). Among the significant findings the gene with the largest log2 fold change was a long non-coding RNA, RPPH1 (P= 4.93x10-21; LFC= -0.451). Genes previously implicated in depression failed to show significant evidence of differential expression (BDNF P=0.250; COMT P=0.403; FKBP5 P=0.074; HTR2A P=0.872; SLC6A4 P=0.851; CACNA1C P=0.756; TPH2 P=0.796).

In the secondary analysis of unmedicated MDD patients, we found evidence for 16 differentially expressed genes (at FDR q< 0.05) when compared to controls (10 up-regulated and 6 down-regulated). The most significant finding was a small non-coding RNA, SCARNA6 (P= 9.88x10-9; LFC= -0.280). Of the significant findings in the unmedicated subset, the gene with the largest log2 fold change was HILPDA (P= 1.50x10-6; LFC= -0.296). Further, we found five significant differentially expressed genes not found in the full set: MT1A (P= 9.05x10-7; LFC= 0.228), HILPDA, TTR (P= 6.36x10-6; LFC= 0.169), OPN1SW (P= 9.33x10-6; LFC= 0.234), MT1JP (P= 2.76x10-5; LFC= 0.138). Overall, the results in the unmedicated subset were very highly correlated with the data from the full set (Pearson's r= 0.84).

Gene-set enrichment analysis (GSEA) of the differential expression findings from our primary analysis implicated 100 biological pathways (significant at FDR q< 0.05) within the Reactome dataset. The top pathway from the GSEA was up-regulation of SRP-dependent cotranslational protein targeting to membrane (R-HSA-1799339; P= 4.48x10-40). GSEA of our unmedicated subset results implicated 140 significant biological pathways from the Reactome dataset. The top finding was the same SRP-dependent pathway as in the primary analysis (R-HSA-1799339; P= 3.23x10-40).

Conclusions: This study provides insight into the underlying etiology of MDD by applying NGS technologies to postmortem DLPFC samples from depressed patients and comparing them with healthy controls. A notable finding from our primary analysis is that six out of the top ten significantly differentially expressed findings were non-coding RNA genes. The top finding, SCARNA5, encodes a special form of small non-coding RNA associated with Cajal bodies in metabolically active cells, including neurons. The function of SCARNA5 is to guide site-specific post-transcriptional modification of spliceosomal small nuclear RNAs (ref 9). Downstream functional changes associated with up- or down-regulation of SCARNA5 have not been established. Interestingly, when focusing our analysis on a subset of unmedicated subjects we found evidence for 16 differentially expressed genes associated with MDD including 11 found in the main analysis and five novel findings. These 133 significantly differentially expressed genes, 16 from the unmedicated at death subset, and associated pathways represent novel candidates for investigating the pathophysiology of MDD.

Keywords: RNAseq, Major Depressive Disorder (MDD), Dorsolateral Prefrontal Cortex.

Disclosure: Janssen Research and Development: Employer, Self.

M118. Effects of a Single Bilateral Infusion of R-Ketamine in the Rat Brain Regions of a Learned Helplessness Model of Depression

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Background: The N-methyl-D-aspartate receptor (NMDA-R) antagonist ketamine is one of the most attractive antidepressants since this drug can produce rapid-onset and sustained antidepressant effects in treatment-resistant patients with major depression and bipolar disorder. Ketamine (Ki= 500 nM for NMDA-R) is a racemic mixture containing equal parts of S-ketamine (esketamine: Ki= 300 nM) and R-ketamine (Ki= 1,400 nM). Recently, we reported that R-ketamine showed greater potency and longer lasting antidepressant effects than esketamine in the animal models of depression (Zhang et al, 2014; Yang et al, 2015). Unlike esketamine, R-ketamine does not induce psychotomimetic-like behavioral side effects in rodents (Yang et al, 2015). Taken together, it is likely that R-ketamine could be a safer antidepressant without psychotomimetic side effects in humans than esketamine (Hashimoto, 2016). Accumulating evidence suggests that the medial prefrontal cortex (mPFC) plays a role in the antidepressant actions of ketamine. However, it is currently unknown whether the specific brain regions might contribute to the rapid and sustained antidepressant effects of ketamine and its enantiomers. In the present study, we examined the effects of a single bilateral infusion of R-ketamine into several brain regions (prelimbic (PrL) and infralimbic (IL) of the mPFC, hippocampal CA3 and DG, the nucleus accumbens (NAc) shell and NAc core, basolateral amygdala (BLA) and central nucleus of the amygdala (CeA)) on depression-like behavior in a rat learned helplessness (LH) model.

Methods: Male Sprague-Dawley rats (200-230 g, 7 weeks old; Charles-River Japan, Co., Tokyo, Japan) were used. To create an LH paradigm, the animals are initially exposed to uncontrollable stress. When the animal is later placed in a situation where the shock is controllable (escapable), the animal not only fails to acquire the escape response, but also often makes no efforts to escape the shock at all. The LH behavioral tests were performed using the Gemini Avoidance System (San Diego Instruments, San Diego, CA). Rats received bilateral microinjections of R-ketamine (2 μg/side) or vehicle. A total volume of 1.0 μL was infused into each side over 15 min, and the injection syringe was left in place for an additional 5 min to allow for diffusion. The coordinates for the cerebral ventricle, DG and CA3 regions of the hippocampus, core and shell of the NAc, IL and PrL portions of the mPFC, BLA and CeA relative to the bregma were determined according to the rat brain atlas of Paxinos and Watson.

Results: A single bilateral infusion of R-ketamine into IL portion of mPFC, CA3, and DG of the hippocampus from LH rats showed antidepressant effects. In contrast, a single bilateral infusion of R-ketamine into PrL portion of mPFC, shell and core of NAc, BLA and CeA had no effect.

Conclusions: This study shows that a single bilateral infusion of R-ketamine into IL of mPFC, and DG, CA3 of hippocampus resulted in antidepressant effects in LH rats,

suggesting that these regions may play a key role in the antidepressant action of R-ketamine.
Keywords: Antidepressant Agents, Infralimbic Cortex, R-(-)-Ketamine, Animal Model.
Disclosure: Nothing to disclose.

M119. *In Vivo* Quantification of Synaptic Density in Depression With 11C-UCB-J PET Brain Imaging

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Background: Major depressive disorder (MDD) is the leading cause of disability worldwide and is characterized by a variety of symptoms including loss of pleasure, fatigue, changes in arousal, including sleep and appetite, and cognitive dysregulation, among others (NIMH 2016). Evidence from postmortem and preclinical studies of cell pathologies in depression strongly implicate reductions in synaptic density in the prefrontal cortex (PFC) and hippocampus in the pathophysiology of this disorder (Feyissa, Chandran et al. 2009, Kang, Voleti et al. 2012, Zhao, Bao et al. 2012, Duric, Banasr et al. 2013) as being associated with affective pathophysiology. The results of the studies using postmortem human brain tissue, and supported by substantial preclinical evidence, show that chronic stress and depression lead to structural changes, which include cell loss, neuronal atrophy and reduced synaptic density. For example, chronic unpredictable stress (CUS) – a rodent model of depression and stress used to study structural and molecular changes – leads to behavioral abnormalities with core symptoms of depression. Relevant to the current study, the CUS animals show reduction in synaptic density and pre-synaptic proteins in the PFC, and exhibit significant symptoms associated with depression, including anhedonia (Li, Lee et al. 2010). This loss in synapses extends to other models of stress paradigms in rodents, showing reductions in dendritic complexity and synaptic density in the PFC (Liu and Aghajanian 2008, McEwen, Eiland et al. 2012, Morrison and Baxter 2012). Exciting advances in positron emission tomography (PET) have recently made it possible to measure synaptic density in vivo via use of the 11C-UCB-J radioligand, which binds with high affinity to the synaptic vesicle glycoprotein 2A (SV2A), a protein found ubiquitously in presynaptic vesicles throughout the brain. Evidence from our group in non-human primate has revealed that SV2A correlates well with other markers of synaptic density employed in postmortem and preclinical work and is thus an excellent marker of synaptic density (Finnema, Nabulsi et al. 2016). In the current study, we aimed to examine PFC synaptic density in individuals with depression as compared to controls with 11C-UCB-J PET, and assess whether decreases in PFC synaptic density are associated with increased depression and cognitive deficits. Furthermore, we conducted in vitro binding studies to measure changes in SV2A in an animal model of depression (CUS).

Methods: The study was approved by the Yale University Institutional Review Board, Radiation Safety Committee, and Institutional Animal Care and Use Committee. Individuals with depression and healthy controls were recruited to participate in one PET scan with 11C-UCB-J to measure target density, and one magnetic resonance imaging (MRI) scan to guide placement of regions of interest for PET as well as to correct for gray matter changes. Ten individuals with MDD (mean age = 39) and 7 healthy controls (mean age = 35) participated in scans, as well as in mood and cognitive assessments. The arterial input function was measured for quantification of volume of distribution (VT), radiotracer was injected as a bolus, and subjects were scanned for 90 minutes. One-tissue compartment modeling was used to quantify VT. For the rodent experiment, the CUS rodents were exposed to stress for 21 days. After that, animals were sacrificed and SV2A levels were measured by western blot analysis (Ota, Liu et al. 2014).
Results: We observed lower synaptic density (i.e., 11C-UCB-J binding) in individuals with depression as compared to control individuals in the dorsolateral PFC (dlPFC, 17% lower) and ventromedial PFC (vmPFC, 15% lower). Interestingly, lower synaptic density in the dlPFC was associated with greater depressive symptomatology ($p = 0.03$) and poorer performance on delayed verbal recall ($p = 0.05$). Furthermore, in rodents, CUS exposure reduced levels of SV2A immunoreactivity in the PFC compared to control rats by 12%, which was similar to reductions in other synaptic proteins commonly used to quantify synaptic density (i.e., synapsin 1 and GluA1).
Conclusions: Given the result in rodents and our previous in vitro and in vivo work with this marker, we conclude that the effect of stress on SV2A is similar to other synaptic proteins and SV2A is thus an excellent target to study illness-related changes in synaptic density in vivo. The preliminary in vivo results in individuals with MDD support the hypothesis that PFC synaptic density is lower in MDD and that this alteration is associated with some of the mood and cognitive deficits observed in the disorder. Normalizing synaptic density through targeted treatments may be a potential mechanism to provide relief for some of the affective and executive dysregulation observed in individuals with MDD.
Keywords: PET, MDD, Synaptic Density, CUS.
Disclosure: Nothing to disclose.

M120. cAMP Signaling in Brain is Decreased in Unmedicated Depressed Patients and Increased by Treatment With a Selective Serotonin Reuptake Inhibitor

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Background: The key role of the cyclic adenosine monophosphate (cAMP) cascade in depression is thought to involve both pathological changes in unmedicated patients as well as a common pathway for various antidepressants to produce their effects. Human postmortem studies in individuals with

depressive disorders have indicated low cAMP signaling. Correspondingly, multiple rodent studies and one human postmortem study showed that various forms of chronic, but not acute, administration of antidepressants upregulate cAMP signaling. Based on these findings, the cAMP theory of depression posits low cAMP signaling in unmedicated patients and, commensurately, upregulation of cAMP signaling as a mechanism of antidepressant treatment. Here, we sought to examine these two theories in patients with major depressive disorder (MDD) using the positron emission tomographic (PET) radioligand 11C-(R)-rolipram, a reversible inhibitor of phosphodiesterase-4 (PDE4). Because of a feedback mechanism, rolipram binding to PDE4 provides a measure of the activity of this enzyme. Essentially, increased cAMP stimulates protein kinase A (PKA), which phosphorylates PDE4. This, in turn, increases both enzymatic activity of PDE4 and rolipram binding affinity.

Methods: 11C-(R)-rolipram PET scans were performed in 44 unmedicated patients during a major depressive episode and 35 healthy controls. Twenty-three of the 44 patients had a follow-up 11C-(R)-rolipram PET scan approximately eight weeks after treatment with an SSRI. Patients were moderately depressed (Montgomery-Åsberg Depression Rating Scale = 30 ± 6) and about half were treatment-naïve. 11C-(R)-Rolipram binding was measured using arterial sampling to correct for individual differences in radioligand metabolism.

Results: We found in unmedicated MDD patients widespread, ~20% reductions in 11C-(R)-rolipram binding compared to controls ($P = 0.001$). SSRI treatment significantly increased rolipram binding (12%, $P < 0.001$) with significantly greater increases observed in older patients ($P < 0.001$).

Of the 23 patients receiving SSRI treatment, 10 had a greater than 50% decrease in MADRS score, and three patients remitted (MADRS < 10). Overall, SSRI treatment decreased MADRS score from 30 ± 6 to 18 ± 11, HDRS-17 from 20 ± 5 to 14 ± 8, and HAM-A from 19 ± 5 to 12 ± 8 ($P < 0.001$ for all three scales, t = 4.7–6.0). Although SSRI treatment significantly increased rolipram binding, there was no correlation between increased rolipram binding and improvement of depressive or anxiety symptoms as assessed by the MADRS, HDRS-17, or HAM-A. Rolipram binding did not correlate with severity of baseline symptoms, and increased rolipram binding during treatment did not correlate with symptom improvement. In contrast to the increased rolipram binding observed after SSRI treatment in the 23 MDD patients, 13 healthy controls who had two rolipram PET scans at similar time intervals but without SSRI treatment showed no change in rolipram binding ($-1 \pm 13\%$, $P = 0.25$, $F = 1.48$, $df = 1,11$).

Conclusions: Taken together with previous findings, the present results support the cAMP theory of depression. We found that the cAMP cascade was downregulated in unmedicated MDD patients currently experiencing a major depressive episode, and upregulated by two months of SSRI treatment. While the lack of correlation between increased rolipram binding and improvement in symptoms raises significant questions about the role of cAMP signaling in MDD, this finding could be due to the heterogeneity of the disorder, the existence of distinct PDE4 subtypes, or interactions between age, gender, and the cAMP cascade. The initial clinical trials of rolipram as an antidepressant found suggestive evidence of anti-depressant efficacy but were discontinued because of nausea and vomiting. In our opinion, the overall strength of prior and current PET studies supports investigating the role of PDE4 subtypes in the pathophysiology of MDD as well as the possibility that subtype selective inhibitors can be an effective treatment for depression without the nausea and vomiting associated with the non-selective inhibitor rolipram. Our results further suggest that age and gender should be taken into account to properly evaluate the effect of new PDE4 inhibitors.

Keywords: Major Depressive Disorder (MDD), PET, Phosphodiesterase-4 (PDE4).

Disclosure: Nothing to disclose.

M121. Withdrawn

M122. Association of Peripartum Synthetic Oxytocin Administration and Depressive and Anxiety Disorders Within the First Postpartum Year

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Background: Due to its potent effects on social behavior, including maternal behavior, oxytocin has been identified as a potential mediator of postpartum depression and anxiety. Synthetic oxytocin is one of the most commonly used medications in the United States, often used for labor induction or augmentation and prevention or treatment of postpartum hemorrhage. Although 22% of the 4 million births recorded in the U.S. in 2014 were induced, little research has examined the potential effects of synthetic oxytocin on postpartum maternal mood or anxiety. The objective of this study was to examine the relationship between peripartum synthetic oxytocin administration and the development of depressive and anxiety disorders within the first year postpartum. We hypothesized that women exposed to peripartum synthetic oxytocin would have a reduced risk of postpartum depressive and anxiety disorders compared to those without any exposure.

Methods: Population-based clinical data available through the Massachusetts Integrated Clinical Academic Research Database (MiCARD) was used to retrospectively (2005-2014) examine this relationship and calculate the relative risk and 95% confidence interval of peripartum synthetic oxytocin for the development of postpartum depressive and anxiety disorders in exposed ($n = 9{,}684$) compared to unexposed ($n = 37{,}048$) deliveries.

Participants were identified at the delivery level, where each delivery was considered a separate unit in our analysis. Deliveries were selected based on the following inclusion criteria: live born singleton deliveries, coded with eligible delivery codes, to females between the ages of 15-50. Multiple gestation deliveries (i.e., twins, triplets, or higher order multiples) were excluded from the study. Deliveries were identified using Current Procedural Terminology (CPT) and International Classification of Diseases 9th Revision (ICD-9)

diagnosis codes. We considered the first delivery to each woman in our dataset as her "index delivery." Depression and anxiety data included diagnosis information and/or documentation of clinically relevant antidepressant or anxiolytic medication prescription. We replicated our analysis on our total sample in two subsets: first, by selecting a random delivery from each woman in our sample; and next, by repeating our analysis on all index deliveries regardless of prepregnancy depressive or anxiety disorder history.

Results: In women with a history of prepregnancy depressive or anxiety disorder, the relative risk of postpartum depressive or anxiety disorder was 36% higher in women who received peripartum oxytocin compared to those who did not (RR: 1.36; 95% CI: 1.20-1.55). In women with no history of prepregnancy depressive or anxiety disorder, the relative risk of postpartum depressive or anxiety disorder was 32% higher in women with oxytocin exposure compared to those not exposed (RR: 1.32; 95% CI: 1.23-1.42).

In index deliveries to women with a history of prepregnancy depressive or anxiety disorder the relative risk of postpartum depressive or anxiety disorder was higher in women who received peripartum synthetic oxytocin compared to those who did not for both vaginal (RR: 1.45; 95% CI: 1.22-1.72) and cesarean (RR: 1.38; 95% CI: 1.00-1.91) deliveries. In deliveries to women with no history of prepregnancy depressive or anxiety disorder, the relative risk of postpartum depressive or anxiety disorder was 19% higher in vaginal deliveries and 25% higher in cesarean deliveries to women with synthetic oxytocin exposure compared to those not exposed (RR: 1.19; 95% CI: 1.07-1.32 and RR: 1.25; 95% CI: 1.05-1.49), respectively.

When selecting a random delivery from each woman, regardless of prepregnancy depressive or anxiety disorder history, the relative risk of postpartum depressive or anxiety disorder was 35% higher in women who received peripartum synthetic oxytocin compared to those who did not (RR: 1.35; 95% CI: 1.26-1.44). Relative risks remained similar when examining all index deliveries, with a 39% increased risk of postpartum depressive or anxiety disorder in deliveries to women with exposure to peripartum synthetic oxytocin compared to deliveries to women with no exposure to peripartum synthetic oxytocin (RR: 1.39; 95% CI: 1.30-1.49).

Conclusions: Contrary to our hypothesis, results indicate that women with peripartum exposure to synthetic oxytocin had a higher relative risk of receiving a documented depressive or anxiety disorder diagnosis or antidepressant/anxiolytic prescription within the first year postpartum than women without synthetic oxytocin exposure. This increased risk was still present when the analyses were restricted to index deliveries or a single random delivery in women who had multiple deliveries, and the risks were higher regardless of delivery mode.

The potent behavioral effects of oxytocin in basic and clinical studies underscore the urgent need for increased study of the potential effects of peripartum manipulation of this hormone and its receptors. There are specific needs for studies on: the longitudinal role of endogenous oxytocin in maternal mood and anxiety, detailed prospective studies of the effects of peripartum treatments on maternal mood, with an emphasis on populations at high risk for maternal mood and anxiety disorders, and clinically relevant animal studies which investigate neural mechanisms of the behavioral effects of exogenous peripartum oxytocin.

Keywords: Oxytocin, Pregnancy, Postpartum Depression, Anxiety Disorders, Peripartum.

Disclosure: Nothing to disclose.

M123. Increased Plasma Levels of Circulating Cell-Free Mitochondrial Dna in Medication-Free Suicide Attempters – Associations With HPA-Axis Hyperactivity

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Background: Preclinical data suggest that chronic stress and/or an activated HPA axis may cause cellular damage and mitochondrial dysfunction. A novel marker of these outcomes may be cell-free plasma mitochondrial DNA (mtDNA). Although leukocyte and salivary mtDNA have been found to be increased in Major Depressive Disorder, no previous studies have measured plasma levels of free-circulating mtDNA in a clinical psychiatric sample. Cell-free mtDNA, as opposed to intracellular mtDNA, may be a more robust marker of the increased cellular stress known to be associated with many psychiatric conditions. During states of stress, be it of metabolic or psychological origin, cells are damaged and may undergo apoptosis potentially resulting in a subsequent leakage of mtDNA from the damaged cells into the bloodstream. The present study sought to determine if free-circulating mtDNA in cell-free plasma is increased in suicide attempters, a group often characterized by depressive symptoms, increased psychological stress and adverse childhood events. We hypothesized that free circulating mtDNA would be elevated in the suicide attempters and would be positively associated with hypothalamic pituitary adrenal-axis hyperactivity.

Methods: Free circulating mtDNA was quantified in plasma samples from 37 suicide attempters (MDD, $n=14$; Dysthymic disorder, $n=5$; Adjustment Disorder, $n=6$; Depression NOS, $n=5$, Anorexia Nervosa, $n=1$; no axis 1 disorder, $n=6$) and 37 healthy controls. Twenty of the suicide attempters had an axis II personality disorder, cluster B being the most frequent specifier ($n=9$). All suicide attempters had undergone a dexamethasone suppression test (DST).

Results: Suicide attempters had highly significantly increased plasma levels of free-circulating mtDNA compared to healthy controls at different time points (pre- and post-DST) (all p-values $<2.98E-12$, Cohen's d ranging from 2.55-4.01). Post-dexamethasone cortisol levels were positively correlated with basal plasma levels of mtDNA (rho $=0.49$, $p<0.003$).

Conclusions: Suicide attempters may have elevated plasma levels of free-circulating mtDNA, which are related to impaired HPA axis negative feedback. This peripheral index is consistent with increased cellular or mitochondrial damage. The specific cells and tissues contributing to plasma levels of free-circulating mtDNA are not known, as is the specificity of this finding for suicide attempters. Future studies are needed in order to better understand the relevance of increased free-circulating mtDNA in relation

to the pathophysiology underlying suicidal behavior and depression.
Keywords: Mitochondrial DNA, Suicide, HPA Axis, Depression.
Disclosure: Nothing to disclose.

M124. Altered Sensitivity to the Rewarding Properties of Cocaine in Adult Female c57bl/6 Mice Exposed to Fluoxetine During Adolescence

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Background: Accumulating preclinical evidence indicates that early-life exposure to psychotropic medications results in long-lasting altered behavioral responses to drugs of abuse – suggesting a risk of enhanced drug liability, later in life. However, to date, these preclinical experimental approaches have been conducted primarily using male subjects. This is surprising given that females, when compared to males, are more likely to be diagnosed with mood-related disorders, and thus, to be prescribed with psychotropic medications, such as antidepressants. Therefore, to examine whether long-lasting alterations to the rewarding properties of drugs of abuse are exhibited as a result of juvenile antidepressant exposure, we exposed adolescent female mice to the selective serotonin reuptake inhibitor (SSRI) fluoxetine (FLX). We selected FLX given that it is the only SSRI approved by the US Food and Drug Administration for the treatment of pediatric depression.
Methods: Female c57bl/6 mice were exposed to FLX in their drinking water (250 mg/L) during adolescence (postnatal days [PD] 35-49), and were later assessed in adulthood (PD 70+) on behavioral responsivity to cocaine (0, 2.5, 5, and 7.5 mg/kg) place conditioning (CPP).
Results: Adult female mice pretreated with FLX during adolescence displayed a decreased preference for environments previously paired with cocaine (2.5, 5.0, and 7.5 mg/kg), when compared to saline-pretreated controls.
Conclusions: Collectively, our data suggest that adolescent exposure to the antidepressant FLX mediates behavioral adaptations that endure into adulthood, and that are indicative of a decreased sensitivity to the rewarding properties of cocaine in female mice.
Keywords: Cocaine, Antidepressants, Female, Fluoxetine.
Disclosure: Nothing to disclose.

M125. An Investigation of Prefrontal GABA and Glutamate Disturbances in Co-Occurring Bipolar Disorder and Alcohol Dependence: 1H-MRS Results From a 2x2 Factorial Design

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Background: Bipolar disorder (BD) and substance use disorders (SUD) co-occur very frequently, and co-occurring BD and SUD are associated with devastating public health costs. There has been very little neurobiological research conducted to guide the development of effective treatments for this treatment-resistant population. The present study was designed to provide an important first Proton Magnetic Resonance Spectroscopy (1H-MRS) study of prefrontal GABA and GLU levels in individuals with co-occurring BD and current Alcohol Dependence (AD), BD alone, current AD alone, and healthy control (HC) subjects.
Methods: Ninety individuals who met DSM-IV criteria for BD I/II and current AD (BD+AD, $n=24$), BD I/II alone (BD, $n=21$), current AD alone (AD, $n=24$), or no diagnosis (HC, $n=21$) were recruited from inpatient/outpatient clinical settings and community advertisements. All participants were required to demonstrate ≥ 1 week of abstinence from alcohol and drugs via serial ethyl glucuronide (EtG) and urine drug screen (UDS) testing. General exclusions included serious medical illness, history of head injury, psychotic disorder, recurrent MDD, past month PTSD, OCD, or eating disorder, current use of benzodiazepines or antidipsotropics, history of delirium tremens or >1 alcohol withdrawal seizure, acute alcohol withdrawal, and daily drug use in the past month. Lifetime drug use disorder was exclusionary for BD and HC, but not BD+AD and AD, participants. BD+AD or BD participants with substantial medication dose changes ≤ 1 week before MRI were excluded. Participants completed a baseline diagnostic visit including Structured Clinical Interview for DSM-IV Axis I Disorders, Montgomery-Asberg Depression Rating Scale, Young Mania Rating Scale, Timeline Followback, EtG, UDS, Barratt Impulsiveness Scale (BIS), and Obsessive Compulsive Drinking Scale (OCDS). Participants then returned approximately 4 days later for an MRI including a Two-dimensional J-resolved Point Resolved Spectroscopy (2D J-PRESS) 1H-MRS acquisition in a 2.5 x 2.5 x 3 cm dorsal Anterior Cingulate Cortex (dACC) voxel. A structural scan was acquired for voxel placement and tissue segmentation, and water unsuppressed 1H-MRS data were acquired for scaling. 2D J-PRESS data were analyzed using the ProFit algorithm and estimated metabolite/water ratios were corrected for within-voxel CSF fraction.
Results: Of the 78 participants who completed the study (BD +AD $n=20$, BD $n=19$, AD $n=20$, HC $n=19$), 40% were female, 30% were smokers, and 40% reported consuming alcohol within 2 weeks of the MRI; mean (SD) age = 38.5 (11.8). Groups did not significantly differ on age ($p=0.27$) or sex ($p=0.91$). There were marginal group differences in smoking ($p=0.08$), but smoking was not associated with dACC GLU ($p=0.70$) or GABA ($p=0.19$) levels. BD+AD and BD groups marginally differed in anticonvulsant use ($p=0.06$), but anticonvulsant use was not significantly associated with GLU or GABA levels (ps >0.50). BD+AD and BD groups did not differ in terms of manic ($p=0.58$) or depressive symptoms ($p=0.82$) or co-occurring anxiety disorders ($p=0.75$). AD+BD and AD participants did not differ in terms of co-occurring drug dependence ($p=0.84$). 2x2 factorial ANCOVA analysis of dACC GABA/water concentrations demonstrated a significant BDxAD interaction ($F=2.91$, $p<0.05$), signifying uniquely low levels of dACC GABA/water in BD+AD relative to BD, AD, and HC (Cohen's D for BD+AD vs. HC = 0.78). This effect nearly doubled when the sample was restricted to individuals who reported consuming alcohol within 2 weeks of MRI (Cohen's

D = 1.53). In the overall sample, there were no significant effects of BD and/or AD on dACC GLU/water levels (BDxAD interaction F = 1.46, $p = 0.231$, Cohen's D for BD +AD vs. HC = 0.33). However, when the sample was restricted to individuals who reported consuming alcohol within 2 weeks of MRI, the BDxAD interaction approached significance (F = 3.83, $p = 0.06$, Cohen's D = 1.01). dACC GABA/water levels were significantly associated with BIS (r = -0.28, $p = 0.02$) and OCDS (r = -0.35, $p < 0.01$) scores across groups. Although GLU/water concentrations were not significantly associated with BIS (r = -0.03, $p = 0.83$) or OCDS (r = -0.18, $p = 0.11$) scores in the full sample, GLU/water levels were significantly associated with OCDS (r = -0.35, $p = 0.05$) scores in individuals who reported consuming alcohol within 2 weeks of MRI.

Conclusions: These data demonstrate that BD+AD have abnormally low levels of dACC GABA and GLU, and that low levels of GABA and GLU are associated with impulsivity and craving. Given replication, future studies can use this information to preferentially evaluate therapeutics in BD +AD known to increase prefrontal GABA and GLU.

Keywords: Bipolar Disorder, Alcohol Dependence, Glutamate GABA, Proton Magnetic Resonance Spectroscopy, Psychiatric Comorbidity.

Disclosure: The present study was funded by NIAAA K23 AA020842 (PI: Prisciandaro). Dr. Anton is supported by NIAAA K05 AA017435.

M126. Vilazodone Inhibits Pro-Inflammatory Gene Expression and Immune Activation Compared to Paroxetine in Late-Life Depression

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Background: A clinical pilot study was conducted to determine whether there is a difference in effect size (ES) and tolerability between Vilazodone (novel compound) and Paroxetine (gold standard) in older depressed patients. Secondly, we examined the pharmacogenomic effects of these compounds on inflammation and immune modulation by assessing changes in gene expression.

Methods: A 12-week, double-blind, randomized clinical trial (RCT) of Vilazodone versus Paroxetine was conducted with 56 non-demented older adults (age 60y and older) diagnosed with Major Depressive Disorder (MDD). Between-group differences in mood, tolerability and safety, as well as genomic markers of inflammation and immune modulation were examined.

Results: ES estimates indicate that the Vilazodone group showed greater improvement in mood (Hamilton Depression Rating Scale (HDRS) scores) compared to Paroxetine (2.25 vs 1.31). Leukocyte gene expression profiles also showed reduced indications of inflammatory biology, including down-regulation of specific pro-inflammatory gene transcripts and bioinformatic indications of reduced Nuclear factor (NF)-κB, activator protein (AP)-1, and cAMP response element binding (CREB) activity in the Vilazodone group versus the Paroxetine group (all $p < 0.05$). Transcript Origin Analyses implicated monocytes and dendritic cells as the primary cellular origins of transcripts down-regulated in the Vilazodone-treated group (both $p < 0.01$).

Conclusions: The Vilazodone group had greater improvement in depression which may be associated with inhibition of pro-inflammatory gene expression and immune modulation. Larger clinical trials are needed to better understand the clinical effects of Vilazodone, and replication of the immunological gene expression findings is also required.

Keywords: Vilazodone, Immune Modulation, Gene Expression, Geriatric Depression.

Disclosure: Forest Research Institute: Research Grant, Self.

M127. Brain Entropy: Intelligence, Personality, and Psychopathology

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Background: Entropy has a fundamental relationship with information and the functioning of all computational systems. Entropy is defined as the number of states available to a system. A system with low entropy has access to fewer states than does one with high entropy. A system with low entropy is more ordered and more predicable than a system with high entropy. Since entropy is related to the functioning of computational systems, there is an emerging theoretical and empirical literature about its role in brain function and dysfunction. We present the results of three integrated studies applying resting state fMRI entropy measurement to understand intelligence, personality, and psychopathology. Brain entropy is an index of an individual's access to brain states at a given time and is measured through the predictivity of brain state over time. Thus, we would expect to observe brain entropic differences between conditions known to be associated with high flexibility (e.g. high intelligence, creativity, novelty seeking) vs. conditions associated with high rigidity (e.g. anxiety, depression, Posttraumatic Stress). The three studies are: Brain entropy and intelligence in 926 adults from the Brain Genomic Superstruct Project, 2. Brain entropy and personality in 926 adults from the Brain Genomic Superstruct Project, and 3. Brain entropy and PTSD in 95 veterans from the NYU Cohen Veterans Data Set.

Methods: Subjects: Study 1 (Entropy and Intelligence) and Study 2 (Entropy and Personality) were conducted with data from the Brain Genomics Superstruct Project (BGSP). The BGSP includes 1570 healthy adult participants between the ages of 18 and 35. The current study utilized data from the 926 participants who completed intelligence and personality assessments. Study 3 (Entropy and PTSD) was conducted with data from the NYU Cohen Veterans Data Set. This data set includes 95 combat veterans, 46 with PTSD and 49 without PTSD.

fMRI Procedures: Brain Genomics Superstruct Project (BGSP). All MRI data were obtained with 3T Trio scanners (Siemens Healthcare, Erlangen, Germany) at Harvard University and Massachusetts General Hospital. MRI scans

for each participant included a high resolution structural scan (T1-weighted multi-echo MPRAGE, TR = 2.2 sec, TE = 1.5/3.4/5.2/7.0 msec, slices = 144, resolution = 1.2 x 1.2 x 1.2 mm) and a resting-state functional scan sensitive to blood oxygenation level-dependent (BOLD) contrast (TR = 3.0 sec, TE = 30 msec, slices = 47, resolution = 3.0 x 3.0 x 3.0 mm, 120 measurements).
NYU Cohen Veterans Data Set: All MRI data were obtained with a 3T Trio scanner (Siemens AG, Erlangen Germany). Anatomical images were acquired with magnetization prepared rapid gradient echo sequence with TE/TI/TR = 2.98/900/2300 ms, 256 x 240 matrix, 256 mm x 240 mm field-of-view, flip angle = 9°, slice thickness = 1 mm and total slice number = 191; resting state fMRI was obtained using an echo-planar imaging sequence (TR/TE = 2000/29 ms, flip angle = 90°), 64 x 64 matrix, pixel size 3.125 mm x 3.125 mm, total slice number = 32, slice thickness = 3.5 mm (without gaps), total volume number = 200. fMRI Entropy Analysis: Brain entropy was calculated using the Brain Entropy Mapping Toolbox (BENtbx) (Wang et al, 2014) for MATLAB (MATLAB Release R2015b, The MathWorks Inc., Natick, MA, United States). The BENtbx utilizes Sample Entropy (SampEn). For a given time series, SampEn is a single number representing the predictability of the series. The entropy of highly predictable series is small, close to 0, indicating a lack of variation or disorder. The entropy of unpredictable series is large, indicating a high amount of variation or disorder. The Sample Entropy process first breaks a series into smaller sets of size m. For example, for m = 2, and the BOLD time series is broken into pairs of consecutive values. Each pair is then compared with every other pair to find the maximum distance (absolute value difference) between any number in the first pair and any number in the second pair. If the distance is less than the threshold r, the two pairs are considered a 'match.' This process is then repeated for sets of size m + 1. Sample Entropy is then the ratio: SampEn = -log A/B: Where, A = number of matches using sets of size m+1 and B = number of matches using sets of size m. For perfectly predictable series, A and B will be equal, and entropy will be 0. As disorder in a series increases, B will become greater than A, and the equation will yield an increasingly large positive number.
Psychometric Measurement:
Study 1: Intelligence was measured with the Shipley Estimated IQ, Vocabulary, and Matrix Reasoning scales.
Study 2: Personality was measured for Behavioral Inhibition, Harm Avoidance, Risk Taking, and Novelty Seeking.
Study 3: PTSD was measured with the Clinician Administered PTSD Scale (CAPS).
Results: Study 1: Shipley Estimated IQ, Vocabulary, and Matrix Reasoning were all associated with higher brain entropy. In particular, Vocabulary was related to higher entropy in the L fusiform gyrus, inferior temporal gyrus, parahippocampal gyrus. Matrix Reasoning was associated with higher entropy in the bilateral superior, medial, inferior frontal gyrus, bilateral orbital gyrus, and R middle frontal gyrus.
Study 2: Harm avoidance and Behavioral Inhibition were associated with lower entropy and Novelty Seeking and Risk Taking were associated with higher entropy.
Study 3: PTSD was associated with lower entropy, particularly in the L hippocampus and parahippocampal gyrus, inferior and middle temporal lobes: and higher entropy in the R precuneus, and R parietal lobe.
Conclusions: Brain entropy may provide a novel approach to understand intelligence, personality, and psychopathology such as PTSD.
Keywords: Functional MRI (fMRI), Entropy, Intelligence Quotient, PTSD.
Disclosure: Nothing to disclose.

M128. Relationships Between Personality Characteristics and mGluR5 Availability Measured Using Positron Emission Tomography With [11C] ABP688 in Healthy Volunteers

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Background: Metabotropic glutamate receptor subtype 5 (mGluR5) is a promising potential therapeutic target in the treatment of psychiatric disorders. Recent studies with positron emission tomography (PET) have identified altered mGluR5 availability in clinical populations including obsessive-compulsive disorder and substance use disorders. mGluR5 modulates neurotransmission and interacts closely with dopamine signaling in the reward system. Accordingly, this receptor has also been implicated in animal models of motivation, anxiety, and novelty-seeking. A recent study found that limbic mGluR5 availability is positively associated with novelty-seeking temperament in humans. This and other personality traits are themselves associated with vulnerability to mental illness, including a well-established link between impulsivity and dopamine-related disorders such as addiction. Differences in mGluR5 expression or function associated with personality traits may therefore contribute to the risk of developing psychiatric disorders. The objective of this study was to explore the relationship between mGluR5 availability and personality traits including impulsivity, reward dependence, and novelty-seeking.
Methods: Healthy volunteers with no personal or family history of psychiatric or substance use disorders were included in this study. At enrolment, participants completed the Barratt Impulsivity Scale (BIS-11) and the Tridimensional Personality Questionnaire (TPQ), which includes Novelty-Seeking, Harm Avoidance, and Reward Dependence components. On a separate visit, participants underwent a 60-minute positron emission tomography (PET) scan using 370MBq [11C]ABP688, a radioligand that binds at an allosteric site on mGluR5. A 45-minute Magnetic Resonance Imaging (MRI) scan was performed for co-registration with PET images. [11C]ABP688 BP(ND) values were calculated using a simplified reference tissue model (SRTM) with cerebellar grey matter as reference region. BP(ND) values were extracted from cortical, limbic, and striatal subregions defined using automated algorithms and masks drawn on standard templates. Correlations between personality traits and regional BP(ND) values were assessed using Pearson's r.

Results: Fifteen participants have completed the study to date (11 females and 4 males, mean age 244.2 years). BIS-11 impulsivity scores were positively correlated with [11C] ABP688 BP(ND) in the grey matter of the occipital lobe ($r = 0.60$, $p = 0.017$), with a marginal relationship in the temporal lobe ($r = 0.45$, $p = 0.095$). No significant associations were observed between BP(ND) and impulsivity in any other brain region, nor with Novelty-Seeking, Harm Avoidance, or Reward Dependence personality traits.
Conclusions: Increased cortical mGluR5 availability, particularly in occipital and temporal regions, was associated with greater impulsivity in this sample of healthy volunteers. This relationship between mGluR5 and impulsivity is consistent with preclinical and clinical evidence of an important role for mGluR5 in modulating dopamine neurotransmission. These preliminary findings extend our understanding of the neurobiology of personality traits in humans.
Keywords: Glutamate, PET Imaging, Impulsivity, Personality, mGluR5 Receptors.
Disclosure: Nothing to disclose.

M129. Generalizability of Clinical Trials Results for Adolescent Major Depressive Disorder

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Background: Despite evidence of low generalizability of clinical trial results for major depressive disorder (MDD) in adults, the generalizability of clinical trials of adolescents with MDD is unknown.
Methods: Data were derived from National Comorbidity Survey–Adolescent Supplement (NCS-A), a large, nationally representative sample of 6,483 adolescents (aged 13-18 years) from the United States population. We applied a standard set of eligibility criteria representative of clinical trials to all adolescents with a DSM-IV diagnosis of MDD ($n = 592$). Our aim was to evaluate the proportion of participants with MDD who would have been excluded by typical eligibility criteria.
Results: More than 8 of 10 respondents with MDD (80.7%) would have been excluded by at least 1 exclusion criterion. In the subsample of participants who sought treatment ($n = 412$), the exclusion rate increased to 88.2%. For both the full sample and the treatment-seeking subsample, the criteria excluding the highest proportion of adolescents with MDD were "significant medical condition", "significant risk of suicide" and "lifetime conduct disorder".
Conclusions: Clinical trials likely exclude a great majority of adolescents with MDD. Broadening study eligibility criteria of treatment trials for adolescent MDD would increase the applicability of their results to community practice.
Keywords: Clinical Trials, Generalizability, Major Depressive Disorder.
Disclosure: Nothing to disclose.

M130. Pain-Induced Alterations in Aversive and Motivational States are Mediated by Upregulation of the Accumbal Kappa Opioid Receptor System

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Background: The mesolimbic pathway, a key region involved in the control of reinforcing and motivational properties of reward, undergoes long term changes in the presence of inflammatory pain. The opioid system plays a prominent role in controlling the activity of dopaminergic neurons within this pathway. Inflammatory pain dysregulates the mu opioid system in the ventral tegmental area (VTA), thus decreasing heroin-induced dopamine release and altering both sucrose and heroin reinforcement and motivation. The kappa opioid receptor (KOR) is expressed on dopaminergic terminals in the nucleus accumbens (NAc) where it locally controls dopamine release. The KOR system can also be altered by pain and is involved in pain-induced alterations in motivation. Indeed, KOR stimulation in the so-called NAc Shell "cold spot" decreases the "liking" of sucrose tasting. Furthermore, activation of the KOR system by dynorphin, its endogenous agonist, mediates aversive behaviors, and pain-induced changes in this system could lead to modifications in these negative effects. In this work, we focused on pain-induced neurobiological modifications in the KOR system in the NAc shell, and the consequences of these alterations on both reinforcing and aversive behaviors in the presence of pain.
Methods: We used an inflammatory pain model (CFA in the hind paw of rats) to assess pain-induced changes in KOR expression and function with GTPgammaS binding and western blot analysis. We also monitored the excitability of dynorphin-containing neurons using ex vivo patch clamp recording. In addition, we conducted in vivo experiments to further dissect the involvement of KOR system in conditions of pain. Microinjections of U50,488 or NorBNI (agonist and antagonist of KOR, respectively) in the NAc were used to assess the role of KOR in pain-induced decrease in motivation for sucrose (using a progressive ratio schedule). We determined whether endogenous dynorphin was necessary for decreased motivation for sucrose by using an chemogenetic silencing approach. An HSV-dynorphin-Gi-DREADD construct was used to silence dynorphinergic neurons in the NAc. We then determined whether CFA treatment was sufficient to potentiate dynorphinergic neuron-mediated place aversion. We expressed a cre-dependent channelrhodopsin (ChR2) virus in dynorphin-IRES-cre mice to induce dynorphin release in the real-time place aversion assay. All experimental protocols in animal studies were approved by the Institutional Animal Care and Use Committee at Columbia University and Washington University in St. Louis.
Results: Our western blot and GTPgammaS results show that 48 hours after the induction of pain, both the expression and function of KORs in the NAc are enhanced. In addition to the increase in receptor function, whole-cell recordings

from dynorphin-cre mice confirmed that pain also potentiates the excitability of dynorphin-containing neurons in the NAc. In order to examine whether this upregulation of the dynorphin-KOR system underlies the pain-induced changes in motivation, we microinjected the long-acting KOR antagonist NorBNI into the NAc. We were able to reverse the previously observed pain-induced decrease in motivation by blocking KOR, highlighting the critical role of this receptor in this pain effect in motivation. In a complementary study, we found that activation of KOR in the NAc by U50,488 is sufficient to decrease motivation for sucrose in a way that mimics an inflammatory pain state. We followed up on these findings by silencing dynorphinergic neurons, using a HSV-dynorphin-Gi-DREADD, and this experiment revealed the necessity of endogenous dynorphin release to mediate the deleterious effects of pain on motivation. Finally, we found that photo-stimulation of dynorphinergic neurons in the ventral NAc shell induces a real time place aversion that is potentiated in the presence of pain (CFA exposure). The activation of these neurons in both control and painful conditions did not alter locomotor activity, which suggests that our effects are specific to motivation, and not a generalized inhibition of locomotion.

Conclusions: Pain induces changes in both the mu and kappa opioid systems, leading to altered monoamine transmission (Dopamine and Serotonin) in the NAc. These adaptations ultimately affect the motivation as well as reward seeking. The study of the mu-opioid system brought us a better understanding of these pain-induced adaptations. However, little work has been dedicated to the dynorphin KOR system in conditions of pain. Here we show that accumbal dynorphin-KOR system is recruited in the presence of pain, which complements our previously-described mu opioid adaptations in the VTA. Furthermore, pain decreases motivation for rewards through this system and increases the aversive potency of dynorphin release in the ventral NAc shell. Characterization of pain-induced changes in neural circuits that regulate motivation and reward is critical in the development of strategies to overcome alterations in mood and reinforcement, particularly in pain. Our present findings highlight the dynorphin-KOR system as a new target for therapeutical improvement.

Keywords: Kappa Opioid Receptor, Inflammatory Pain, Motivation, Reward and Aversion.

Disclosure: Nothing to disclose.

M131. Subjective Effects of Psilocybin Predict Next-Day Differences in Default Mode Network and Medial Temporal Lobe Functional Connectivity

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Background: Psychedelics, or classic serotonin-2a receptor agonist hallucinogens (e.g. psilocybin and LSD), are being investigated for their therapeutic potential in patients with mood disorders and addiction. Strong psychoactive effects during psychedelic sessions, including decreases in a sense of self and increases in ratings of connectedness, positive mood, ineffability, and timelessness (operationalized as a "mystical experience") can be followed by sustained therapeutic changes, including improvement of depressive symptoms and reduction or cessation of addictive behaviors. Brain imaging studies using several different imaging modalities have identified changes in the activity and connectivity of regions of the posterior and ventral default mode network (DMN) during acute psychedelic effects. While these acute effects of psychedelics on the brain have been associated with subjective effects of spiritual experience and a decreased sense of self, little is known about the sustained effects of psychedelics on brain activity and connectivity after the acute effects of psychedelics have subsided. Thus, knowledge about the neurobiological basis of sustained therapeutic changes after psychedelic sessions is limited. The current double-blind, placebo-controlled study aimed to examine the relationship between acute psilocybin subjective effects and subsequent (next-day) differences in resting-state DMN connectivity.

Methods: 18 volunteers (Age = 25-70, M = 54.2, SD = 12.6; 10 F/8 M) in a study of the effects of psilocybin on meditation were randomized to receive either a placebo ($n = 9$) or a high dose (25 mg/70 kg) psilocybin ($n = 9$) capsule before a laboratory session. Groups were matched on age ($\chi 2 = 0.9$, $p =$ n.s.), sex ($t = 1.01$, $p =$ n.s.), number of previous hallucinogen uses ($t = 0.07$, $p =$ n.s.), and estimated lifetime hours of meditation ($t = 1.40$, $p =$ n.s.). Self-report measures of subjective experience (including the Mystical Experience Questionnaire, or MEQ, and the Challenging Experience Questionnaire, or CEQ) were assessed at the end of the session (after the effects of psilocybin had subsided). Seven minutes of resting-state fMRI data were collected the day after the session using a 3T Achieva Philips MRI scanner and echo planar imaging (TR = 2s, 37 slices, resolution = 3mm3, slice ga$p$ = 1mm, matrix = 80x80). Resting state data were preprocessed and normalized to the MNI template using SPM, screened for gross motion (Power et. al, 2012), and artifact-corrected using volume sensoring (Power et al, 2014) and 36-parameter motion correction (Satterthwaite et. al, 2013) which includes aCompCor (Behzadi et. al, 2007). Seed-based functional connectivity analyses were applied to resting-state data using the CONN toolbox. Resting-state data were parcellated using regions of interest (ROIs) defined in the Brodmann area atlas as well as additional amygdala and hippocampus ROIs defined in the Anatomy toolbox in SPM. Self-report measures and functional connectivity of DMN regions to all other ROIs were compared between placebo and psilocybin groups and correlated within the psilocybin group. Functional connectivity results were corrected for multiple comparisons using false discovery rate (FDR, $p < 0.05$).

Results: Participants who received psilocybin scored significantly higher than those who received placebo on the MEQ and the CEQ. There were no main effects of drug condition on functional connectivity, however in those who received psilocybin, there was a significant association between MEQ scores and connectivity of DMN regions (including the posterior cingulate and lateral parietal cortices) to both executive (dorsal lateral prefrontal cortex) and limbic (amygdala) brain regions. There was also a significant association between CEQ scores and connectivity of medial temporal lobe regions, including the amygdala,

entorhinal cortex, and hippocampus in those who received psilocybin.
Conclusions: Long-term therapeutic change observed after mystical and challenging experiences with psilocybin may be related to DMN, executive, and limbic connectivity change. Sustained change in DMN connectivity is consistent with therapeutic changes observed after psychedelic sessions. The relationship between challenging experiences, limbic connectivity, and therapeutic outcomes should be further investigated.
Keywords: Hallucinogens, Functional MRI (fMRI), Serotonin 2A, Resting State Functional Connectivity, Individual Differences.
Disclosure: Nothing to disclose.

M132. Effects of Inhaled, Vaporized Cannabis on Functional MRI Signal and Behavior in a Simulated Driving Program

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Background: As cannabis (CNB) begins to be decriminalized, medical CNB use is allowed in multiple U.S. states, and perception of its harmfulness falls, CNB use is rising and it will become increasingly common to publicly encounter persons who recently used the drug. An area of concern is that ever-greater numbers of CNB users and further drug legalization will increase the risk of driving while intoxicated from recent CNB use. Lab studies of acute dosing support that CNB use deleteriously affects driving and cognitive test performance. This fMRI pilot project tested the following hypotheses using an fMRI simulated driving paradigm to study brain and behavioral effects of different doses of acutely-administered vaporized marijuana. 1. CNB-related brain alterations would be most visible in cannabinoid-rich areas and those implicated in prior fMRI driving tasks. 2. There would be dose-related effects on brain and behavioral responses. 3. Experienced vs occasional CNB users would manifest relatively fewer brain and behavioral adverse effects at each dose.
Methods: We used pre-rolled NIDA herbal CNB cigarettes. Doses were placebo (0% THC), low dose (3.4% THC), and mod/high dose (5.7% THC). CNB was placed in a 'Volcano' brand vaporizer chamber and heated to 400°F. CNB was administered to subjects via vaporizer bags, using a published paced inhalation paradigm, in a randomized, counterbalanced, double-blinded fashion, immediately before subjects completed one of several, comparable, simulated driving paradigms. Scenarios were programmed using the National Advanced Driving Simulator's MiniSim software. We used a custom-designed, MRI-compatible, fiber-optic, in-scanner steering wheel & gas/brake pedal. fMRI data were collected using a 3T Siemens Allegra scanner at the IOL Olin Neuropsychiatry Research Center. Functional imaging volumes were identified using localizer images. The echo-planar image (EPI) gradient-echo pulse sequence was (TR/TE 1500/28ms, flip angle 650 FOV 24 cm x 24 cm, 64 x 64 matrix, 3.4 mm x 3.4 mm in plane resolution, 5 mm effective slice thickness, 30 slices). We quantified functional connectivity using Independent Component Analysis (ICA), similar to approaches used in our prior fMRI driving publications.
Results: fMRI analysis revealed previously documented driving-related circuits detected in our prior work with alcohol-intoxicated driving, and found differences between placebo and acute CNB exposure in regional recruitment of brain regions across most of these networks. For regions in cerebellar- (A), ($f = 0.31$), motor- (B) ($f = 0.30$), visual- (C) ($f = 0.30$) and rostral ACC/orbitofrontal- (D) ($f = 0.32$) networks, CNB dose modulated regional recruitment. Our prior work has linked these networks to action planning, collision avoidance & monitoring traffic respectively. Dose-effects were widespread across various brain regions known to have notable THC receptor concentrations, and brain activity was modulated by frequency of CNB use (average group difference effect size across these circuits is $f = 0.33$, with slightly higher peak effects). Regular users generally showed less activation. Dose-related behavioral effects were seen mainly on ability to stay accurately in lane, (weaving).
Conclusions: Despite the relatively modest sample sizes and relatively low THC doses, we were able to demonstrate significant acute CNB-driven changes at the brain and behavioral level that were modulated variously by dose and drug use patterns. These pilot results provide encouragement for conducting future larger-scale studies of underlying cognitive and brain mechanisms, and relating them to THC/metabolite concentrations in blood and oral fluids.
Keywords: Simulated Driving, Marijuana, Functional MRI (fMRI).
Disclosure: Astellas: Consultant, Self.

M133. New Frontier in Neuroimaging: Imaging Upright Moving Subjects With a Wearable, Low-Dose PET Scanner

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Background: Whole brain neuroimaging has been limited to completely motionless persons, which exclude many natural, and upright behaviors and tasks. EEG and fNIRS allow head motion but have limited resolution and exclude deep brain structures critical to many behaviors and tasks. An advance in PET detector technology has enabled us to build a prototype ring of PET detectors that is lightweight and therefore allows substantial head movement during active behavioral tasks. Because the detectors are very close to the head, it allows all the advantages of Standard PET such as specific ligand targeting, at a 1/10th of the dose or less.
Methods: We built and tested a single ring PET scanner prototype with a ring of 12 detector Silicon Photomultiplier (SiPM) based detector modules, that was large enough to fit around the head, and supported it from above, allowing free head motion that did not load weight onto the participant. We obtained phantom and human brain images during

modest head turning, at 25% and at 10% of standard dose. In addition, we performed simulation testing using Simulation System for Emission Tomography (SimSET) and GATE to estimate the sensitivity of head-mounted PET scanners with the truncated sphere geometry, as well as simulation of different shapes such as simple rings vs. helmet type design, both with and without the presence of detector modules at the top of the head and under the chin.
Results: Data acquired with our device showed reliable brain images, including during head turning. Normalized activity levels in anatomical regions of interest were not significantly different than those acquired with the same participants using Standard PET. One percent dose was sufficient in producing brain imaging results specific to motor and visual activation tasks.
Conclusions: In sum, the Helmet_PET is an Ambulatory Microdose brain imager that could have considerable impact in the field of mental health, stroke, traumatic brain injury and other functions, during active behavior. When combined with different neurotransmitter radioligands as well as with dynamic imaging techniques to increase temporal resolution, researchers should be able to see brain changes over time during more natural behavioral tasks.
Keywords: Positron Emission Tomography Imaging, Mental Health, Neurological Disorders, Novel Tool.
Disclosure: Nothing to disclose.

M134. Newmeds Data on Duplicate and Sequential Enrollment in Clinical Trials and Online Registry to Reduce Duplicate and Sequential Enrollment

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Background: There is emerging evidence, and growing concern, that subjects simultaneously and consecutively enroll in clinical trials, a practice that can lead to false or negative studies and has led to death and serious adverse events. As part of the Innovative Medicines Initiative sponsored NEWMEDS project, one of the largest ever-international academic-industry research collaborations aimed at improving the efficiency of clinical trials in schizophrenia and depression, we examined the extent to which subjects dual enroll in studies of antipsychotic and anti-depressant medications, motivations of persons who enroll concurrently in more than one clinical trial and developed a cross sponsor precompetitive data sharing online global registry to help prevent duplicate enrollment across trials and indications.
Methods: Data was from NEWMEDS repository of patient level data on 34,237 subjects from 96 clinical trials in schizophrenia and depression. Apparent duplicate enrollment by region was examined by matching subjects on available demographic information. For two studies, data were re-analyzed after removing duplicate subjects to examine their impact on efficacy results. In addition, we did 20 in-depth interviews, using opportunity sampling, with study participants, study personnel and study monitors to understand why persons duplicately enroll.
Results: The results of the analysis of duplicate and sequential enrollment showed apparent duplicates by region as follows: North America, 8.6%, Western Europe 4.8%, India 14.1%, Eastern Europe, 6.5%, Australia 7.8%. Also examined was data from all seven studies from one drug development program that showed that duplicates per study ranged from 11% to 14.8%. Data from one antidepressant study and one antipsychotic study suggest that even as few as 5% of subjects is sufficient to change study results from being statistically significant to being non-significant. Based on the interviews, the reasons for duplicate enrollment were categorized into the following main categories: (1) I know better, it is a silly requirement; (2) Investigational drug, not me I am not a criminal; (3) I am on a study for a cream, this is a study for a pill; it is not the same. They don't mean me; (4) They miscalculate, previous study ended 100 days ago; (5) They don't understand the question and are embarrassed to ask; (6) They want to be paid for an additional study; and (7) They, or their accompanying family member, are desperate and want to increase the chances of getting active treatment.
Conclusions: The data provides some minimum estimate of duplicate enrollment, as we did not have data on the entire universe of clinical trials. However, we could not estimate false positives. Results suggest that like many trials, these trials included duplicate patients. Having data on additional variables would have reduced the chance of false positives. The interviews suggest that there is a broad range of reasons for duplicate enrollment running the gamut of outright fraud to the desire to get needed treatment. Duplicate enrollment can be minimized by using a global clinical trial participant registry such as the NEWMEDS DupCheck tool, which can be used to screen out duplicate patients before enrollment. It uses either data manually entered into system or data automatically streamed via EDC/IVR/IRT. System can also be used to re-analyze completed trials after removing duplicate patients.
Keywords: CNS Clinical Trials, Clinical Trial Methodology, Data Sharing.
Disclosure: Abraham Pharmaceuticals: Consultant, Self; Minerva Pharm: Advisory Board & Consultant, Self; Pierre Fabre: Consultant, Self; Spatz Medical: Consultant, Self; Ethypharm: Consultant, Self; Intracellular: Consultant, Self; Kamada: Consultant, Self; MedAvante: Advisory Board, Self; DupCheck: Founder/owner of site, Self.

M135. Amphetamine Induced Endogenous Serotonin Release in the Human Brain: A Pet Study With [11C] cimbi-36

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Background: PET techniques have enabled the estimation of dopamine and opioid release in the human brain, however the extension of these methods to other neurotransmitters has proved to be challenging. The development of [11C] cimbi-36, a 5-HT2 agonist radioligand, has encouraged us to evaluate its suitability to detect endogenous 5-HT release. A previous attempt to image human brain serotonin release

with [11C]cimbi-36, using the SSRI citalopram as serotonin releasing agent, has been disappointing. However, the ability of single dose SSRI administration to induce robust serotonin release in the cortical projection areas has been questioned. We therefore decided to test the utility of d-amphetamine combined with [11C]cimbi-36, as a method to image human serotonin release. D-amphetamine is a safe experimental challenge in human subjects, that does release dopamine, and has been shown to increase extracellular serotonin levels serotonin in pre-clinical experiments.

Methods: Five male healthy volunteers received [11C]cimbi-36 PET scans before and after a single oral dose of 0.5mg/kg of D-amphetamine, and a single structural MRI scan of the brain. Dynamic PET data were acquired over 90 minutes post injection of the radioligand, and corrected for attenuation, scatter and subject motion. Arterial blood samples were collected during each PET scan and a metabolite corrected arterial plasma input function was constructed. PET emission data was quantified via a 2-tissue compartment model (2TCM) and the total volume of distribution (VT) was derived for the frontal cortex and the cerebellum for each PET scan. The frontal cortex [11C]cimbi-36 binding potential (BPND), a measure of 5-HT2A receptor availability, for each PET scan was calculated as VTfrontal/VTcerebellum -1. D-amphetamine induced serotonin release was quantified as the percent reduction in the frontal cortex BPND in the post-dose scan compared with the baseline scan. Statistical inference was tested by means of a paired students t-test evaluating a reduction in post-amphetamine [11C]cimbi-36 BPND.

Results: Following D-amphetamine administration, [11C]cimbi-36 BPND in the frontal cortex was reduced by an average of 10% ($p = 0.07$). See Table 1.

Conclusions: Our data indicate that [11C]cimbi-36 may be sensitive to the change in synaptic serotonin concentration in the human brain. More data will be required to confirm these observations. The combination of [11C]cimbi-36 and D-amphetamiine challenge has the potential to enable the evaluation of the human brain 5-HT system in neuropsychiatric disorders.

Keywords: PET Imaging, Serotonin, Amphetamine.

Disclosure: Imanova: Employee, Self; Opiant Pharmaceuticals: Consultant, Self; Gedeon Richter: Speaker, Self; GlaxoSmithKline: Shareholder, Self.

Table 1

	BPND Baseline	BPND post-amphetamine	Delta BPND
Subject 1	1.16	0.95	18%
Subject 2	0.94	0.95	-1%
Subject 3	1.18	1.06	10%
Subject 4	0.93	0.96	-3%
Subject 5	1.33	0.96	28%
		Mean	10%
		SD	13%
		p	0.07

M136. Identifying Patterns of Fear and Extinction Learning Across Anxiety, Eating and Compulsive Disorders

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Background: Translationally based paradigms involving fear and extinction learning are widely used to investigate the physiology of normal and pathological fear or anxiety. As an alternative to categorical psychiatric diagnoses, the NIMH Research Domain Criteria (RDoC) initiative encourages the study of clinically relevant behaviors with a known relationship to specific neural circuits. In this study we applied both approaches to data from an established psychophysiological paradigm designed to assess behaviors related to fear circuitry. First we compared healthy controls (HC) to three diagnostically defined groups that, while clinically different, also share significant symptoms of fear and anxiety: obsessive compulsive disorder (OCD); anorexia nervosa (AN); and social anxiety disorder (SAD). Second we conducted a latent class analysis (LCA) of the combined data from all four groups to identify intrinsic patterns of response. Our goals were to compare outcomes of these approaches and to explore whether identifying intrinsic fear-related response patterns may independently, or in juxtaposition with the categorical analyses, provide new insights about pathophysiology.

Methods: An established discriminant paradigm (UCS = shock, visual CS) was used to assess fear learning, extinction, extinction recall and fear renewal in 102 medication free adults with principal diagnoses of SAD ($N = 41$), OCD ($N = 41$), or AN ($N = 20$), and 64 matched HC. Acquisition took place in one context, Extinction and Extinction Recall in another, and Renewal in the original context (ABBA design). Outcome was measured with skin conductance response (SCR). For the categorical analyses, mean CS+/CS- differences in SCR scores over each phase were obtained from mixed effects models incorporating diagnosis, block and stimuli (CS+, CS-) and adjusting for race and gender. These estimated means were used to perform statistical contrasts of each disorder group to HC. For the LCA, subjects were clustered, based on SCR score at each time point, and a Bayesian Information Criterion (BIC) was used to determine the optimal number of clusters. Mixed effects models with cluster (but not diagnosis) included as a predictor, were used to test for group differences in SCR response. Significance was set at $p < 0.05$ (two tailed) for all contrasts.

Results: In the diagnostic based analyses both SAD and OCD demonstrated significantly greater Fear Renewal as compared to HC (OCD vs HC, $t = 2.53$, $p = .012$; SAD vs HC, $t = 2.16$, $p = 0.031$). There was also a trend for OCD to show reduced Extinction Recall ($t = -1.71$, $p = .087$). The best fitting LCA model indicated four Response Types, none of which were congruent with the DSM-IV – based diagnostic categories (i.e. each Response Type included participants from all four diagnostic groups and vice versa). A mixed effects analysis revealed a significant interaction ($p < .0005$) between Response Type and CS+ vs CS- difference for all

phases except Habituation. Though all of the Response Types demonstrated successful Habituation and Acquisition, their individual trajectories were quite different, particularly during the later, extinction related phases of the paradigm. Half of participants (Response Type 1, Nonfearful) had the expected response in all phases. Another third (Type 2, Extinction Recall Deficit) differed only in having a moderate Extinction Recall deficit. The remaining 15% were evenly split between two unexpected response patterns. One (Type 3, Recall failure/Sensitization) had great difficulty achieving and maintaining extinction. Their CS+ vs CS- difference during Extinction Recall was significantly greater than that of all other groups ($p < .003$), and it was unchanged or greater relative to their own response during Acquisition (i.e. sensitization). The other (Type 4, Threat Sensitivity) demonstrated extreme arousal once exposed to the mere threat of shock (prior to first shock in Acquisition). Both of these latter groups also had significantly greater CS+ vs CS- differences during Fear Renewal (all two-way p values $< .009$) than either Type 1 (Nonfearful) or Type 2 (Extinction Recall Deficit), which did not differ from each other.

Conclusions: Both our categorical and LCA analyses are consistent with the literature in suggesting that at least a subset of individuals with pathological anxiety demonstrate significant difficulties maintaining extinction of learned fear. Two previous studies reported extinction recall deficits in OCD and both delayed extinction and increased fear renewal have been associated with social anxiety. The finding of several trans-diagnostic behavioral response patterns is consistent with the RDoC framework and the hypothesis of diagnostic heterogeneity with respect to circuitry function. Two of these patterns (Type 3, Extinction Recall Failure, Type 4, Threat Sensitivity) may have implications for treatment outcome, particularly in the application of cognitive-behavioral therapy which relies on extinction learning, strengthening extinction recall and, in exposure treatments, an initial willingness to engage with feared situations.

Keywords: Extinction Learning, Research Domain Criteria (RDoC), Fear Renewal, Extinction Recall.

Disclosure: Nothing to disclose.

M137. The Long Shadow of Early Life Stress: Preadolescent Maternal Stress Impacts Stress Physiology in Offspring

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Background: Early life stress (ELS) can pave the way for dysregulated physiologic stress response and increased risk for affective disorders in adulthood. These enduring effects of stress are of particular interest among women during pregnancy; their own ELS may influence their response to stress and in turn impact fetal development. This represents a potential avenue for transgenerational transmission of stress. In rodents, pre-gestational maternal stress resulted in accentuated HPA response in offspring. In humans, maternal childhood trauma was associated with higher placental corticotropin-releasing hormone (CRH) levels. In humans, impact of maternal ELS on offspring stress response, particularly stress timing relative to maternal puberty, is unknown. We hypothesize that maternal adverse childhood experiences (ACEs) experienced prior to the pubertal window may eventually lead to a sub-optimal milieu for the developing fetus, altering development of the fetal HPA axis and subsequently stress reactivity in the offspring.

The aim of this study was to assess stress reactivity, specifically cortisol response, among women with high or low exposure to preadolescent ACEs, as well as their offsprings' cortisol response. This is part of a larger study assessing stress response among women across pregnancy and postpartum.

We hypothesized that maternal preadolescent ACE will predict 1) maternal cortisol response during a postpartum separation task, and 2) her infant's cortisol response to the separation stressor.

Methods: Women were healthy controls recruited at 8-17 weeks gestational age; those with current psychiatric illness assessed via SCID, or with history of preterm birth were excluded. Women completed a demographic and health questionnaire, including age of first menses, and the Adverse Childhood Experience Questionnaire (ACE). A separation task was performed six months postpartum; the mother was separated from her infant while the infant underwent a laboratory stressor (exposures to loud sound bursts and physical restraint). Salivary cortisol samples were collected from mother and infant at six timepoints. For statistical analyses, women were grouped into low ACE (0-1 adverse events) or high ACE (2 or more adverse events); preadolescent ACEs were those occurring up to two years prior to menses onset. Cortisol was log transformed to achieve normality. Linear regressions were used to assess impact of maternal preadolescent adversity on maternal and infant cortisol response as follows: H1) Assessed log transformed maternal cortisol response after subtracting baseline (average cortisol levels at T-15 and T-0) with race as a covariate. H2) Assessed log transformed infant maximum cortisol response to the separation task with race as a covariate.

Results: Twenty-five women and 39 infants provided cortisol samples during the separation task.

1) Maternal preadolescent ACE had a significant main effect on maternal cortisol response. Women with high levels of preadolescent ACE had 42% lower cortisol response, on average, during the postpartum separation task compared to low ACE women ($p = 0.01$).

2) Infants of mothers in the high versus low preadolescent ACE group showed a blunted cortisol response ($p = 0.02$). When race was added to the model, infants of high ACE African American women showed 71% lower cortisol response than high ACE Caucasians ($p = 0.009$). Infant sex did not influence these associations.

Conclusions: Women who experienced high levels of preadolescent adversity, as well as their offspring, had blunted cortisol response to stress. This effect was not secondary to depression, as participants were psychiatrically and medically healthy. Among infants, the blunted response was particularly strong among offspring of African American

mothers in the high ACE group. Our findings are consistent with recent animal models, and suggest transgenerational effects of stress, i.e. stress experienced by a woman during her preadolescence can "reach forward" to impact her offspring's stress reactivity decades later. A critical area for further study is timing of stress relative to puberty. Puberty is a critical window of brain development, when sex-specific changes in HPA responsivity begin to emerge. As neurosteroids regulate HPA function, examining the interplay among reproductive hormones, ELS timing, and stress response across the lifespan will be an important area of research.

Keywords: Pregnancy, Transgenerational, HPA, Cortisol, ACE.

Disclosure: Nothing to disclose.

M138. Discovery of Brain-Penetrant COMT Inhibitors for the Treatment of Cognitive Impairment

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Background: Cognitive impairment is a core feature of many psychiatric and neurological disorders and currently available treatments are inadequate. Dopamine in the prefrontal cortex is critical for the regulation of cognitive function and therapeutic interventions that increase dopamine in the cortex are thought to improve cognition. The FDA-approved catechol-O-methyltransferase (COMT) inhibitor tolcapone has shown promise as a potential cognitive enhancer across multiple clinical indications. Although tolcapone has been approved as a treatment for the motor symptoms of Parkinson's Disease, it has multiple features that prevent its widespread use as a cognitive enhancer including 1) low brain penetrance 2) poor pharmacokinetics (PK) that requires 3X/day dosing 3) a black box warning for liver toxicity. The goal of our current research is to develop novel brain-penetrant COMT inhibitors, with good PK (1X/day dosing), and improved safety profiles compared to tolcapone. Here we describe multiple COMT inhibitors with promising preclinical in vivo profiles that may be suitable for clinical trials testing their cognitive enhancement capabilities.

Methods: To determine the in vivo activity of our novel compounds, we measured the concentrations of dopamine metabolites in cerebrospinal fluid (CSF) and compared the results to the known effects of tolcapone. Male Sprague Dawley rats were administered compounds (IP or PO) and either 1 or 4 hours later, they were anesthetized and placed into a stereotaxic frame. CSF was withdrawn. Following CSF withdrawal, blood was taken via cardiac puncture, rats were transcardially perfused, and then the brain was removed. Drug levels were measured in CSF, plasma, and brain tissue. The dopamine metabolites homovanillic acid (HVA) and 3,4-Dihydroxyphenylacetic acid (DOPAC) were measured in CSF. HVA levels decrease and DOPAC levels increase following COMT inhibitor treatment.

Results: We synthesized multiple COMT inhibitors from two distinct chemical series with significant in vivo activity. Our most promising compounds inhibited in vivo dopamine metabolism within an order of magnitude of the effect seen following tolcapone treatment. These compounds have slightly lower in vitro potency compared to tolcapone, but much greater brain-penetrance and more favorable PK characteristics. Current synthesis efforts are focused on increasing the potency of the compounds while maintaining the high brain-penetrance and good PK.

Conclusions: The COMT inhibitor tolcapone has shown promise as a potential cognitive enhancer in short-duration studies across multiple clinical indications. Unfortunately, tolcapone has multiple compound-specific negative attributes that would hinder its utilization as a long-term cognitive enhancer. Our current program is focused on developing a potent, brain-penetrant COMT inhibitor with similar in vivo effects as tolcapone without the toxicity risk. We currently have multiple compounds with good in vivo potency as measured by the change in the concentration of dopamine metabolites in the CSF. Next steps involve confirming similar behavioral efficacy to tolcapone in preclinical assays.

Keywords: Cognitive Enhancement, Dopamine, Prefrontal Cortex, COMT Inhibitor.

Disclosure: UCB: Research Grant, Self.

M139. Anhedonia, Depression, Anxiety, and Craving for Opiates in Opiate Dependent Patients Stabilized on Oral Naltrexone or an Extended Release Naltrexone Implant

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Background: Naltrexone is a μ-opioid receptor antagonist that blocks opiod analgesic and euphoric effects. Opioid craving, depression, anxiety, and anhedonia are common among opioid dependent individuals, and concerns have been raised that naltrexone increases them due to blocking endogenous opioid. Here we present information that addresses these concerns in a secondary analysis of data from a naltrexone study.

Methods: Opioid dependent patients ($N = 306$) were enrolled in a three cell (102ss/cell) randomized, double blind, double dummy, placebo-controlled 6-month trial comparing extended release implantable naltrexone, oral naltrexone (50 mg/day), and placebo (oral and implant). Monthly assessments of affective responses used a Visual Analog Scale for opioid craving, the Beck Depression Inventory, Spielberger Anxiety Test, and the Ferguson and Chapman Anhedonia Scales. Outcomes were analyzed using mixed model analysis of variance (Mixed ANOVA) with repeated measures using Tukey test for between group comparisons.

Results: Depression, anxiety, and anhedonia were elevated at baseline but reduced to normal within the first 1-2 months for patients who remained in treatment and did not relapse. No significant differences between groups were seen other

than increased anxiety prior to dropout at week 2 among oral naltrexone/placebo patients.
Conclusions: Naltrexone blockade of opiate receptors did not induce anhedonia or depression among patients that remained in treatment and did not relapse. These reductions occurred regardless of medication group and were most likely due to treatment success. Oral naltrexone 50 mg may increase anxiety, but its relationship to dropout is unclear since it was seen only at week two.
Keywords: Naltrexone, Opiates, Addiction.
Disclosure: Nothing to disclose.

M140. GWAS Meta-Analysis Reveals Novel Loci and Genetic Correlates for General Cognitive Function: A Report From the Cogent Consortium

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Background: The complex nature of the human cognitive phenotype has resulted in cognitive genomics lagging behind many other fields in terms of gene discovery using genome-wide association study (GWAS) methods. There were two major aims of the current study: (1) conduct a large-scale GWAS meta-analysis of general cognitive function in 24 independent cohorts ($N = 35{,}298$), to identify SNP-based and gene-based loci associated with cognition; and (2) determine the extent of genetic correlation between general cognitive function and published neurobehavioral phenotypes of interest. These aims were executed within the context of the Cognitive Genomics Consortium (COGENT), an international collaborative effort designed to study the molecular genetics of cognitive function.
Methods: To date, COGENT has acquired individual-level neuropsychological, demographic, clinical and SNP array data from 24 studies (comprised of 35 sub-cohorts) with 35,298 individuals (46.8% females, mean age of 45.3 ± 8.6 years) of European ancestry drawn from the general population. Genotype data underwent common QC and imputation procedures, resulting in ~8M high-quality SNPs. The GWAS phenotype was general cognitive function ("g"), derived from the first principal component of a PCA performed on an average of 8 ± 4 neuropsychological tests. Allelic association analysis was conducted with imputed allele dosages using Plink 1.9, except for 8 sub-cohorts including related individuals, which were analyzed with BOLT-LMM. GWAS results were combined for meta-analysis using the inverse variance weighted Z-score method in METAL, with subsequent gene-based analysis using MAGMA. Additionally, we utilized individual SNP lookups and polygenic score analyses (using the LD score regression method) to identify genetic overlap with other relevant neurobehavioral phenotypes.
Results: Our primary GWAS meta-analysis identified two novel SNP loci associated with cognitive performance at the genomewide significance level ($P < 5E\text{-}8$). On chromosome 2, intronic SNP rs76114856 in the CENPO gene was genome-wide significant ($P = 6.58E\text{-}9$). On chromosome 1, a cluster of six SNPs located in a lincRNA, RP4-665J23.1, were also genome-wide significant (top SNP, rs6669072, $P = 2.77E\text{-}8$). In addition, a large 1.4Mb region at chromosome 17q21.31, coextensive with a known inversion polymorphism, harbored 101 nearly-significant SNPs (top SNP, rs916888, $P = 8.18E\text{-}8$). Gene-based analysis, as well as integration with prior GWAS studies of cognitive performance and educational attainment (CHARGE, UK Biobank, and SSGAC) yielded several additional significant loci. Finally, we found robust polygenic correlations between cognitive performance and educational attainment, several psychiatric disorders, smoking behavior, and a novel genetic association with the personality trait of openness.
Conclusions: These data provide new insight into the genetics of neurocognitive function that are applicable to genetic research of neuropsychiatric illness. Novel genes implicated include CENPO, TP53, ATXN7L2, ARPP21, and RPL31P12. Our results also support several loci derived from the recent CHARGE meta-analysis of general cognitive function, the UK Biobank GWAS of verbal numerical reasoning, and the SSGAC analysis of educational attainment. Polygenic overlap analyses demonstrate the significance of understanding the genetics of general cognitive function to unraveling the etiology of numerous psychiatric illness and health-relevant conditions.
Keywords: Genetic Variability, Polygenic Risk Score, Cognitive Functioning, GWAS, Schizophrenia Genetics.
Disclosure: Nothing to disclose.

M141. Top-Down Control of Attentional Behavior by Long-Range Fronto-Posterior Cortical Circuit

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Background: Understanding the neural circuits mediating attention processing may provide insight into the pathophysiology of attention deficit symptoms frequently observed in neurodevelopmental and psychiatric disorders. Human imaging studies suggest that a key aspect of attention processing is to improve efficiency in information processing through a shift in the information flow between certain brain areas. Frontal cortex has been implicated in implementing a top-down control of attention by voluntarily directing one's attention in anticipation of an expected event. However, the precise neural network mediating the top-down control of attention by frontal cortex has been largely unknown. Previous functional connectivity studies in human and pharmacological and chemogenetic studies in rodent (Koike et al,Neurophsychopharmacology 2016) demonstrated that dorsal anterior cingulate cortex (dACC) as a key area within frontal cortex for top-down control of attention. Here we combined an attentional behavioural assay and genetic manipulation techniques to identify specific neural circuits projecting from dACC to mediate top-down control of attentional behavior.
Methods: We used Bussey-Saksida TouchScreen-based 5-choice serial reaction time task (5CSRTT) to assess attention performance, processing speed, and response control in C57BL/6 mice. To identify the functional localization of the task-specific dACC projections implicated in the top-down

control, an activity- and tamoxifen-dependent neuron labeling method was used to fluorescently label dACC circuits activated during the 5CSRTT. Specifically, we virally expressed the activity- and tamoxifen-dependent Cre recombinase (AAV-E-SARE- ERCreER) (Kawashima et al, Nature Methods 2013) together with cre-dependent Channelrhodopsin fused with eYFP (AAV-EF1a-DIO-ChR2-eYFP) in the dACC, and then injected an ER agonist, 4-hydroxytamoxifen, immediately after 5CSRTT. Fluorescently labeled projections from dACC were then quantified across the whole brain regions. To examine the causal contribution of neural circuits projecting from the dACC, we virally expressed an inhibitory designer receptors exclusively activated by designer drug (DREADD) in the specific circuit (fronto-posterior cortical projection neurons) which was identified by activity-dependent labeling approach to demonstrate its necessity for the visual attention performance.
Results: Task-dependent fluorescent labeling of dACC projections revealed that dACC neurons projecting to cortical and subcortical regions were diffusely activated in the control home cage condition. In contrast, 5CSRTT-exposed mice revealed reduced 5CSRTT-dependent labeling in projections to subcortical areas while leaving the projections to visual cortex remain labeled, suggesting a relative increase in the activation of fronto-posterior cortical circuit from dACC to visual cortex by the attentional task. To demonstrate the causal contribution of this fronto-posterior cortical circuit to attentional behavior, we directly manipulated the neuronal activity of this top-down circuit by using inhibitory DREADD during 5CSRTT and found that the inhibition of fronto-posterior cortical circuit activity disrupted their attention performance without affecting other outcomes: motivation, response control, and motor activity.
Conclusions: The observed shift in 5CSRTT-dependent activation of dACC out-puts from subcortical to specific fronto-posterior cortical circuits suggests specific circuit bases for top-down attentional processing. The observed attention task-dependent shift in dACC out-puts may be a consequence of task-dependent activation of local inhibitory neurons in frontal cortex (Kim et al, Cell 2016) which may specifically suppress task-irrelevant dACC neuronal activity while leaving relevant circuits remain active. Our results also demonstrated the functional necessity of long-range dACC neurons projecting to visual cortex for 5CSRTT performance, especially an attention behavior, providing a potential therapeutic target for attention deficits at the circuit level. Our study provides a key template for future studies to modulate this fronto-posterior cortical circuit to mitigate attention deficits in animal models of neurodevelopmental and psychiatric disorders.
Keywords: Attention, Anterior Cingulate Cortex, 5CSRTT, Top-Down |Control.
Disclosure: Taisho Pharmaceutical Company: Self.

M142. Increasing Minority Participation and Diversity in ACNP Meetings and Membership: Barriers and Best Practices

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Background: Neuropsychopharmacology science, its application in disease prevention and medicine, and drug regulation and policy, should address and benefit the diversity of the population. That includes diversity by race, ethnicity, sexual orientation and gender identity. A better understanding of diversity can be informed by all areas of science encompassed by neuropsychopharmacology. These include research focusing on molecular genetics, neuropharmacology, and behavioral pharmacology, as well as individual and population-based human investigations. The consequences of the science can be far reaching as society wrestles with issues ranging from medications development to better serve diversity of needs, the more equitable availability of medicines, diagnostic issues such as the social and science-based rejection of homosexuality as a disease, and issues generally presumed to be "social" such as violence, aggression, and racial discrimination in the criminal justice system. Indeed, it is increasingly recognized that addressing diversity-related issues can be enhanced by the involvement of scientists who reflect the diversity of the population. Among 823 active ACNP members in 2014, the numbers of those who self-identified as "Black," "Hispanic," "Pacific Islander," and "Native American" were 7, 28, 0, and 0, respectively, representing 4.3% of the total membership. The number who self-identified as being female was somewhat more encouraging at 181 (22%). In the past decade, ACNP established the Minority Task Force and the Women's Task Force in order to promote its efforts to increase diversity participation and membership. This poster will provide trends including the most recent data on meeting participation and ACNP membership, with a comparison to similar data from the College on Problems of Drug Dependence (CPDD) which accelerated its diversification efforts in the early 1990s.
Methods: Historical data summarized by the ACNP Minority and Women's task forces and those collected by the Underrepresented Populations Committee (URPop) of CPDD are being evaluated. Those will be updated by including available data from the 2015 ACNP and 2016 CPDD conference registration listings and membership data. In addition, the authors have begun to collect expert opinion data on "impediments and barriers," and "what works" to increase meeting participation and membership from members of the ACNP task forces and CPDD's URPop. Data are also being collected from the National Institute on Drug Abuse (NIDA) Office of Diversity and Health Disparities (ODHD) program.
Results: Preliminary examination of data indicate that the participation of women in ACNP has been increasing at an encouraging rate in the past 1-2 decades, whereas progress in increasing racial and ethnic diversity in meeting attendance and, especially, in ACNP membership is not evident. For example, only 2 of the 65 (3.1%) underrepresented

minority (URM) travel awardees from 2008-2014 are current Associate or Full members. Similarly, preliminary assessment of data from CPDD indicates that specific programs, such as minority travel awards, can contribute to increased participation in annual meetings but that progress in increasing minority membership has been slower. Summaries of identified 'Impediments and Barriers' and 'What Works' will be presented in the poster. An important outcome is that there are gaps in certain measures of diversity such as ethnicity, sexual orientation, and gender identification.

Conclusions: There is broad concurrence of opinion that increasing diversity of participation and membership is vital to increase the excellence, relevance, and process by which neuropsychopharmacology science advances. Diversity also strengthens communications and improves policy recommendations flowing from the science. It is also clear that the motivation to make strong, visible and rapid progress is driven by the self-reported beliefs that this is important for society at large, is fair and is right. This dimension is encouraging with respect to ACNP because discussions among the members and leadership indicate strong support for increasing diversity. These discussions also suggest the potential benefits of more systematic evaluation of feelings and attitudes among ACNP members to better understand the impediments and barriers and to improve progress in diversification of the organization. Such understanding is vital to the development, selection and implementation of appropriate interventions, policies, and practices. Despite the challenges, there is evidence that there are tools and practices that are effective in promoting change and their increasing adoption by ACNP and support by appropriate organizations (e.g., NIDA) is recommended. For example, there appears to be little difficulty in utilizing all available travel awards; increasing these with increased support is a relatively reliable path to increased diversity in meeting attendance. On the other hand, increasing membership will require more active efforts to recruit potential members and encourage and support their applications. Identification of barriers may lead to recommendations for modifications in the application process. More comprehensive data collection and dissemination will be important to better monitor and guide progress. Although there is much progress to be made and much we do not know, there appears to be sufficient progress and knowledge to begin the process of establishing core best practices.

Keywords: Racial/Ethnic Minority Elderly, Population-Based, Ethics.

Disclosure: Nothing to disclose.

M143. Development of Amygdala Functional Connectivity During the Perinatal Period Using Fetal Functional Magnetic Resonance Imaging

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Background: Alterations of amygdala functional connectivity are a characteristic of many mental health disorders. In particular, this circuit appears to be highly sensitive to early life adversities and shows large-scale changes across childhood through adolescence. While most MRI studies demonstrating the effect of early life adversities and alterations of amygdala functional connectivity have been conducted in children and older subjects, emerging reports in infants are beginning to highlight the vulnerability of the amygdala to prenatal psychological stress exposure. However, normal developmental trajectories of amygdala functional connectivity in the perinatal period (3rd trimester and neonatal period) have not been mapped out, making it difficult to establish critical periods of vulnerability to prenatal stress. As such, we interrogated the emergence of amygdala networks during the perinatal period using longitudinal fetal functional magnetic resonance imaging.

Methods: This study was approved by the Yale University Human Investigation Committee, and pregnant women signed consent for the protocol. Ten typically-developing fetuses underwent longitudinal resting-state fMRI at 30-32 weeks postmenstrual age (PMA), at 34-36 weeks PMA, and after birth, at 40-44 weeks PMA. Imaging was performed on a 3.0 T scanner (Siemens Skyra) using a 32-channel body for fetal imaging or 32-channel head coil for neonatal imaging. Four or five functional runs, each 5 minutes in length, were collected per participant at each time point, resulting in 20-25 minutes of data for each fetus.

Standard functional connectivity preprocessing adapted for fetal imaging were performed using BioImage Suite. Fetal motion was corrected for using a custom fetal motion correction algorithm. Several covariates of no interest were regressed from the data including linear and quadratic drift, six rigid-body motion parameters, mean cerebral-spinal-fluid (CSF) signal, mean white-matter signal, and overall global signal. The data were temporally smoothed with a zero mean unit variance Gaussian filter (approximate cutoff frequency = 0.12Hz). Frame censoring was used to remove frames of high motion, of low signal-to-noise ratio, and of low motion correction quality. To minimize confounds, 50-80% of the data was censored for each fetus, leaving approximately 5 to 10 minutes of usable data per fetus. Functional images were warped into a fetal template based on post-mortem MRIs using a low-grade non-linear transformation. Standard methods were used to process the neonatal data.

The left amygdala seed region was defined on the reference brain. The time course of the amygdala seed in a given participant was then computed as the average time course across all voxels in the amygdala. Next, this time course was correlated with the time course for every other voxel in the gray matter to create a map of r-values, reflecting amygdala-to-whole-brain connectivity. These r-values were transformed to z-values using Fisher's transform yielding one map representing the strength of correlation to the amygdala seed for each participant.

Results: At 30-32 weeks PMA the amygdala functional network consisted of only local connectivity to the amygdala. At 34-36 weeks PMA, the amygdala functional network demonstrated several ipsilateral connections to the insula cortex, auditory cortex, middle temporal lobe, and inferior frontal lobe. Finally, after birth at 40-44 weeks PMA, the amygdala functional network consisted cross-hemispheric connectivity to the contralateral amygdala in addition to

these ipsilateral connections. These results suggest that amygdala functional connectivity develops across the third trimester and that significant cross-hemisphere connections are not present until very late in gestation.
Conclusions: During the perinatal period, left amygdala connectivity is first characterized by local circuitry, then begins to connect to ipsilateral regions in the frontal and temporal lobes, and finally develops connections to the contralateral amygdala. The developmental trajectory of these fundamental connections subserving neurobehavior may explain the prenatal vulnerability of this circuitry. Future work should investigate how alterations in these developmental trajectories mediate mental health outcomes.
Keywords: Amygdala, Functional Connectivity, Fetal fMRI.
Disclosure: Nothing to disclose.

M144. Studying GABA Neurophysiology by Simultaneous [18F]Flumazenil-Positron Emission Tomography and Magnetic Resonance Spectroscopy

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Background: The major inhibitory system in the brain, the GABAergic system, is hypothesized to be abnormal in various neurodevelopmental disorders (e.g., autism spectrum disorder) and neurogenetic disorders (e.g., fragile X syndrome). Few studies have explored the GABAA receptor distribution by positron emission tomography (PET) or GABA levels by magnetic resonance spectroscopy (MRS) in these disorders. Simultaneous acquisition of both PET and MRS data has not been possible until recently. This pilot study aims at demonstrating the feasibility of simultaneous acquisition of GABAA receptor binding potentials by PET (with [18F]Flumazenil ([18F]FMZ) as radiotracer) and GABA levels by MRS in normal human volunteers.
Methods: Participants. Healthy volunteers aged 21-45 consented according to the local IRB.
Image acquisition. Subjects were scanned on GE SIGNA PET/MR (Waukesha, WI). PET data were acquired in list mode (0-60min p.i), dynamically reconstructed into 27 time-frames (12×15s, 3×1min, 3×3min, 9×5min) and corrected for photon attenuation using both scanner-specific 8-channel headcoil correction and a MR-measured head atlas-based attenuation correction maps. During PET data acquisition, a series of MR structural sequences were acquired, including (a) T1-weighted MP-RAGE sequence, (b) T2-weighted fast-spin-echo sequence. For the MRS measurement of GABA, MEGA-PRESS was performed on the left dorsolateral prefrontal cortex and bilateral thalami with voxel sizes of approximately 20cc and TE/TR = 80/2000ms, 15min acquisition time.
Image and data analysis. The dynamic PET and structural MR data were normalized to MNI (Montreal Neurological Institute) space (PMOD 3.7, Switzerland). Time-activity curves were extracted using pre-defined volumes-of-interest (VOIs) based on the Hammers atlas. A reference tissue model (Ichise model; MRTM0) was used to calculate binding potentials (BPND) with pons as the reference region. Mean values between the two subjects for each VOIs were calculated. The MEGA-PRESS edited spectrum was obtained by subtracting the editing OFF spectrum from the editing ON spectrum. GABA level was estimated from the integrated 3ppm peak area in the edited spectrum divided by the Cre peak area.
Results: We have successfully acquired [18F]FMZ-PET, MRS, and structural MRI data concurrently with a simultaneous PET/MR imaging system in three healthy male volunteers (mean age 31). Highest uptake was observed in the neocortical regions (BPND = 4.5 ± 1.0) and limbic system (BPND = 3.8 ± 0.6), intermediate in the cerebellum (BPND = 2.8 ± 0.8), thalamus (BPND = 2.0 ± 0.3), and basal ganglia (BPND = 1.9 ± 0.6), and low uptake in the brainstem (BPND = 0.25 ± 0.07). The GABA+ peak at 3 ppm (J-coupled to GABA spins at 1.9ppm and coedited macromolecules) and combined glutamate+glutamine (Glx) peaks at 3.75ppm are clearly observed in the MEGA-PRESS edited spectrum. The estimated GABA/Cre ratio is 9.7%, in agreement with the level measured using MEGA-PRESS on healthy human subjects.
Conclusions: We have successfully acquired [18F]FMZ-PET and MR data concurrently with our PET/MR imaging system in three healthy volunteers. We will proceed with implementing this protocol in patient populations with neurodevelopmental disorders (autism spectrum disorder, idiopathic intellectual disability, fragile X syndrome) in the near future.
Keywords: PET Imaging, Magnetic Resonance Spectroscopy, GABA, Simultaneous PET-MR.
Disclosure: Nothing to disclose.

M145. Cognitive Training, the Brain, and Decision Making

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Background: Increased preference for immediate over delayed and for risky over certain rewards is a hallmark of addiction. Motivated by evidence that enhanced cognitive control can shift choice behavior away from immediate and risky rewards, we tested whether training executive cognitive function could influence choice behavior and brain responses.
Methods: In this randomized controlled clinical trial, 128 young adults participated in 10 weeks of training with either a commercial web-based cognitive training program or web-based video games (which do not specifically target executive function or adapt the level of difficulty throughout training). Pre- and post-training, participants completed cognitive assessments and functional magnetic resonance imaging (fMRI) during performance of validated decision-making tasks: delay discounting (choices between smaller rewards

now vs. larger rewards in the future) and risk sensitivity (choices between larger riskier rewards vs. smaller certain rewards).
Results: Contrary to our hypothesis, we found no evidence that commercial cognitive training influences neural activity during decision-making, nor did we find effects of cognitive training on measures of delay discounting or risk sensitivity. Participants in the commercial training condition did improve with experience and practice on the specific tasks they performed during training, but participants in both conditions showed similar improvement on standardized cognitive measures over time.
Conclusions: Commercial adaptive cognitive training in healthy young adults appears to have no benefits above those of standard video games for measures of brain activity, choice behavior, or cognitive performance.
Keywords: Cognitive Enhancement, Functional Neuroimaging, Cognitive Training.
Disclosure: Nothing to disclose.

M146. Social Exposure Robustly Enhances the Activation of Oxytocin-Sensitive Reward Pathways by a Melanocortin Agonist

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Background: Oxytocin (OT) enhances several aspects of social cognition, and the OT system is an important therapeutic target for improving social function in disorders such as autism. Melanocortin 4 receptor (MC4R) agonists stimulate local release of OT in the hypothalamus and potentiate OT release in distal target brain areas in response to a physiological stimulus. Both OT and MC4R agonists rescue social deficits in mouse models of autism and facilitate partner preference formation in socially monogamous prairie voles. In previous experiments Melanotan II (MTII), an MC4R agonist, only evokes detectable OT release in the nucleus accumbens (NAcc) in response to hypertonic saline, a physiological stimuli known to evoke endogenous OT release. Given this priming effect of MTII, we predict MTII will enhance OT release in response to social exposure, thereby modulating neural activity of OT-sensitive brain regions mediating social behaviors, including NAcc and prefrontal cortex (PFC).
Methods: We used immunohistochemistry (IHC) targeting the immediate early gene c-fos to assess neural activity in female prairie voles in response to central MTII infusion in 2 contexts, MTII alone and MTII with exposure to a novel stimulus male. First, ICV MTII (3nmol, 2μL) or aCSF (vehicle control, 2μL) was administered to adult female prairie voles, followed by immediate return to an empty homecage. Ninety minutes later animals were perfused and brains processed for Fos IHC. In the second experiment, following IVC infusion of MTII or aCSF as before, female prairie voles were exposed to a novel adult male for 30 minutes, and perfused 60 minutes after the end of social exposure. The social exposure period was videotaped and behavior was scored. In a third experiment, we added an additional group of animals receiving a co-infusion of MTII and a selective OT receptor antagonist (MTII 3nmol + OTA 5ng, 2μL). ICV MTII and aCSF dosage in this third experiment remained identical to experiment 2. These 3 groups were then exposed to a novel adult male for 30 minutes and perfused 60 minutes later, as before. All brains were again processed for Fos IHC. Quantification of Fos-positive cells was performed for the following brain regions: NAcc, PFC, paraventricular nucleus of the hypothalamus (PVN), lateral septum (LS), basolateral amygdala (BLA) and central amygdala (CeA).
Results: In the homecage condition, A 2-way repeated measures ANOVA revealed a significant main effect of brain region ($p<0.0001$), and treatment ($p=0.04$) but the interaction between region and treatment was not significant ($p=0.47$). Bonferroni corrected post-hoc tests showed that MTII infusion resulted in a significant increase in Fos-positive cells in the BLA ($p=0.002$) and CeA ($p=0.03$). No significant differences were observed in the NAcc, PFC, LS, or PVN. In the second experiment, which included a social exposure component, MTII resulted in a significant increase in Fos positive cells (main effect, $p<0.0001$). Further, a significant main effect of brain region ($p<0.0001$) and a significant interaction between treatment and brain region ($p<0.0001$) were observed. Bonferroni corrected post-hoc tests showed significant effects of MTII treatment in the BLA and CeA, as in the first experiment, but also in the NAcc, LS, PFC and PVN (all ps <0.05). These regions are known to play an important role in social cognition and social attachment and are part of what has previously been described as the pair bonding network (PBN). Effect size analyses showed the effect of social interaction (by comparing the effect size of MTII in the homecage condition to the second experiment including the social exposure component) to be strongest in LS, NAcc, and PFC (all Cohen's D values $>$ 1). In the third experiment, we replicated previous social contact findings which show PBN activation enhancement following MTII administration, in the BLA, CeA, NAcc, LS, PFC and PVN (all Bonferroni corrected ps <0.05). In addition, we show that OTA significantly reduces the effect of MTII on Fos-active cells in the same regions (all Bonferroni corrected ps <0.05). Interestingly, the brain regions most strongly influenced by the social exposure component (LS, NAcc and PFC) also showed the strongest effect of OTA (all Cohen's D values $>$ 0.65). In both social exposure experiments, no significant differences were observed in any of the behaviors quantified.
Conclusions: We conclude that social contact, a presumed facilitator of central OT release, changes neural activation in the pair bonding network in response to MC4R stimulation. We demonstrate that MTII-enhanced neuronal activation in multiple regions of the pair bonding network is blocked by co-administration of a selective OT receptor antagonist. It is therefore likely that the OT-priming effect of MTII is responsible for a majority of enhanced neuronal activation observed in regions of the pair bonding network. Future experiments will examine the ability of peripherally administered MTII to enhance neuronal activation in the context of social exposure. Demonstration that MTII is indeed a peripherally administerable compound with the ability to affect central OT signaling would represent a significant advance in the study of social cognition, as few compounds, if any, can affect these processes in such a profound way. These findings have important implications for ongoing

clinical studies examining the efficacy of an MC4R agonist in enhancing OT-dependent social cognition in autistic patients.
Keywords: Oxytocin, Autism, Social Cognition.
Disclosure: Nothing to disclose.

M147. Vitamin E Analogue Rescues Ionizing Radiation Injury to the Fronto-Limbic Circuit in Primate Brain

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Background: Ionizing radiation causes characteristic and well recognized side effects including problems thinking clearly, difficulty managing previously easy tasks, poor memory, confusion, personality changes, headaches similar to migraines (SMART attacks), and, in the case of cancer therapy, additional residual symptoms stemming from the original tumor. The fronto-limbic circuit of the primate brain includes brain regions that could be targets of radiation damage and also be responsible for the coincident behavioral symptoms.
Methods: A vitamin E analogue, γ-tocotrienol (GT3), has been found to be radioprotective in mice and nonhuman primates (NHP) in acute radiation syndrome (ARS) models We therefore hypothesized that GT3 could mitigate radiation-dependent molecular changes within the fronto-limbic circuit. To test this hypothesis, we irradiated NHPs with an LD25-50/60 dose (viz, lethality of 25-50% within 60 days) of 60Co γ-radiation and treated the animals with a single dose of GT3 24 h prior to irradiation. Specific subdomains in this circuit were isolated for transcriptome analysis, including the orbital frontal cortex; the Area 25 Medial Frontal Cortex; the Anterior Cingulate Cortex, (ACC); the Insular Cortex; the Amygdala; the Hippocampus; and the Entorhinal Cortex.
Results: We found that irradiation to NHPs caused significant transcriptome changes in all of the tested subdomains of the fronto-limbic circuit. However, GT3 best mitigated radiation-dependent changes in the Anterior Cingulate Cortex. Genes most affected were involved in oxidative phosphorylation and proinflammatory signaling. These data thus support the hypothesis that GT3 can mitigate radiation-dependent transcriptomic changes within the fronto-limbic circuit in NHPs.
Conclusions: We further suggest that this radioprotective drug effect might prove useful for treating the most troublesome side effects of ionizing radiation to the brain in humans.
Keywords: Radiation Injury Non-Human Primate, Vitamin E, Non-Human Primate.
Disclosure: Nothing to disclose.

M148. PGE1 Delivered by Liposomes Antagonized Dendritic Spine Loss and Reduction of VEGF and VEGF-R2 in Frontal Cortex and Hippocampus of Diabetic Rats

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Background: Diabetes (type 1 and 2) is a common metabolic disorder that can lead to functional and structural neurological complications such as cognitive impairment, dementia and reduced volume of hippocampus and frontal cortex. Diabetes related impairments in cognition and neural structure and function could be the consequence of a progressive damage at vascular level. Given that prostaglandins (cyclic oxygenated fatty acids) exert a potent positive action on vascular endothelium in many tissues, we used one of these molecules (PGE1) to prevent or ameliorate the negative effects of diabetes in vascular district in a rat model of diabetes (streptozocin treated rats). Since prostaglandins are rapidly metabolized by different enzimes we included PGE1 into liposomes made with phosphatidilcholine and Poly-L-lysine.
Methods: Liposomes (1μg/kg) were intraperitoneally administered (twice a week for three months) to streptozotocin (70 mg/Kg) treated rats. Healthy control rats and diabetics rats treated with saline have been used as control; all rats were sacrificed after three months. The glycemia was checked one time a week and 1 UI insulin retard administered once a week.
Results: In diabetes rats the dendritic spines density, VEGF (vascular endothelial growth factor) and VEGF-R2 (tyrosine kinase receptor of VEGF) expression levels were markedly reduced in the hippocampus and frontal cortex. As expected, the morphology and some molecular parameters of gastrocnemius muscle, lungs and kidneys showed a dramatic alteration effects almost completely reversed by PGE1 treatment.
Conclusions: The results suggest that PGE1 treatment has great efficacy in antagonizing the neurochemical and molecular consequences elicited by experimental diabetes both in brain and periphery. The reduced expression of dendritic spines densities, associated to the impairment of learning and memory present in this pathology, are consistent with the structural brain damage and functional decline present in diabetes patients.
Keywords: PGE1, Diabetes, VEGF-R2, Rodents.
Disclosure: Nothing to disclose.

M149. Rivastigmine Transdermal Patch Treatment for Moderate to Severe Cognitive Impairment in Veterans With Traumatic Brain Injury: A Double-Blind, Placebo-Controlled Multicenter Study (RiVET Trial)

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Background: Traumatic brain injury (TBI) represents one of the most significant health risks related to military duty. Even in peacetime, military personnel have higher rates of TBI than civilians with the risk of TBI increasing significantly during war-time. Since 2000, 348,000 service members were diagnosed with TBI. The most common injuries inflicted in the conflicts in Afghanistan and Iraq are caused by improvised explosive devices (IEDs), rocket propelled grenades, and land mines, with approximately 60% of blasts to which military personnel are exposed

resulting in brain injuries. Not surprisingly, TBI is often called the "signature wound" of the Iraq and Afghanistan wars. With the improvement in acute care provided to these patients, treatment of long-term sequelae of TBI is paramount. Despite significant variation in the severity of TBI, data consistently indicate deficits in cognitive functions such as memory, attention, speed of information processing, and executive functions among these individuals. Approximately 10-15% of people with mild TBI have persistent cognitive and behavioral complaints, and 65% of those with moderate to severe TBI experience long-term cognitive difficulties. It is well recognized that cognitive impairment is one of the most debilitating consequences for individuals who attempt to fully function in society.

Research findings both in animal models and humans suggest that cholinergic function is chronically deficient among subjects with TBI, and that cholinergic deficits may be a significant contributor to post-traumatic cognitive impairments—particularly memory impairments. As such, cholinergic deficits may be a useful target for the pharmacotherapy of TBI-related cognitive impairments. However, despite recommendations by VA-sponsored consensus groups advocating the use of cholinesterase inhibitors in the treatment of posttraumatic memory deficits in Veterans with TBI, no standard for pharmacological treatment of memory impairments among Veterans with TBI has been established.

Herein we will present the first multicenter clinical trial, conducted in five VA Medical Centers, evaluating the efficacy and safety of rivastigmine transdermal patch, an intermediate-acting cholinesterase inhibitor, in Veterans suffering from moderate to severe posttraumatic memory impairment following TBI.

Methods: This is the first phase II, randomized, multi-site, double-blind, placebo-controlled 12-week trial evaluating the effect on cognition and the safety of rivastigmine 9.5 mg/24 hours (10cm2) transdermal patch in Veterans with closed, non-penetrating TBI who present with moderate to severe memory impairment (NCT01670526). The study consisted of a one-week single-blind, placebo run-in phase, and a 12-week double-blind acute treatment phase (Phase I). During this phase, there was an initial 4-week titration period (5cm2 patch for 4 weeks increased to 10 cm2 patch thereafter) followed by an 8-week continuation phase. Following the 12-week acute treatment phase, participants continued in the double-blind phase (Phase II) for an additional 14 weeks or until study treatment period ended.

Participants were Veterans with a history of closed head trauma(s) (non-penetrating) at least 12 months prior to study enrollment, based on ICD 9 CM diagnosis code 854.0, and who met or exceeded modified ACRM criteria for mild TBI as determined by TBI diagnostic assessment. Participants were required to have a deficit in verbal memory, as assessed by the Total Recall index (Trials 1-3) of the Hopkins Verbal Learning Test, Revised (HVLT-R). Persistent cognitive deficit was defined as a Total Recall index (Trials 1-3) that is at least 20% lower than an intelligence-adjusted "expected score," based on the WAIS-IV Information and Vocabulary subtests. Participants who exhibited invalid data on cognitive testing utilizing TOMM and MSVT were excluded.

The primary outcome variable was defined as the proportion of responders who had at least 5-word improvement on HVLT-R Trials 1-3. Secondary outcomes included PASAT, COWAT, Digit Span and Letter-Number Sequencing subtests of WAIS-IV, Trail Making, BVMT-R, Q-LES-Q, SDS, NFI, PCL-M, BDI-II, University of California San Diego Performance-Based Skills Assessment (UPSA-B), CGI, CSSRS and treatment-emergent adverse events.

Results: Ninety-six participants were randomized. The last study participant visit occurred on May 25, 2016. Database lock occurred on July 6, 2016. Demographic characteristics of study sample include: mean age 40.7 years; 95.7% males; 68.1% Caucasian. Baseline mean HVLT-R Total Recall index was 20.0, corresponding to severe impairment (2nd percentile compared to HVLT-R normative sample) for the participants' mean age. Study codes have not been broken; pre-planned statistical analyses will be completed by September 26, 2016 and presented at the meeting.

Conclusions: This trial provides the largest dataset to date of Veterans with TBI and posttraumatic memory deficits enrolled in a pharmacological clinical trial. Its results will lead to a better understanding of the role that rivastigmine transdermal patch could play in the care of these patients

Keywords: Posttraumatic Memory Deficit, TBI, Cholinergic Function.

Disclosure: Neuroquest VP Clinical Affairs: Consultant, Spouse; BioPharma Connex: Consultant, Spouse; Arcadia: Consultant, Spouse; Transition Therapeutics Inc.: Consultant, Spouse; INSYS Therapeutics: Consultant, Spouse. Study Supported by the Clinical Science Research and Development Service and the Cooperative Studies Program, Department of Veterans Affairs Office of Research and Development.

M150. Safety and Tolerability of Valbenazine (NBI-98854) in Subjects with Tardive Dyskinesia: Results of Long-Term Exposure Data From Three Studies

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Background: Valbenazine (NBI-98854) is a novel and highly selective vesicular monoamine transporter 2 (VMAT2) inhibitor that is being investigated for the treatment of tardive dyskinesia (TD) in adults. It has been evaluated in several double-blind, placebo-controlled (DBPC) clinical trials with long-term extension phases and a 1 year open-label study. Based on data from these studies, long-term safety and tolerability are currently being analyzed in subjects who received up to 48 weeks of valbenazine treatment.

Methods: The pooled long-term exposure (LTE) population includes valbenazine-treated subjects from the following studies: KINECT (NCT01688037: 50 mg/day, 6-week DBPC period, 6-week open-label treatment period); KINECT 3 (NCT02274558: 80 or 40 mg/day, 6-week DBPC period, 42-week double-blind extension period); KINECT 4

(NCT02405091: 80 or 40 mg/day, 48-week open-label treatment). For the LTE analyses, the 50-mg dose group was pooled with the 40-mg groups. Outcomes evaluated in the pooled LTE population included: treatment-emergent adverse events (TEAEs), serious adverse events (AEs), discontinuations due to AEs, and AEs leading to dose reduction; vital signs and electrocardiograms (ECGs); laboratory evaluations; psychiatric status, as measured using the Positive and Negative Syndrome Scale (PANSS), Calgary Depression Scale for Schizophrenia (CDSS), Young Mania Rating Scale (YMRS), and Montgomery-Asberg Depression Rating Scale (MADRS); and extrapyramidal symptoms, as measured using the Barnes-Akathisia Rating Scale (BARS), and Simpson-Angus Scale (SAS). Interim results for these outcomes, analyzed using descriptive statistics, are presented.
Results: At the time of data cut-off for the current analysis, 427 subjects were included in the LTE population (KINECT, $n = 46$; KINECT 3, $n = 220$; KINECT 4, $n = 161$), all of whom had a psychiatric diagnosis of schizophrenia/schizoaffective disorder (71.7%) or mood disorder (28.3%). In the LTE population, 85.5% of subjects were taking an antipsychotic medication (atypical only, 69.8%; typical only or typical + atypical, 15.7%). The mean duration of valbenazine exposure was approximately 6 months. The most common TEAEs (80 and 40 mg, combined) were somnolence (4.7%), fatigue (3.7%), and dry mouth (2.3%). Serious AEs were reported in 12.6% of subjects (80 mg, 14.3%; 40 mg, 10.7%); discontinuations due to AEs occurred in 13.1% of subjects (80 mg, 11.7%; 40 mg, 14.7%); and AEs leading to dose reduction occurred in 6.3% of subjects (80 mg, 7.0%; 40 mg, 5.6%). Mean changes in vital signs and ECGs were not clinically significant. Moreover, no clinically significant increases in ECG parameters were observed, including the 81% of subjects who were taking medications with a known potential to prolong QTc. No notable mean changes in laboratory parameters, including liver function tests and metabolic parameters, were observed. Evaluation based on psychiatric scales indicated that subjects with schizophrenia/schizoaffective disorder (PANSS, CDSS) or mood disorder (YMRS, MADRS) did not have worsening of symptoms. No evidence of clinically significant drug-induced extrapyramidal symptoms (BARS, SAS) was observed.
Conclusions: Valbenazine appeared to be well tolerated in adults with TD who received up to 48 weeks of treatment. The types of TEAEs observed in the LTE population were consistent with TEAEs that were reported in the 6-week DBPC trials. Long-term valbenazine did not have any notable effects on cardiac or hepatic function, and psychiatric status remained stable. Additional ongoing analyses, to be presented at the meeting, will further clarify the safety and tolerability of valbenazine in adults with TD. This novel VMAT2 inhibitor shows promising potential for the long-term treatment of TD.
Keywords: Tardive Dyskinesia Clinical Trials, Safety, Tolerability, Movement Disorders.
Disclosure: Neurocrine Biosciences, Inc.: Consultant, Self; Synchroneuron: Consultant, Self; Medicure: Consultant, Self; Laboratorios Farmaceuticos Rovi: Consultant, Self; Novartis: Speakers Bureau, Self.

M151. Efficacy of Valbenazine (NBI-98854) in Subjects With Tardive Dyskinesia: Results of a Long-Term Extension Study (KINECT 3 Extension)

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Background: Tardive dyskinesia (TD) is a persistent movement disorder associated with chronic exposure to dopamine receptor blockers such as antipsychotics. Valbenazine (NBI--98854) is a novel and highly selective vesicular monoamine transporter 2 (VMAT2) inhibitor that is in development for the treatment of TD. The efficacy and safety of valbenazine have been evaluated in several randomized, double-blind, placebo-controlled (DBPC) Phase 2 studies. KINECT 3 (NCT02274558) was a Phase 3 study of once-daily valbenazine (80 or 40 mg) that included 6 weeks of DBPC treatment, followed by a 42 week valbenazine extension (VE) period and a 4-week follow-up. This study met the primary efficacy endpoint for the 6-week DBPC period, defined as a significant difference between valbenazine 80 mg and placebo at Week 6 for the change from baseline in Abnormal Involuntary Movements Scale dyskinesia total score (or "AIMS score"; sum of AIMS items 1-7): valbenazine 80 mg, -3.2; placebo, -0.1; $P < .0001$. Preliminary analyses of the AIMS score and other outcomes have been conducted for the 42 week VE period to evaluate the long-term effects of once-daily valbenazine 80 and 40 mg on TD.
Methods: KINECT 3 included adult subjects with moderate or severe drug-induced TD and diagnosis of schizophrenia/schizoaffective disorder or mood disorder. Subjects were required to be psychiatrically stable prior to study entry; concomitant medications to treat the psychiatric disorders were allowed. Subjects who completed the 6-week DBPC period were eligible to participate in the 42-week VE period. Those initially randomized to placebo were re-randomized 1:1 to valbenazine 80 or 40 mg/day; those initially randomized to valbenazine 80 or 40 mg/day continued at the same dose. Outcomes assessed in the intent-to-treat (ITT) population for the VE period included AIMS score change from baseline to Week 48 and Clinical Global Impression of Change-Tardive Dyskinesia (CGI-TD) score at Week 48. The CGI-TD includes scores ranging from 1 ("very much improved") to 7 ("very much worse"). AIMS scoring was based on the consensus of two blinded, central video AIMS raters. Preliminary results for the VE period, analyzed using descriptive statistics, are presented here. Additional and updated results will be presented at the meeting.
Results: A total of 198 subjects entered the VE period of KINECT 3 (80 mg, $n = 101$; 40 mg, $n = 97$). At the time of data cut-off for this current analysis, Week 48 AIMS data were available for 58 subjects (80 mg, $n = 28$; 40 mg, $n = 30$). Improvements in TD symptom severity were observed in this group of subjects, as indicated by mean AIMS score change from DBPC baseline at Week 48 in both valbenazine dose groups (80 mg, -4.9; 40 mg, -3.0). Mean AIMS scores at Week 48 (80 mg, 6.3; 40 mg, 6.9) and Week 52 (80 mg, 9.7; 40 mg, 8.8) indicated that TD symptoms worsened during

the 4-week period following discontinuation of valbenazine treatment. Mean CGI-TD scores at Week 48 were available for 78 subjects (80 mg, $n = 40$; 40 mg, $n = 38$) at the time of this analysis. CGI-TD scores in these subjects were consistent with clinically meaningful global improvements (80 mg, 2.0; 40 mg, 2.4).

Conclusions: The effectiveness of valbenazine in reducing TD symptoms appears to have been persistent among subjects who received long-term treatment with valbenazine 80 or 40 mg. In addition to the long-term safety profile (reported separately), these results indicate that long-term valbenazine therapy may be beneficial for reducing and managing TD symptoms. Additional ongoing analyses, to be presented at the meeting, are expected to further elucidate the effects of valbenazine in subjects with drug-induced TD who received up to 48 weeks of treatment.

Keywords: Movement Disorders, Presynaptic Uptake, Antipsychotic Treatment.

Disclosure: Neurocrine Biosciences, Inc., Full Time Employee, Self.

M152. Metallo-Beta-Lactamase Domain-Containing Protein 1 (Mblac1) is a Specific, High-Affinity Target for Ceftriaxone

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Background: Beta-lactam antibiotics are among the most widely prescribed antibacterial agents. In addition to their antibacterial properties, these agents (e.g. ceftriaxone (Cef)) have been shown to exhibit functional effects in animal models of Amyotrophic Later Sclerosis (ALS), stroke, ischemia, and addiction. The mechanisms proposed to support their CNS and behavioral actions involve a reestablishment of homeostatic glutamate signaling through an elevation of astrocytic glutamate transporter mRNA and protein expression. Thus, levels of GLT-1 (Slc1a2), the primary astrocytic glutamate transporter, and of the cystine/glutamate exchanger (Xc-), and their functional activities are increased following chronic in vitro or in vivo exposure to Cef. To date, however, a specific, high-affinity, CNS-expressed target for Cef has yet to be identified. Recently (Hardaway et al, 2015), we identified the C. elegans protein SWIP-10 as a metallo-beta-lactamase domain-containing protein that regulates glutamate-dependent, dopamine neuron excitability. The SWIP-10 ortholog, Mblac1, retains all residues involved in the hydrolytic function of metallo-beta lactamases, and is expressed in brain, as revealed by the immunoblotting of wildtype and recently developed Mblac1 KO mice. Here, utilizing extracts from transfected cells and mouse brain, we provide evidence of a specific, high-affinity Cef interaction with Mblac1. Our studies suggest the presence of a novel, Cef-sensitive metabolic pathway subserved by Mblac1. The demonstration of Cef:Mblac1 interactions establish a path to the rational design of novel agents that may modulate extracellular glutamate homeostasis for therapeutic ends.

Methods: Two complementary approaches were established to assess the potential binding of Cef to Mblac1. First, we generated Cef-conjugated Sepharose beads to perform affinity chromatography of extracts from an inducible cell line expressing Mblac1, immunoblotting flow-through and bead eluate to determine Mblac1 binding. Specificity and high-affinity Cef binding to Mblac1 was inspected through pre-incubations of cell lysates with free Cef 20 minutes prior to bead interactions. Second, we implemented Back-Scattering Interferometry (BSI) to demonstrate direct, dose-dependent binding between Mblac1 and Cef in cell extracts, documenting specificity through parallel BSI studies of extracts from uninduced cells, with inactive antibiotics, and with induced extracts subjected to heat denaturation. Finally, we assessed the role of native Mblac1 in Cef binding with BSI studies on mouse brain extracts, evaluating specificity via immunodepletion.

Results: Mblac1 was efficiently extracted from tetracycline-induced cell lysates via Cef-conjugated CNBr-Sepharose beads. Little or no Mblac1 binding was obtained with beads lacking Cef conjugation. Cef interactions were significantly attenuated through preincubations with free Cef (10μM). In BSI studies, we obtained evidence for specific Cef binding to Mblac1, with high-affinity ($KD = 2.2\mu M$) binding interactions that were absent in heat-denatured extracts or extracts from uninduced cells. Additionally, BSI studies revealed a lack of Mblac1 interaction with the beta-lactam antibiotic, cephalosporin C, a compound previously shown to lack the ability to induce glutamate transporter activity in cultured brain slices. Importantly, we did not detect binding activity for Cef in brain extracts where Mblac1 had been removed from preparations via immunodepletion using Mblac1 antibodies.

Conclusions: Our studies provide evidence for the specific, high-affinity binding of Cef, a neuroprotective and behaviorally active beta-lactam antibiotic, to the metallo-beta-lactamase domain containing protein, Mblac1. Whereas studies assessing the endogenous function of Mblac1 are ongoing, the role of the C. elegans Mblac1 ortholog, SWIP-10, has been found to function in glial pathways that confer glutamate-dependent modulation of dopamine neurons. This activity is consistent with a functional attribution of Mblac1 as engaged in mammalian brain glutamate homeostasis. Importantly, our binding studies were performed with Mblac1 derived from both transfected cells and native preparations. With our recent development of Mblac1 KO mice, we are positioned to determine how Mblac1 acts in vivo, and to determine the mechanisms supporting the neuroprotective and behavioral actions of chronic Cef treatment. Moreover, these studies establish a path to the development of novel, Mblac1-targeted small molecules that may be useful for brain disorders linked to disrupted CNS glutamate signaling.

Keywords: Ceftriaxone, Glutamate Homeostasis, Drug-protein Interactions.

Disclosure: Nothing to disclose.

M153. p38α Mapk Inhibitors as Potential Treatments for Autism Spectrum Disorder: Insights From the SERT Ala56 Genetic Mouse Model

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Background: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder presenting with deficits in language and communication, social interactions, and repetitive, restricted behaviors. Currently, there are no FDA approved pharmacotherapies to treat these core symptoms. Although the molecular mechanisms responsible for the etiology of the disorder are unknown, elevated blood serotonin (5-HT) levels are found in a significant number (25-30%) of ASD patients. Prior studies have revealed five rare, ASD-associated coding variants in the 5-HT transporter (SERT, SLC6A4) that lead to elevated SERT function (Sutcliffe et al. 2005). In vivo expression of SERT Ala56 in a knock-in mouse model results in multiple ASD-like phenotypes, including hyperserotonemia, repetitive behaviors and deficits in social interactions and communication (Veenstra-VanderWeele et al, 2012). Notably, SERT Ala56 mice display a p38 MAPK-dependent hyperphosphorylation of SERT within the CNS, consistent with our prior findings that activation of p38α MAPK elevates SERT function in vitro and in vivo (Zhu et al. 2006, Zhu et al. 2010). These findings suggest that elevated 5-HT clearance in SERT Ala56 mice and resulting ASD-like phenotypes are due to unopposed p38α MAPK phosphorylation of the transporter. We hypothesized that pharmacologic inhibition of p38α MAPK might attenuate or reverse one or more phenotypes in the SERT Ala56 model. Here we report our efforts to normalize 5-HT clearance and reverse ASD-like features of adult SERT Ala56 mice with novel, selective and CNS penetrant p38α MAPK inhibitors.
Methods: All experiments utilizing murine models were conducted under a protocol approved by the Institutional Animal Care and Use Committee at both Vanderbilt University and Florida Atlantic University. To assess whether MW108 or MW150 attenuates p38 MAPK-mediated stimulation of SERT activity, we monitored [3H] 5-HT uptake in neuroectodermal SK-N-MC cells that stably express WT human SERT with or without treatment with the p38 MAPK activator anisomycin (0.1 nM for 15 min). To explore the utility of p38α MAPK inhibition in vivo, we acutely pretreated (30 min) adult (8-16 wks) male, SERT Ala56 mice or WT littermates with MW150 (5 mg/kg, i.p.,) or vehicle, followed by tests of 5-HT receptor sensitivity and social interactions. Cohorts were also treated chronically with MW108 (5-10 mg/kg, i.p., QD, 1 week) or MW150 (5-10 mg/kg, i.p., QD, 1 week) prior to undergoing biochemical and behavioral assessment. We assessed 5-HT2A receptor sensitivity using 1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane (DOI, 1.0 mg/kg, i.p.)-induced head twitch assays and quantified 5-HT1A receptor sensitivity using 8-hydroxy-2-(di-n-propylamino)-tetraline (8-OH-DPAT, 0.1 mg/kg, s.c.)-induced hypothermia assays. To monitor social behavior, we examined pair-wise interactions between animals in the Tube Test. In vivo chronoamperometry was utilized to ascertain whether MW150 treatment normalizes 5-HT clearance in SERT Ala56 mice. p38α MAPKloxP/loxP:ePet1:Cre:SERT Ala56 mice generated through classical breeding strategies of previously reported mouse lines, were subjected to the Tube Test.
Results: Acute treatment (5 min pretreatment, 1 and 10 nM) with MW108 or MW150 blocked the ability of anisomycin to enhance SERT activity in transfected SK-N-MC cells, consistent with a role for p38α MAPK in SERT stimulation. Acute MW150 treatment failed to elicit a reversal of 5-HT2A receptor hypersensitivity or social deficits in SERT Ala56 mice. Chronic treatment with MW108 treatment eliminated the 5-HT2A receptor hypersensitivity of SERT Ala56 animals and attenuated, albeit non-significantly, 5-HT1A receptor hypersensitivity. Similarly, chronic MW150 treatment was found to attenuate SERT Ala56-mediated increases in 5-HT1A and 5-HT2A receptor sensitivity. Chronic treatment with both MW108 or MW150 resulted in the normalization of social behavior in SERT Ala56 mice. Further, chronic treatment with MW150 resulted in the normalization of hippocampal 5-HT clearance in SERT Ala56 mice. Specific genetic elimination of p38α MAPK in 5-HT neurons of SERT Ala56 mice was found to normalize social behavior in the Tube Test.
Conclusions: These are the first studies, to our knowledge, that document pharmacologic reversal of ASD-like phenotypes through the inhibition of p38α MAPK signaling. Further, our studies support a unique requirement of p38α MAPK expressed by CNS 5-HT neurons in regulating social behavior in SERT Ala56 mice. The ability to reverse receptor and behavioral phenotypes through treatments given to adult animals argues that ongoing p38α MAPK signaling, rather than irreversible consequences of altered 5-HT signaling during development, are important for multiple deficits induced by the SERT Ala56 model. We speculate that insights gained from our studies may apply to other means of elevating SERT function, as with immune signaling-induced p38α MAPK activation (Zhu et al, 2010). Although our findings derive from a preclinical model, the reversal of ASD-like traits in our preclinical model encourage a continued evaluation of p38α MAPK inhibitors for their potential as pharmacotherapies in the treatment of ASD.
Keywords: Autism Spectrum Disorder, Serotonin, p38 MAPK, Serotonin Transporter.
Disclosure: These studies were supported by the following NIH grants: MH078028, MH094527, MH094604, NS007491, U01AG043415, the Simons Foundation and the PhRMA Foundation.

M154. Less Dyskinesia at Motor-Equivalent Doses of Triple-Deuterated L-Dopa vs. L-Dopa After Chronic Administration in Rats

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Background: Treatment with L-DOPA may, possibly via toxic metabolites, contribute to motor and non-motor complications, particularly dyskinesia, in Parkinson´s disease

(PD). Replacement of hydrogen by deuterium in the α-,β-,β-positions has been shown to reduce the metabolic breakdown rate of dopamine (DA) via monoamine oxidase (MAO) and DA-β-hydroxylase, thus avoiding potential adverse excess of norepinephrine and/or toxic 3,4-dihydroxyphenylacetaldehyde (DOPAL) levels. The triple deuterated L-DOPA (D3-L-DOPA) exhibited similar peripheral plasma concentration kinetics in rats as L-DOPA but significantly elevated and sustained striatal DA concentrations, a decreased DOPAC/DA ratio and a reduced norepinephrine response compared to L-DOPA.

Here we compared dyskinesia at motor equivalent doses of D3-L-DOPA vs. L-DOPA in rats after 3-weeks administration. All animals were pretreated with a peripheral dopadecarboxylase inhibitor as in our above mentioned previous experiments.

Methods: A: To assess the motor equivalent doses, L-DOPA-induced rotation was used to establish a dose-response curve for the acute effects of L-DOPA vs. D3-L-DOPA. The total number of contralateral rotations during 3 hours following administration of incremental doses of L-DOPA and D3-L-DOPA (3-8 mg/kg) was measured (0-180 minutes post-injection). L-DOPA (3.2, 6.4 and 8 mg/kg) and D3-L-DOPA (3.2, 4.8 and 6.4 mg/kg) were administered s.c. ($n = 5$-6/group).

B: To assess dyskinesia during chronic treatment the abnormal involuntary movement scale (AIMS) test was performed [3]. The maximal score for each of the three AIMS subtypes (axial, orofacial, lingual) was 4. The rats were scored every 20 minutes for 180 minutes after drug administration (9 observations/animal, 6 sessions in 3 weeks). After unilateral striatal 6-OH-dopamine lesions doses of 8 mg/kg/d L-DOPA, 8 mg/kg/d D3-L-DOPA and 5 mg/kg/d D3-L-DOPA (the motor equivalent dose in the dose finding part A) were administered for 3 weeks. The cylinder test was used to quantify the motor effect by change of left paw contacts (% of total) in the cylinder.

Results: A: The dose-response curve for D3-L-DOPA was shifted to the left, demonstrating an increased potency. The equipotent dose of D3-L-DOPA vs. L-DOPA was graphically determined at 50% of the rotational response (EC50) induced by the highest doses administered and found to correspond to ≈ 60% of a given L-DOPA dose.

B: Effects of D3-L-DOPA and L-DOPA on development/expression of AIMs. There was significantly less ($p < 0.05$) dyskinesia, assessed by the median cumulative AIMS scores for the entire treatment period, after D3-L-DOPA 5 mg/kg vs. L-DOPA 8 mg/kg (the motor equivalent dose). In the same animals the change of left paw contacts (% of total) was determined. The motor improvement after 8 mg/kg L-DOPA tended to be less than after 5 mg/kg D3-L-DOPA and significantly less than after 8 mg/kg D3-L-DOPA, confirming the previously determined motor equivalent dose ratio of 60% D3-L-DOPA vs. 100% L-DOPA.

Conclusions: The major conclusion of this study is that the triple deuterium substitutions in the L-DOPA molecule allows a significant dose reduction without loss of motor efficacy and reduced dyskinesia, indicating a wider therapeutic window for D3-L-DOPA vs. L-DOPA. Consequently, D3-L-DOPA may be the first drug to appear superior to L-DOPA for the treatment of PD.

Keywords: Parkinson's Disease, Triple-Deuterated L-DOPA, L-DOPA, Dyskinesia.

Disclosure: Nothing to disclose.

M155. BDNF-Trkb Signaling in Oxytocin Neurons Contributes to Sex-Specific Social Behaviors

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Background: Brain-derived neurotrophic factor (BDNF) is an activity-dependent neurotrophin that is strongly regulated by social experience. BDNF and its receptor TrkB are expressed in the paraventricular hypothalamus (PVH), the site of neuroendocrine cells secreting oxytocin (OXT), a neuropeptide critical for the regulation of social behaviors. Deficits in BDNF and OXT gene expression have been extensively linked to adverse social experiences, especially during early development. Transcription of Bdnf is controlled by several promoters, which drive expression of multiple transcripts encoding an identical protein. BDNF derived from promoters I and II is highly expressed in the hypothalamus and is critical for the regulation of aggression in male mice. While BDNF governs sex-typical social behavior in males, it is unknown how BDNF influences sex-specific female social behavior, particularly reproduction and maternal care.

Methods: We generated mice in which production of BDNF from promoters I and II was specifically disrupted (Bdnf-e1 and Bdnf-e2). To examine abnormal social behaviors in females, we tested postpartum Bdnf-e1 and –e2 dams for impairments in maternal care using a pup retrieval paradigm. Bdnf-e1 virgin females were tested for mating abnormalities and pup-directed aggression. We used quantitative PCR to analyze Oxt and Oxtr gene expression in the hypothalamus. To assess alterations in OXT neuron function following loss of promoter I-derived BDNF, we examined the distribution and activation of OXT neurons in the PVH of Bdnf-e1 females. Finally, we genetically ablated TrkB in hypothalamic neurons producing OXT to directly test how disruption of BDNF signaling in these neuroendocrine cells impacts maternal behavior.

Results: Disruption of BDNF produced from promoters I and II resulted in abnormal reproductive and parenting behaviors in female mice. In a pup retrieval paradigm, postpartum BDNF-e1 and Bdnf-e2 females displayed deficits in pup carrying and nesting. Bdnf-e1 virgin females showed heightened pup-directed aggression and significant mating impairments. Deficits in social behaviors were accompanied by a 50% decrease in Oxt, but not Oxtr, gene expression. Disruption of BDNF signaling in OXT neurons partially phenocopied deficits in maternal care observed in Bdnf-e1 and –e2 females.

Conclusions: BDNF-TrkB signaling influences OXT neuron function to regulate sex-specific social behaviors in female mice. These studies highlight BDNF as a key molecular player in modulating sexually-dimorphic hypothalamic circuits that govern complex social interactions.

Keywords: BDNF, Oxytocin, Social Behavior, Maternal Behavior, Hypothalamus.
Disclosure: Nothing to disclose.

M156. Withdrawn

M157. CPP-115 Target Engagement: Inhibition of Cortical GABA Transaminase Detected by Magnetic Resonance Spectroscopy Studies in Healthy Human Brain

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Background: The modulation of gamma-amino butyric acid (GABA) levels in human brain is a major focus for developing pharmacotherapies designed to treat a range of psychiatric illnesses, substance abuse disorders, and neurologic diseases. GABA-transaminase (GABA-T) is the enzyme primarily responsible for GABA catabolism, and its irreversible inhibition by suicide inhibitors leads to elevated intracellular cerebral GABA concentrations. Vigabatrin is a commonly used GABA-T inhibitor used to treat seizure disorders including epilepsy, complex partial seizures, and infantile spasms. Clinical proton (1H) magnetic resonance spectroscopy (MRS) studies have demonstrated vigabatrin-induced GABA concentration increases of ~100% higher than baseline values in epilepsy patients (Mattson, et al, Epilepsia, 1994), although a more moderate GABA increase of ~60% was reported in medication-free healthy controls (Weber, et al, 1999). Unfortunately, high doses of vigabatrin (3-4g/day) are required to achieve therapeutic effects, and chronic vigabatrin dosing in humans is associated with significant adverse effects, including visual field defects and central nervous system disturbances. Hence, vigabatrin typically is administered as an adjuvant medication in seizure disorders, and as monotherapy in patients where the potential benefits outweigh associated risks. Compared to vigabatrin, clinical trials for CPP-115, a novel GABA-T inhibitor, show that significantly lower drug dosages (80 mg/day) afford comparable pharmacokinetic data, improved tolerability and a more favorable toxicity profile. The pharmacodynamic effects of CPP-115 in human brain have yet to be evaluated, and the present study was designed to employ non-invasive 1H MRS procedures to measure cortical GABA changes in healthy adults receiving oral daily doses of CPP-115 or placebo.
Methods: Subjects/Study Design: The University of Utah IRB approved the present study, which met criteria for investigations in human subjects. A cohort of six healthy adult male subjects (mean age ± SD = 34.2 ± 16.8 years; age range = 19 – 57 years) was enrolled into a double-blind, randomized, placebo-controlled study. Subjects received either a single oral daily 80 mg dose of CPP-115 ($n = 4$) or placebo ($n = 2$) for 6, 10, or 14 continuous days, with both placebo and CPP-115 administered as taste-matched, artificially sweetened beverages. Subjects underwent a baseline 1H MRS scan at day-1 with study drug or placebo initiated at day1. 1H MRS scans subsequently were performed at day7 and day13, with scans occurring 2.5 hours post-drug administration at approximately the same time-of-day as day-1 measures. The fourth and final 1H MRS measurement (follow-up) occurred within the day20 to day23 window, allowing for a minimum 7-day washout period from the final dose day.
1H MRS Measures: All MRI/MRS measurements were performed using a 2.89 T Siemens (Erlangen, Germany) Verio MRI scanner, employing a 12-channel phased-array head coil for RF signal reception. Following imaging and static magnetic field (B0) shimming procedures, 1H MRS data were acquired from two separate single voxels measuring 25 x 25 x 30 mm3, positioned bilaterally within the parietal-occipital cortex (POC) and supplementary motor area (SMA) cortical regions. A metabolite-edited 1H MRS sequence (MEGAPRESS) was used was used for determining POC and SMA GABA concentrations, whereas a two-dimensional (2D) J-resolved 1H MRS sequence was used to measure other POC and SMA metabolites including N-acetyl aspartate (NAA), total creatine (tCr), choline compounds (Cho), glutamate (Glu), glutamine (Gln), and myo-inositol (Ins).
Results: For all subjects receiving CPP-115 ($N = 4$), day7 POC and SMA GABA concentrations were 57 – 104 % and 52 – 133 % higher than baseline levels, respectively. The corresponding day13 POC and SMA GABA levels were 57 – 113 % and 70 – 108 % higher than baseline values, respectively. The POC and SMA GABA levels returned to within 2 - 21 % of baseline levels following CPP-115 clearance (follow-up measures). Subjects receiving placebo ($N = 2$) showed highly stable GABA levels across all four MRS scanning time points, with mean coefficients of variation (SD ÷ mean) of 8.2 % and 5.0 % calculated for the POC and SMA regions, respectively. Concentration changes were not detected for other 1H MRS-visible metabolites including NAA, tCr, Cho, Glu, Gln, and Ins.
Conclusions: These data demonstrate the utility of 1H MRS for monitoring CPP-115 inhibition of GABA-T and, at significantly lower doses (~fiftyfold), CPP-115 mediated GABA concentration increases are up to 121% greater than the changes reported in previous vigabatrin 1H MRS studies of healthy brain (Weber, et al, 1999). The return to baseline GABA levels following drug clearance indicates reversible and stable GABA-T resynthesis. Changes in brain metabolite concentrations were limited to GABA, suggesting that CPP-115 has negligible effect on neuronal function (stable NAA), membrane synthesis/turnover (stable Cho), brain energetics (stable tCr), and glutamatergic metabolism and/or neurotransmission (stable Glu and Gln). Future 1H MRS studies should aim to characterize the acute GABA changes associated with CPP-115 administration, and dose-ranging 1H MRS studies will be essential for optimizing drug safety and tolerability. Similar MRS studies ultimately will be critical for correlating CPP-115 induced GABA changes with clinical response in patient populations.
Keywords: Cortical GABA Transaminase, CPP-115, Proton Magnetic Resonance Spectroscopy.
Disclosure: Nothing to disclose.

M158. Effects of Propranolol on Verbal Problem Solving and Conversational Reciprocity in Autism Spectrum Disorder: A Double-Blind, Single-Dose Psychopharmacological Challenge Study

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Background: Autism spectrum disorder (ASD) is characterized by impairments in social communication and restricted, repetitive behaviors. However, all currently approved pharmacotherapeutic agents target behavioral disturbances, not these core features. Identification of a pharmacotherapeutic agent targeting core features would represent a significant advance. Some evidence has suggested a dysregulated noradrenergic system in ASD. Furthermore, the beta-adrenergic antagonist propranolol has widely been used off-label for performance anxiety and text anxiety, and comorbid anxiety is common in ASD. As a result, we wished to explore the effects of propranolol on core aspects of ASD. Our initial pilot trials had suggested benefits on language tasks. Therefore, we examined in a larger population of ASD patients the effects of propranolol on verbal tasks, while also examining effects on structured social interaction, in a single-dose psychopharmacological challenge study, while exploring the impact of baseline anxiety and autonomic function on treatment response.
Methods: Twenty individuals with ASD (age 21.4 ± 4.6 std dev, 19 males), diagnosis confirmed with the Autism Diagnostic Interview-Revised, with a full scale IQ of at least 85 (110.8 ± 14.6 std dev) participated. Subjects participated in two testing sessions in a within-subject crossover design. The Spence Children's Anxiety Scale (SCAS) was administered at baseline, and psychophysiological measurements of autonomic activity were recorded. For the psychophysiological measurements, electrocardiography was recorded for 5 minutes after 3 minutes of acclimation with a BIOPAC MP150 acquisition unit (BIOPAC Systems, Inc., Goleta, CA), R-R intervals were extracted, and heart rate variability was calculated (pNN50 and RMSSD). At the first session, subjects were randomized to receive a single dose of 40 mg propranolol or placebo, in a double-blinded manner. Sixty minutes after the dose, subjects completed a series of behavioral tasks. At the second visit, at least 24 hours later, the procedure was repeated with the drug not administered at the first visit. As with our previous work, one of two versions of an anagram task was administered after drug administration, with 20 sets of letters that subjects were asked to unscramble to form a word, with a maximum allowed time of 120 seconds per task, and solution latencies were recorded. For the conversational reciprocity task, the General Social Outcome Measure-Conversational Reciprocity (GSOM-CR) was utilized, where several social skills were assessed during a semi-structured conversation between the examiner and the subject on one of two topics, including monitoring performance on staying on topic, sharing information, reciprocity, transitions/interruptions, nonverbal communication, and eye contact, each on a scale of 0-2 (for total possible score of 12).
Results: For the anagrams, there was no difference between drug conditions in number solved, but ANCOVA with drug order included as a covariate revealed significantly better performance for solution latency for solved anagrams after administration of propranolol as compared to placebo ($F(1,16) = 7.35$, $p = 0.02$, $\eta p2 = 0.32$). Early discontinuation resulted in missing data for the anagram task in two participants. All participants completed the GSOM-CR. Performance was significantly better for the total GSOM-CR score with propranolol as compared to the placebo condition ($t(19) = 2.36$, $p = 0.03$, $d = 0.40$). Drug order had no effect on the GSOM-CR, and was not included as a covariate. Among the anxiety assessments, only the baseline separation anxiety subscale score on the SCAS was positively related with response to propranolol for mean solution latency ($F(2,15) = 4.37$, $p = 0.03$, $R2 = 0.37$). A negative relationship was also observed between baseline RMSSD ($F(2,15) = 3.96$, $p = 0.04$, $R2 = 0.35$) as well as pNN50 ($F(2,15) = 4.04$, $p = 0.04$, $R2 = 0.35$) and response to propranolol for mean solution latency. No such relationships were observed for the GSOM-CR.
Conclusions: The results of this single dose, double-blinded psychopharmacological challenge study begin to suggest promise for propranolol in ASD. These findings are in need of support in the serial dose clinical trial setting, along with assessment of impact on more clinically salient outcomes. Assessment in younger patients and individuals with varying functional levels is also needed, along with an expanded set of biomarkers to understand the impact and predict response in these individuals. It is also unknown as to whether these effects would be greater or would diminish in the serial dose setting. However, the potential for the availability of an inexpensive therapeutic agent with a well-established safety profile that affects the core features of ASD warrants further exploration. Further efforts at understanding who is most likely to respond will also be critical. For this purpose, we have recently obtained funding from the Department of Defense to perform a clinical trial on this agent in an expanded population of ASD patients.
Keywords: Autism, Noradrenergic, Propanolol, Social Communication.
Disclosure: Nothing to disclose.

M159. Self-Directedness is Associated With Global Resting-State Connectivity in Healthy Adults

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Background: Personality is typically described as a dynamic set of characteristics possessed by a person that uniquely influence cognitive processes, motivation, and behavior. Evidence suggests that variation in specific personality traits contribute to an array of psychiatric illnesses and may reflect variability in brain circuitry. The present study sought to examine whether dimensions of personality were individually or collectively associated with functional connectivity of the brain at rest.
Methods: A sample of 125 healthy adults (55M/70F; Mean age = 36.45 ±13.59) completed the Temperament and Character Inventory (TCI) and underwent resting state

functional magnetic resonance imaging (rs-fMRI). In accordance with our prior work we calculated a measure of global connectivity (GC), defined as the first component of a principal component analysis of 266 nodes distributed throughout the brain. Next, linear regression was used to examine whether the 7 dimensions of the TCI either individually or collectively could be used to predict GC.
Results: Linear regression revealed that self-directedness was the only TCI dimension that was significantly predictive of GC ($b = .41$, $t(124) = 2.66$, $p = .009$). Specifically, those with low levels of self-directedness demonstrated the lowest levels of GC. This effect remained significant even after controlling for a range of demographic and cognitive variables.
Conclusions: The present results suggest that low self-directedness, a personality trait that has been shown to confer risk for a range of psychiatric disorders, may be associated with variations in the connectivity patterns of the brain. Additional work examining the specific patterns of connectivity across the 266 nodes in currently underway.
Keywords: fMRI Resting State, Temperament, Self-Directedness.
Disclosure: Nothing to disclose.

M160. Monoamine Oxidase-A Genetic Variants and Childhood Abuse Predict Impulsiveness in Borderline Personality Disorder

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Background: Impulsivity is a core feature of borderline personality disorder (BPD) and antisocial personality disorder (ASPD) that likely arises from combined genetic and environmental influences. The interaction of the low activity variant of the monoamine oxidase-A (MAO-A) gene and a history of childhood abuse has been shown to predict aggression and global antisocial behavior in clinical and non-clinical populations. Although impulsivity is a risk factor for aggression in BPD and ASPD, little research has investigated potential gene-environment ($G \times E$) influences impacting impulsivity in these conditions.
Methods: Full factorial analysis of variance was employed to investigate the influence of MAO-A genotype, childhood abuse, and diagnostic group on Barratt Impulsiveness Scale-11 (BIS-11) subscale scores in 61 individuals: 20 subjects with BPD, 18 subjects with ASPD, and 23 healthy controls. Genomic DNA was extracted from peripheral leukocytes with MAO-A genetic polymorphisms determined using standard PCR procedures.
Results: A group × genotype × abuse interaction was present ($F2, 49 = 4.4$, $p = 0.018$), such that the low expression MAO-A allele in combination with a history of childhood abuse predicted greater BIS-11 motor impulsiveness in BPD. In all models of BIS-11 impulsivity subtypes, ASPD and BPD subjects reported greater impulsiveness than healthy controls. Additionally, BPD subjects reported higher BIS-11 attentional impulsiveness versus ASPD participants ($t(1,36) = 2.3$, $p = 0.025$).
Conclusions: These preliminary results suggest that MAO-A genetic variants in combination with a history of early adverse experiences may increase risk of trait impulsiveness in BPD. Results additionally indicate that impulsiveness may be expressed differently in BPD and ASPD.
Keywords: Monoamine Oxidase-A, Genetics, Borderline Personality Disorder, Antisocial Personality Disorder.
Disclosure: Nothing to disclose.

M161. Anthranilic Acid – A Potential Biomarker and Treatment Target for Schizophrenia

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Background: Dysregulation of Trp - Kyn pathway is a recent hypothesis of mechanisms of schizophrenia. In particular, over-production of kynurenic acid (KYNA), one of the three immediate downstream metabolites of kynurenine (Kyn) along tryptophan (Trp) – Kyn pathway, has been considered as a new target for therapeutic intervention in schizophrenia. Up-regulation of KYNA formation was suggested to occur at the expense of down-regulation of 3-hydroxyKyn (3-HK), the other immediate downstream metabolite of Kyn. We were interested to assess the fate of the third immediate downstream Kyn metabolite, anthranilic acid (AA).
Methods: Serum AA concentrations were evaluated by HPLC-mass spectrometry method in patients with schizophrenia and control subjects. Study was approved by Tufts Medical Center IRB.
Results: We found 5-fold increase of AA and 3-fold decrease of 3-HK concentrations in serum of schizophrenia patients.
Conclusions: Impact of AA elevation in schizophrenia might be mediated by mitochondrial enzymes downregulation described in schizophrenia, and upregulation (found in anterior cingulate brains of schizophrenia) of formation of 3-hydroxy AA, a potent generator of free radicals and glutamatergic agonists. Present data warrant further studies of AA as biological marker of, at least, a subgroup of schizophrenia patients and as a potential new target for therapeutic intervention.
Keywords: Anthranilic Acid, Schizophrenia, 3-hydroxy-kynurenine.
Disclosure: Nothing to disclose.

M162. Microstructural White Matter Abnormalities in Youth With Psychosis Spectrum Symptoms: A Longitudinal Perspective

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Background: Psychosis is a complex brain disorder that typically develops in adolescence or early adulthood {Casey, 2014;Insel, 2010;Rapoport, 2012} and has enormous impact on both public health costs and functional disability {Zeidler, 2012;Wu, 2005}. Early intervention requires valid and

reliable methods of identifying youths at high risk for developing psychosis. Youths with sub-theshold psychotic symptoms often exhibit subtle neurobiological abnormalities similar to those found in schizophrenia {Pettersson-Yeo, 2013; Barbato, 2012; Kelleher, 2012}. Hence, many contemporary investigations focus on individuals at high risk for developing psychosis, or at-risk mental states, in both help-seeking {Cannon, 2008}, and community-based samples {Calkins, 2015; Calkins, 2014}. Many of the deficits in cortical microcircuitry in psychosis are found to some extent in at-risk youths including disrupted neurotransmission {Egerton, 2014; Allen, 2015}, lower gray matter volume {Satterthwaite, 2016; Chan, 2011; Nenadic, 2015; Bois, 2015}, and disrupted white matter microstructure {Karlsgodt, 2009; Katagiri, 2015; Bloemen, 2010; Carletti, 2012}.

Diffusion tensor imaging (DTI) has facilitated in vivo study of brain white matter microstructural organization. DTI maps and characterizes the diffusion of water molecules through brain tissue, a process that is affected by microstructural properties of the local surrounding tissue {Basser, 1994;Le Bihan, 2001}. Given recent convergent evidence that psychosis is likely a product of abnormal neurodevelopment, it is likely that WM microstructure is aberrant in at-risk youths. Indeed, at-risk individuals tend to show disrupted WM integrity as compared to healthy individuals (for review see {Samartzis, 2014}). Yet, other studies find elevated FA in at-risk youths {Schmidt, 2015} or no difference between at-risk and healthy individuals {Thomas, 2008;Peters, 2010}. These inconsistencies likely stem from relatively small samples of individuals at risk, heterogeneity of disease course and DTI methodology.

Methods: We use a community-based approach to measure white matter integrity in 377 non-help-seeking youth with psychosis spectrum symptoms (PS) and 374 healthy youth (HC). To evaluate clinical course and potential predictors of the progression of early psychosis spectrum symptoms, we re-evaluated a subsample of PS and HC individuals approximately two years later. Thus, clinical symptoms and diffusion tensor imaging were measured at two time points for a subsample of 50 PS and 120 HC. Initial DTI data were collected as part of the Philadelphia Neurodevelopmental Cohort using 64 diffusion-weighted directions. Acquisition and processing descriptions are published{Roalf, 2016; Satterthwaite, 2014}. Statistical analyses were performed using non-linear models and accounted for sex, race, data quality, and maternal education.

Results: PS individuals ($n = 377$) showed lower whole brain FA compared to healthy youth ($n = 374$; $p < .01$, FDR corrected) at baseline. PS individuals ($n = 50$) with persistent or worsening clinical features over two years showed lower whole brain FA, MD and RD at baseline (ps < 1.0x10-3 FDR corrected) compared to healthy youth. At follow-up, individuals with persistent symptoms again showed lower whole brain FA and MD (ps < 1.0x10-4 FDR corrected), however region-specific declines in FA emerged within the corticospinal tracts (CST), cingulum bundle (CGH) and the inferior frontal occipital fasciculus (IFO). Association with clinical features are also examined.

Conclusions: Our preliminary work indicates that youth with persisting early psychosis spectrum symptoms exhibit subtle, but significant, abnormalities in brain white matter organization that persist or increase over time. Given the clinical evidence implicating abnormal neurodevelopment in the pathogenesis of schizophrenia, and the potential utility of brain structure and function to predict illness vulnerability, DTI holds promise for understanding neurodevelopmental contributions to schizophrenia pathophysiology. These abnormalities may reflect aberrant brain development that predisposes an individual to the illness, and quantification of these features will likely enhance our ability to detect those truly at risk for developing psychosis.

Keywords: Clinical High Risk, Psychosis, Diffusion Tensor Imaging, Longitudinal.

Disclosure: Nothing to disclose.

M163. One-Year Symptom Trajectories in Patients With Stable Schizophrenia Maintained on Antipsychotics vs. Placebo: A Meta-Analysis

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Background: Antipsychotic treatment plays a critical role in alleviating the acute symptoms of schizophrenia, while maintenance treatment is routinely recommended to prevent relapse. However, definitions of relapse differ substantially between investigations, which in turn compromises validity when directly comparing multiple studies. In the context of data aggregation, total scores (i.e., a continuous variable) on clinical rating scales such as the Positive and Negative Syndrome Scale (PANSS) and Brief Psychiatric Rating Scale (BPRS) provide a more generalizable outcome than relapse, a dichotomous variable that can vary one definition to the next. To date, none have compared symptom trajectories between antipsychotic and placebo treatments in the maintenance phase. Thus, we conducted a systematic review and meta-analysis of randomized controlled trials (RCTs) comparing symptom trajectories between antipsychotic and placebo treatments in stable patients with schizophrenia.

Methods: MEDLINE, Embase, and Cochrane Central Register of Controlled Trials (CENTRAL) were systematically searched up to January 2016. RCTs consisting of antipsychotic and placebo treatment arms in patients with stable schizophrenia and reporting PANSS or BPRS total scores at more than one-time point were included. PANSS or BPRS total scores were collected for each study for up to 52 weeks from randomization, weekly for the first 4 weeks and at 4-week intervals thereafter. Meta-regression analyses were employed using a mixed model to compare symptom trajectories between the two treatment groups. The primary and secondary outcomes were standardized total scores and percent score changes, respectively.

Results: A total of 11 studies involving 2,826 patients ($n = 1{,}618$ for antipsychotic treatment; $n = 1{,}208$ for placebo treatment) were included in the meta-analysis. All studies were parallel-group RCTs conducted in a double-blind fashion with study duration ranging from 24 weeks to 64 weeks. All except for one study examined second-generation antipsychotics. Meta-regression analyses revealed significant interactions between group and time (Ps < 0.0001); both standardized total scores and percent

score changes remained almost unchanged in patients continuing antipsychotic treatment, whereas symptoms continuously worsened over time in those switching to placebo treatment.
Conclusions: The present meta-analysis shows that patients with stable schizophrenia who continue antipsychotic treatment demonstrate ongoing symptom stabilization over a one-year interval, in contrast to a trajectory of continuous symptom exacerbation observed in patients receiving placebo. This highlights the clinical benefits of antipsychotic maintenance treatment in schizophrenia, as well as the necessity of immediate and ongoing monitoring for clinical worsening in the face of antipsychotic discontinuation.
Keywords: Antipsychotics, Maintenance Treatment, Placebo, Schizophrenia, Symptom Trajectory.
Disclosure: Canadian Institutes of Health Research (CIHR): Fellowship grant, Self; Centre for Addiction and Mental Health (CAMH) Foundation: Fellowship grant, Self; Japanese Society of Clinical Neuropsychopharmacology: Fellowship grant, Self; Astellas Foundation for Research on Metabolic Disorders: Fellowship grant, Self; Dainippon Sumitomo Pharma; Manuscript fee, Self.

M164. Global Impact of Activation of VTA Dopamine Neurons as Measured by Opto-fMRI

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Background: VTA dopamine neurons are critically involved in many aspects of cognition and motivated behavior. Dysfunction of VTA dopamine activity has also been implicated in various psychiatric disorders such as addictive disorders, schizophrenia, ADHD, and mood and anxiety disorders. As a result, human functional magnetic resonance imaging (fMRI) studies on these disorders have heavily focused on blood oxygen level dependent (BOLD) activity in VTA innervated limbic regions. However, whether VTA dopamine neuron activity and subsequent dopamine release directly contribute to these BOLD signals in downstream areas is not clear. Hence, the current study aimed to determine the causal relationship between phasic activation of VTA dopamine neurons and global fMRI responses in the forebrain.
Methods: We used optogenetics combined with fMRI (opto-fMRI) to transiently activate VTA dopamine neurons and simultaneously measure fMRI activity. We used two types of fMRI contrasts: BOLD and cerebral blood volume-weighted (CBVw). We used BOLD contrast because of its relevance to human BOLD fMRI studies. Because BOLD signals can be influenced by non-specific contributions from large blood vessels, we also used CBVw contrast that reduces sensitivity to large blood vessels and enhances spatial specificity to neural activity.
Th::Cre rats (that express Cre recombinase under the control of the tyrosine hydroxylase promoter) and wild-type rats were unilaterally injected in the VTA with recombinant adeno-associated viral vector constructs containing the gene encoding channelrhodopsin under the Cre promoter (AAV5-Ef1α-DIO-ChR2-eYFP), and optical fibers were implanted dorsal to VTA. After > 6 weeks from viral infusion surgeries, fMRI experiments were conducted in lightly anesthetized rats (0.7 – 1.0 % isoflurane). BOLD and CBVw functional images were acquired while VTA was optically stimulated transiently at 20 Hz for 20 s (2.5 – 7.5 mW).
Results: We found that that optical stimulation of VTA dopamine neurons in Th::Cre rats increased BOLD signals in the ventral striatum that receives considerable innervation from VTA dopamine neurons. However, regions that receive sparse or no VTA innervation such as the dorsal striatum and the globus pallidus were also activated. In fact, the predominant activation in the forebrain was observed in the dorsal striatum. Similar to BOLD activity, CBVw fMRI activation in the forebrain was strongest in the dorsal striatum. Weaker increases in CBVw fMRI activity were observed in limbic and non-limbic regions including the ventral striatum, ventral pallidum, orbitofrontal cortex, amygdala as well as global pallidus, thalamus, and hippocampus. There was no significant change in BOLD or CBVw activity throughout the brain of wild-type rats upon optical VTA stimulation.
Conclusions: The current study establishes causation between VTA dopamine activity and fMRI responses in the forebrain. The most striking observation of our study was the robust BOLD and CBVw signal increase in the dorsal striatum compared to other regions. This finding provides evidence for a functional interaction between phasic activity of VTA dopamine neurons and basal ganglia systems that receive sparse or no projections from the VTA. This mode of interaction might provide a novel circuit-level framework to reassess existing theoretical models about striatal-dependent normal functions and disorders such as schizophrenia, addictive disorders, and mood disorders.
Keywords: Optogenetics, Basal Ganglia, Schizophrenia, Addiction.
Disclosure: Nothing to disclose.

M165. Risk-Based Error Prevention Using Virgil Flags in Panss Administration and Scoring

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Background: Sponsors of clinical trials seeking ways to improve oversight efficiency and ensure the quality of study data are adopting technology-based solutions such as centralized risk-based monitoring (RBM). Another aspect of data quality is improving accuracy in the scoring of rating scales, a goal which also lends itself to a risk-based technology solution capable of identifying scoring errors in efficacy measures before the data are submitted. The Positive and Negative Syndrome Scale (PANSS) is a complex scale prone to administration and scoring errors that can contribute to poor interrater reliability and inaccurate clinical trial results. The Virgil Investigative Study Platform, an electronic clinical outcome assessment (eCOA) platform that collects electronic source (eSource) data, is designed to standardize administration of diagnostic and outcome

measurements and improve data quality by providing raters with clinical guidance in real time, including "flags" to alert the rater to scoring inconsistencies while administering the PANSS. In the present study, we examined the extent to which occurrences of these flags are associated with error rates in PANSS scoring in previous schizophrenia trials.
Methods: 768 paper-based assessments aggregated from eight randomized, double blind, placebo-controlled schizophrenia trials were reviewed by the same central cohort of blinded clinicians who identified scoring errors by reviewing audio recordings and worksheets from each assessment. The scoring errors were then compared against the Virgil flags to determine how often a flag would have been triggered in assessments that had two or more errors. 18 Virgil flags, developed to identify the potential risk of PANSS scoring errors, range from within-visit scoring inconsistencies (e.g., a difference of more than two points between related items) to between-visit alerts (e.g., same response on all items from previous visit). Each flag was examined to determine its association with scoring errors.
Results: 565 assessments (74%) had two or more scoring errors, indicating that scrutiny of PANSS ratings scale administration is essential. The flags that occurred with higher frequency in these problematic ratings included: depressed or low mood rated two or higher without verbal confirmation from the subject (71%); inconsistencies between distrustfulness and active social avoidance (33%); inconsistencies between lack of insight and delusions (17%); and between-visit total score change of greater than 25% (12%). Assessments with zero or one error triggered significantly fewer flags compared to those with two or more errors.
Conclusions: Risk-based analysis is useful in identifying potential scoring errors and technology can assist in preventing problematic ratings by directing the rater's focus to the most critical data elements that need attention. The Virgil eCOA platform with real-time clinical guidance, scoring anchors, and item descriptors minimizes scoring inconsistencies to reduce error variance and improve signal detection.
Keywords: Risk-based Monitoring, eCOA, PANSS Inconsistencies, Schizophrenia, Endpoint Reliability.
Disclosure: Columbia University College of Physicians and Surgeons: Affiliation, Self; MedAvante, Inc.: Affiliation, Self.

M166. A Phase 1 Open Label Safety and Tolerability Study of SEP-363856, a Novel NON-D2 Mechanism of Action Molecule, in Patients With Schizophrenia

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Background: SEP-363856 is a novel non-D2 mechanism of action compound, which shows broad efficacy in animal models of schizophrenia. The molecular target(s) responsible for the profile of effects is unknown, but may include agonism at 5 HT1A and TAAR1 (trace amine associated receptor 1) receptors. SEP-363856 exhibits clear, functional central pharmacodynamic signals both in rat, nonhuman primate and man. Clinical experience to date demonstrates an excellent pharmacokinetic (PK) profile (dose-proportional, ~20-hour half- life), robust rapid eye movement (REM) sleep suppression, and a safety profile supporting further clinical development. The primary objectives of the current study were to gain longer-term safety and tolerability data for open-label SEP-363856 in patients with schizophrenia.
Methods: A Phase 1, 2-part, randomized, single blind, placebo controlled, ascending multiple oral dose and open-label study assessed the safety, tolerability, and PK of SEP 363856 in adult subjects with schizophrenia. In the open-label study, adult patients ($N = 16$) diagnosed with schizophrenia were admitted to the clinic and completed a washout of their prior antipsychotic medications. After successful washout, subjects were dosed with SEP-363856 (75 mg/day) for 28 days. Patients remained in the clinic for the first 2 weeks of dosing and outpatient for the remaining 2 weeks of dosing. Safety assessments included incidence of adverse events, clinical laboratory measures, and movement disorder scales (BARS, AIMS and M-SAS). The effect of SEP-363856 on the positive and negative syndrome (PANSS) scale and clinical global impression-severity (CGI-S) was also assessed.
Results: A total of 14 subjects completed the 28-day open label study. Two patients discontinued the study after 2 weeks due to multiple mild/moderate AE's. No exacerbations of schizophrenia symptoms were observed in any subject. There were no clinically significant findings related to laboratory results, ECGs, neurological examinations, or movement disorder scales. In addition, treatment with SEP-363856 demonstrated improvement in efficacy measures (PANSS total score, CGI-S) compared with baseline. Furthermore, ad hoc subgroup analyses showed a significantly greater decrease from baseline in PANSS total scores at the end of the 28-day treatment period in subjects who had less frequent hospitalizations per year of illness compared with subjects who had more frequent hospitalizations per year of illness. The most commonly reported AEs included somnolence, dizziness, nausea and headache which were mild to moderate in intensity.
Conclusions: Results from this study demonstrate an acceptable safety and tolerability profile of SEP-363856 (75 mg/day) up to 28 days in patients with schizophrenia. Convergent translational evidence across rodent, nonhuman primate and man supports potential of this novel mechanism of action molecule as a treatment for schizophrenia. Phase 1 studies in normal healthy volunteers and in patients with schizophrenia continue to validate the safe and well behaved profile of SEP-363856 and support further investigation of clinical utility as a novel non-D2 mechanism of action antipsychotic.
Keywords: Novel, Mood Disorder, Schizophrenia.
Disclosure: Sunovion (Koblan): Employer, Self.

M167. Placental Gene Expression and the Interaction Between Obstetrical History and Genetic Risk for Schizophrenia

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Background: Early life events influence later susceptibility to many adult diseases and may contribute to define the environmental context in which genes enhance risk for complex disorder like schizophrenia. Here we analyze the role of intrauterine and perinatal environment and placental gene expression in modulating the association of schizophrenia with genomic risk.
Methods: We evaluated the relationship between genomic risk, intrauterine and perinatal complications (Early Life Complications, ELCs) and schizophrenia in samples from USA, Europe and Eastern Asia (total $N = 946$). Genomic risk was measured with risk profile scores (RPS) based on GWAS-significant alleles, while ELCs exposure was assessed with the McNeil-Sjöström Scale. We tested whether genes overlapping the RPS loci interacting with ELCs are enriched in placenta and differentially expressed in placental samples from complicated pregnancies, in 8 independent placental datasets. Finally, we evaluated whether GWAS SNPs marking loci containing genes highly expressed and dynamically modulated in placenta (PlacRPS genes) drive the interaction between RPS and ELCs, and performed pathway analyses on PlacRPS genes.
Results: In the USA sample, genetic risk score is associated with schizophrenia only in the context of exposure to ELCs ($p = 6.62e\text{-}09$); similar results are found in the Italian sample ($p = 0.0005$), and the relationship between RPS and ELCs is further replicated in the sample of Japanese patients ($p < 0.05$). The gene-set based on RPS loci interacting with ELCs is highly expressed in multiple placental tissues ($p < 0.001$) and dynamically regulated in placental samples from complicated, in comparison with normal, pregnancies ($p < 0.05$). These differences are significantly greater in placentae from male compared with female offspring ($p < 10\text{-}8$). The interaction between RPS and ELCs is largely driven by PlacRPS genes ($p = 0.002$); RPS constructed from the remaining loci do not interact with ELCs (NonPlacRPS, $p = 0.60$). Pathways and biological functions associated with NonPlacRPS genes are reminiscent of previous analyses about schizophrenia risk-genes, while PlacRPS genes implicate an orthogonal biology, with roots in the fetal/placental response to hypoxic stress.
Conclusions: Our data suggest that the most significant schizophrenia GWAS variants contribute to risk at least partly by converging on a developmental trajectory sensitive to ELCs and altered placental gene expression. The sex-associated effects on placental transcription suggest that the male preponderance of schizophrenia may arise from gene-environment interactions that influence placental biology. These results highlight placental health as a new public health frontier for primary prevention, particularly in high-risk males.
Keywords: Schizophrenia, Obstetric Complications, Polygenetic Risk Score, Placenta, Gene–Environment Interaction.
Disclosure: Nothing to disclose.

M168. Neuroleptic-Induced Striatal Blood Flow Changes and Neurocognitive Functioning in Schizophrenia

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Background: Decline and lasting impairment of cognitive functioning is a core and disabling feature of schizophrenia. Modest and variable cognitive gains with antipsychotic treatment have been reported for both typical and atypical agents, and how this may be linked to medication-induced neurophysiological alterations remains unclear, preventing a mechanistic understanding of this therapeutic effect. In light of prior work suggesting that neuroleptic-induced basal ganglia blood flow increases may be an important marker of antipsychotic response, we sought to determine whether cognitive changes with treatment correspond to concurrent, independently measured striatal regional cerebral blood flow (rCBF) differences.
Methods: Fifteen patients (mean age 30.4+/-10.7, six female) with schizophrenia or schizoaffective disorder were included. During a blinded, balanced two-arm medication withdrawal protocol, each participant underwent both cognitive function testing and positron emission tomography (PET) scanning in separate sessions. During both arms, cognition was measured with the Wechsler Abbreviated Scale of Intelligence, and rCBF was measured with [15O]water PET, which was performed after at least three weeks of standard, stable atypical antipsychotic monotherapy or placebo. For each [15O]water session, two 60-second-long resting-state emission scans, separated by 6 minutes, were obtained for each subject and were corrected for attenuation and background activity, registered, scaled to correct for variations in injected dose and tracer delivery, and averaged together. Three regions of interest (ROIs; bilateral caudate, putamen and ventral striatum) were delineated on a T1 weighted MRI image collected separately for each individual and coregistered to the PET data to generate mean ROI rCBF values for each collected scan. Association analyses were performed using SPSS.
Results: As previously reported, striatal rCBF was significantly greater with active neuroleptic medication than placebo in all regions ($p < 0.05$). However, there was not an overall significant cognitive advantage in the estimated full-scale scores on active treatment. An inverse association between changes in PET and cognitive measurements was identified such that only individuals with less antipsychotic-induced increase in caudate rCBF showed numerical gains on neurocognitive measurements ($p = 0.01$). Post-hoc testing identified the verbal and not performance subscale as the

underlying factor ($p = 0.001$). This relationship was unchanged by controlling for order effects, age, sex, total symptom rating or chlorpromazine equivalent dose.
Conclusions: Variability in cognitive responses to antipsychotic medication withdrawal may be related to concomitant, independently measured neural activity changes in the caudate, a structure closely linked to neurocognitive functioning. These data suggest that striatal blood flow may be a behaviorally meaningful phenotype in schizophrenia treatment, and merit larger studies to further identify potential striatal mechanisms underlying neurocognitive performance changes during antipsychotic treatment and withdrawal.
Keywords: Cognition, PET, Antipsychotic.
Disclosure: Nothing to disclose.

M169. Connectome-Wide Association of Resting fMRI With Dopamine Functioning in Patients With Schizophrenia and Healthy Adults

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Background: Dopamine (DA) has widespread actions throughout the brain, including modulatory effects on executive functioning, motor control, and reward circuitry. The DA system is implicated in a number of human pathologies, including psychosis, addiction, Parkinson's disease, and ADHD. In psychotic illness in particular, DA plays a central role in the pathophysiology, and DA receptor blockade remains the current mainstay of antipsychotic treatments. Despite the widespread effects of DA and its importance in psychotic disorders, few studies have examined its relationship to intrinsic functional connectivity. Here, we took two approaches to characterize this relationship. First, in healthy adults, using resting state fMRI and [18F]-fluorodopa (FDOPA) positron emission tomography (PET), a method for directly measuring regional presynaptic DA synthesis and storage capacity in vivo, we performed a connectome-wide association analysis (CWAS) to identify brain regions where functional connectivity was associated with FDOPA uptake (Ki). Second, in patients with psychosis, we performed the same CWAS analysis to evaluate the effects of DA blockade with neuroleptic medications compared to placebo treatment.
Methods: In the first of two independent studies, we sought to examine the relationship between presynaptic DA tone and intrinsic brain connectivity. Seventy-six healthy adults (mean age 39.6 ± 13.4 years, 36 males) were scanned with both 3T resting fMRI (TR/TE = 2000/24ms, 184 images, 12 minutes) and FDOPA PET (16 mCi by IV bolus, 27 dynamic scans over 90 minutes). fMRI preprocessing steps included motion correction, normalization to MNI space, censoring of corrupted volumes, anatomic Component Correction, and bandpass filtering ($0.008 < f < 0.1$ Hz). For PET scanning, FDOPA tracer uptake (Ki) was measured using the Patlak-Gjedde method and a cerebellar reference region. A CWAS analysis was then performed to determine voxels where resting state connectivity was associated with FDOPA Ki values, measured from a midbrain region of interest. Post-hoc analyses were then carried out using the significant regions from the CWAS results as seeds in a connectivity analysis to determine brain regions contributing to the findings.
In the second study, 14 inpatients with a history of psychosis (mean age 27.8 ± 8.5 years, 11 males) completed a double-blind, crossover study of active atypical antipsychotic medication vs. placebo, four weeks of each in a counterbalanced design. In each arm of the study, patients completed the same resting fMRI scanning detailed above. Preprocessing steps for these fMRI data were identical to the first analysis and a CWAS analysis was performed to evaluate the within-subject effect of antipsychotic treatment (i.e. treatment vs. placebo); here changes between DA receptor blockade and placebo were taken to be an indicator of the relationship between DA functioning and intrinsic brain connectivity, at least in the setting of psychotic illness. As in the first study, post-hoc analyses were also carried out using significant regions from this active vs. placebo CWAS result as seeds in a functional connectivity analysis to determine which brain regions contributed to the findings.
All results were corrected for multiple comparisons ($p < 0.05$) based on 10,000 Monte Carlo simulations and a $p < 0.005$ uncorrected voxel-wise threshold.
Results: In the group of healthy adults, the CWAS analysis to examine the effects of presynaptic DA tone revealed that midbrain FDOPA Ki was significantly associated with resting state connectivity of the bilateral caudate extending into the ventral striatum. A post-hoc seed-based connectivity analysis using this striatal region as an ROI showed striatal connectivity with motor and dorsal attention networks to be significantly associated with midbrain FDOPA Ki, such that increased functional connectivity between the striatum and these regions decreased with increasing Ki.
In the group of patients with schizophrenia, a CWAS analysis examining the pharmacologic effects of antipsychotic medication revealed that the connectivity pattern of a region in the right caudate extending into the ventral striatum that was similar to the region identified in the first FDOPA-resting fMRI study was significantly related to the within-subject difference between antipsychotic treatment and placebo. Similar to the results found with FDOPA, a post-hoc seed-based analysis of this region also showed that striatal connectivity with regions in both motor and dorsal attention networks were significantly associated with antipsychotic treatment, such that connectivity while on neuroleptics was significantly reduced compared with placebo.
Conclusions: Here, we show that the whole-brain intrinsic functional connectivity pattern of the striatum is both associated with midbrain DA tone in healthy adults and altered by antipsychotic treatment in patients with psychosis. The striatal region identified in both studies is known from both preclinical and clinical studies to be a central hub of DA innervation. Additionally, these results highlight the roles of both DA and the striatum in motor and attentional networks, as well as the effects of DA receptor blockade on these systems. Further characterization of this relationship, in both health and disease will not only help to characterize

the role of DA in the human brain but can also serve as biomarkers for diagnosis and treatment.
Keywords: Schizophrenia, Dopamine, Antipsychotics, PET Imaging, Resting State Functional Connectivity, Ventral Striatum, Caudate.
Disclosure: Nothing to disclose.

M170. Complex Biomarker Identification From Structural Magnetic Resonance Images in Schizophrenia Using Independent Component Analysis

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Background: Clinical and cognitive domain based subtyping in schizophrenia (Sz) has been critiqued due to the lack of neurobiological correlates. Structural imaging measures show reliable differences between cases and controls, with some findings of relationships with symptoms. However, relationship between patterns of gray matter differences and symptom severity measures have not yet been examined. We present a data driven framework using source based morphometry (SBM) to detect clinical subgroups from the gray matter concentration (GMC) of patients with schizophrenia only (Sz).
Methods: We used the structural imaging data from three legacy multisite datasets that included Sz. Each dataset including diagnosis, age at time of scan, gender, illness duration, positive and negative symptom scale (PANSS) rating scores and current medications were shared by each participating group according to the sites protocol when available. The majority of Sz were on anti-psychotic medications, either typical, atypical or a combination. All Sz were clinically stable at the time of scanning. A total of 382 Sz (mean age = 36.4, SD = 11.65, range: 18-64, 274 males/108 females) from three independent studies formed the aggregated dataset, which totaled to 10 scanning sites. The aggregated multisite dataset were regressed for age, gender and site voxelwise.
The developed methodology consists of the following steps: SBM decomposition, selection of two components namely the insula/superior temporal gyrus and the superior/middle/medial frontal gyrus which showed case/control difference and had the maximum effect size from our previous SBM study, simultaneous sorting of loadings by absolute value for these components. Loadings greater than quartiles (Q1 and Q3) for both components were found. Subject IDs corresponding to sorted loadings from both components were then intersected to obtain subgroup loaded on both the components. Exclusive subgroups loaded on each component were also found. Reconstruction of components for each subgroup was then done using Group information guided ICA (GIG-ICA).
Results: We observed two clinical subtypes of subjects, one heavily weighted on each of these two components (57 subjects on insula component and 55 subjects on superior temporal gyrus). We observed a third, overlapping clinical subtype weighing heavily on both of these components (43 subjects). These subsets of subjects were characterized by significant differences in PANSS positive clinical symptoms ($p = 0.005$). The PANSS general clinical symptom of the overlapping subgroup was significantly correlated with the loading coefficients of the superior/middle/medial frontal gyrus regions ($r = 0.35$; $p = 0.02$). The covarying patterns GMC in the two dichotomous clinical subtypes showed complex and varying directionality patterns on two different components. The reconstructed subgroup-specific component maps showed subtle variations by group. The subgroups (S1 and S3) component maps showed additional regions of precentral gyrus, anterior cingulate and medial frontal gyrus in the first component compared to the overlapping group, while for the second component the overlapping group showed additional regions of cingulate gyrus, middle temporal gyrus and inferior frontal gyrus.
Conclusions: These subtypes were defined based on paired values of structural measures, and correlated with differences in the PANSS positive symptom scores. The varying GMC directionalities support the idea that schizophrenia is a heterogeneous disorder having complex relations with different brain structures, warranting personalized treatment and targeted drug development.
Keywords: Schizophrenia, Subtypes, Neuroimaging.
Disclosure: Nothing to disclose.

M171. Sex Similarities and Differences in the Mam Model of Schizophrenia in Mice

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Background: Schizophrenia is a very complex, psychiatric disease with still an unknown neurobiological etiology and ineffective therapeutic interventions. A leading theory for its etiopathology is the neurodevelopmental hypothesis, which supports the notion that genetic and/or toxic events during gestation disturb the normal development of the nervous system. Based on this hypothesis, a well-characterized animal model in rats is generated by the administration of the DNA methylating mitotoxin, methylazoxymethanol acetate (MAM), in pregnant rats on gestation day (GD) 17, referred as the MAM model. The offspring of injected rat dams show core behavioral, histological and neurochemical phenotypes of schizophrenia. Our goal in this study is to establish the MAM model in mice, and study sex differences in their 'schizotypic-like' characteristics and cognitive effects.
Methods: Pregnant mice were injected with MAM (26mg/kg) or saline on GD 16 (MAM-E16) or 17 (MAM-E17) male and female offspring (MAM- or saline-treated) were examined during adulthood (> 90-days old). Initially, experiments were performed in both MAM-E16 and MAM-E17-mice. However, we observed that MAM-E16-treated mice had a stronger phenotype, therefore, most experiments used MAM-E16 treated mice. Both male and female mice were tested in the following experiments to establish schizotypic-like characteristics: a) locomotor activity in response to systemic administration of the NMDA

receptor antagonist, dizocilpine (MK-801) (0.2mg/kg), b) pre-pulse inhibition (PPI) of the acoustic startle reflex, c) analysis of gross anatomical features of the prefrontal cortex (PFC) and the hippocampus (HPC) using the Nissl staining technique, and d) parvalbumin (PV) protein expression, using fluorescent immunohistochemistry. Moreover, we assessed cognitive function with the contextual fear-conditioning paradigm and long-term potentiation (LTP) in the CA3-CA1 synapses of HPC following tetanic stimulation, by recording field excitatory postsynaptic potentials (fEPSPs). Additionally, trait anxiety levels were measured using the elevated plus maze (EPM) task. All experiments were approved by the Institutional Animal Care and Use Committee of the University of Crete and were conducted in accordance with the European Union ethical standards. Statistical significance was ascertained by two-way or repeated ANOVA.

Results: Female MAM-treated mice showed enhanced hyperlocomotion and stereotypic behavior compared to saline-treated mice, after acute administration of MK-801, ($n = 8$ in each group, two-way ANOVA, $p = 0.02$ for MAM-E16 and $p = 0.05$ for MAM-E17), significant reduction of the horizontal and vertical length of HPC, a trend towards thinning of PFC ($n = 5$ in each group, $p = 0.02$), reduced PV expression in the PFC ($p = 0.03$) but not in dorsal HPC ($p = 0.07$).On the other hand, male MAM-E16-treated mice, compared to saline-treated mice, showed decreased PPI ($n = 12$ in each group, $p = 0.03$), statistically significant thinning of the PFC along with decreased number of neurons ($n = 6$ in each group, $p = 0.02$), a trend towards reduced HPC volume ($n = 6$ in each group, $p = 0.07$), as well as decreased PV expression in the PFC ($n = 5$ in each group, $p = 0.01$).

Upon examining the cognitive function, MAM-E16 female mice displayed a trend for impaired contextual fear memory formation ($p = 0.06$), while male MAM-E16 mice significantly showed reduced freezing 24hrs after contextual fear training. The above results suggest a possible deficit in the underlying neurophysiological mechanisms of HPC. Indeed, the fEPSPs recorded in the CA3-CA1 synapses of HPC could not remain potentiated for more than 30min following theta-burst stimulation, revealing a trend for decreased expression of LTP, for both male and female mice ($n = 7$, $p = 0.01$). Female mice, only, also showed increased freezing behavior, after the delivery of the electric shock ($p = 0.008$), indicating altered processing of fear stimuli. In parallel, female mice only exhibited high levels of trait anxiety, during adulthood, as the EPM task revealed (Open/Closed Arms entries, $p = 0.03$).

Conclusions: Our results demonstrate that both male and female mice, prenatally treated with MAM on E16, display several core schizophrenia-like deficits, suggesting that the MAM model could be used as a neurodevelopmental model of schizophrenia in mice. We further reveal cognitive deficits in both male and female mice treated with MAM, but only female mice exhibit comorbidity with increased trait anxiety and altered fear memory processing.

Keywords: Hippocampal Volume, Medial Prefrontal Cortex, Long-Term Potentiation, Schizophrenia-Like Behavior, Anxiety.

Disclosure: Nothing to disclose.

M172. Disease Severity and the Modulatory Effect of Tolcapone on Cognitive Information Processing in Patients With Schizophrenia

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Background: Abnormal prefrontal cortical function is considered one of the most prominent research findings in schizophrenia, and studies have been focused in understanding the mechanisms involved in the regulation of prefrontal physiology. Some features of these prefrontal abnormalities are also detected in unaffected siblings, thus indicating a possible familiar mechanism in schizophrenia. Of potential susceptibility genes considered as risk factors, a functional polymorphism in the catechol-O-methyltransferase (COMT) gene (Val108/158 Met) has been a widely held contender because of the postulated role of dopamine deficit in the pathophysiology of schizophrenia. Since COMT plays a major role in regulating DA in cortical areas, reducing COMT activity in the prefrontal cortex may represent a major tool to improve cortical function. Tolcapone, a centrally acting COMT inhibitor that readily crosses the blood brain barrier (BBB), has been shown to improve cognition and cortical information processing in healthy volunteers as well as in mouse models. We also found that the use of Tolcapone in patients with schizophrenia, in which frontal-cortical function is impaired, may represent a valuable therapeutic tool to improve cortical efficiency. In the current is study, we investigated whether the COMT inhibitor Tolcapone or placebo administered three times a day for one week would be effective in patients with schizophrenia. We postulated that patients with more evident negative symptoms may have a more efficient response to Tolcapone treatment.

Methods: Thirty-one Caucasian patients with schizophrenia enrolled to participate in this double-blinded, placebo controlled, cross-over study and underwent BOLD fMRI while performing the 2-Back working memory task. Tolcapone 100 mg (or Placebo) was administered three times a day on the first day of the trial, and Tolcapone 200mg (or Placebo) was administered three times a day for the next 6 days. The two arms (Placebo/Tolcapone) were separated by a one-week wash-out period. On the seventh day, subjects underwent bold fMRI three hours after the last dose of the compound. A contrast map between Placebo and Tolcapone working-memory BOLD response was generated with a first-level analysis for each subject. We then performed a one sample t-test of this difference map across all subjects in the 2-Back study to obtain a group activation cluster ($p < 0.05$ uncorrected) in left and right Dorsolateral Prefrontal Cortex (DLPFC). The mean BOLD activation of all voxels in left and right DLPFC clusters was then extracted for each patient's difference (Placebo-Tolcapone) contrast map and a correlation analysis was done.

Results: The statistical analysis indicated a significant correlation between the Tolcapone-induced difference in the mean bold activation right DLPFC activation (Placebo-

Tolcapone) and antipsychotic dose ($r = 0.36$; $p = 0.0491$) only in the right DLPFC. The effect of Tolcapone seems to be more evident in patients which present more prominent negative symptoms (placebo > tolcapone right DLPFC vs. Negative Symptoms, $r = 0.45$, $p = 0.0116$). The effect of Tolcapone on negative symptoms showed a significant reduction in symptoms with the drug.

Conclusions: These results suggest that Tolcapone is effective in modulating prefrontal cortical information processing in patients with schizophrenia. These data support the use of COMT inhibitors as potential tools to improve cognitive efficiency in schizophrenia and suggests a particular role of this class of compounds for the treatment of negative symptoms.

Keywords: Functional MRI (fMRI), Schizophrenia, COMT Inhibitor.

Disclosure: Nothing to disclose.

M173. Advancing the Clinical Development of ITI-007 for the Treatment of Schizophrenia – Safety and Tolerability Data

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Background: Existing treatments for schizophrenia are often limited by side effects, such as movement disorders, metabolic disturbances and cardiovascular events. These and other side effects frequently result in non-adherence, lead to relapse and result in re-hospitalization. Efforts to find effective, yet tolerable medications for the treatment of schizophrenia continue.

ITI-007 is a first-in-class investigational agent in clinical development for the treatment of schizophrenia. ITI-007 60 mg has been shown to be effective in reducing symptoms of schizophrenia in two late-stage clinical trials at time of abstract submission. Further in both studies, ITI-007 was well-tolerated with favorable metabolic, motoric and cardiovascular profiles similar to placebo. A third trial has recently been completed and results will be presented.

As a selective and simultaneous modulator of serotonergic, dopaminergic and glutamatergic systems, ITI-007 represents a new pharmacological approach to the treatment of schizophrenia and other neuropsychiatric disorders. ITI-007 is a potent 5-HT2A antagonist, a mesolimbic/mesocortical dopamine phosphoprotein modulator (DPPM) with activity as a pre-synaptic partial agonist and post-synaptic antagonist at dopamine D2 receptors, a mesolimbic glutamate GluN2B receptor phosphoprotein modulator downstream from dopamine D1 receptor activation, and a serotonin reuptake inhibitor.

Methods: An ongoing late-stage schizophrenia development program for ITI-007, dosed once daily in the morning, includes three randomized, double-blind, placebo-controlled clinical trials in patients with acute schizophrenia: ITI-007-005, ITI-007-301, and ITI-007-302.

In the Phase 2 trial ITI-007-005, 335 patients were randomized to receive one of four oral treatments once daily for 4 weeks: 60 mg ITI-007, 120 mg ITI-007, 4 mg risperidone (positive control) or placebo in a 1:1:1:1 ratio. In the first Phase 3 trial ITI-007-301, 450 patients were randomized to receive one of three oral treatments once daily for 4 weeks: 60 mg ITI-007, 40 mg ITI-007, or placebo in a 1:1:1 ratio. In the second Phase 3 trial ITI-007-302, 696 patients were randomized to receive one of four oral treatments once daily for 6 weeks: 60 mg ITI-007, 20 mg ITI-007, 4 mg risperidone (positive control) or placebo in a 1:1:1:1 ratio. The primary outcome measure for each study was the Positive and Negative Syndrome Scale (PANSS) total score. Safety measures included observed and reported adverse events, 12-lead electrocardiograms (ECGs), 3-positional vital sign assessments, clinical laboratory assessments (hematology, serum chemistry and urinalysis), Barnes Akathisia Rating Scale (BARS), Simpson-Angus Rating Scale (SAS), Abnormal Involuntary Movement Scale (AIMS), and Columbia - Suicide Severity Rating Scale (C-SSRS).

Results: In both the Phase 2 trial ITI-007-005 and the first Phase 3 trial ITI-007-301, ITI-007 60 mg met the primary endpoint and demonstrated efficacy with statistically significant superiority over placebo at Day 28 as measured by the PANSS total score. The second Phase 3 trial ITI-007-302 was recently completed and available data will be presented. In both completed studies, ITI-007 demonstrated a motoric, metabolic, and cardiovascular profile similar to placebo. There were no clinically significant differences from placebo in rates of akathisia or extrapyramidal symptoms. ITI-007 did not significantly increase body weight or blood levels of prolactin, glucose, insulin, or lipids. The only treatment-emergent adverse events, considered at least possibly related to ITI-007 which occurred at rates greater than 5% and at least twice the rate of placebo, and observed in both studies were predominantly mild sedation and somnolence. Furthermore, high treatment completion rates were observed for patients randomized to ITI-007 in these studies, and rates of discontinuation due to adverse events were low and similar to placebo. In both studies, key measures of cardiovascular function (including heart rate, QTc intervals and other ECG parameters) were similar between ITI-007 and placebo. The second Phase 3 trial, ITI-007-302, is ongoing and available data will be presented at this conference.

Conclusions: ITI-007 administered orally once daily in the morning with no dose titration required was well-tolerated with a favorable safety profile. Positron emission tomography data from patients with schizophrenia (ITI-007-008) found that ITI-007 60 mg was associated with a mean striatal dopamine D2 receptor occupancy of approximately 40%. Together these data suggest that ITI-007 has demonstrated efficacy in two separate well-powered studies with relatively low striatal D2 receptor occupancy - lower than the occupancy range required by most other antipsychotic drugs for the treatment of schizophrenia. The unique mechanism of action of ITI-007 likely contributes to the favorable safety and tolerability profile observed to date, with reduced risk for hyperprolactinemia, akathisia, extrapyramidal symptoms, and other motoric side effects. ITI-007 also lacks off-target pharmacological interactions that may contribute to cardiovascular and metabolic liability of other treatment options. As such, ITI-007 represents a unique approach to schizophrenia treatment.

Keywords: Antipsychotics, Schizophrenia, Phase III Trial.

Disclosure: Intracellular Therapies, Inc: Employee, Self.

M174. Selective Benefit of Low Dose Methotrexate on Positive Symptoms of Schizophrenia in a Randomized Double-Blind Placebo-Controlled 12-Week Feasibility Trial

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Background: There is growing evidence that inflammatory processes are involved in the pathogenesis of schizophrenia. Autoimmune disorders are over-represented in patients and relatives and changes in the concentration of circulating cytokines appear consistent in meta-analyses. However, attempts to improve symptoms of schizophrenia with anti-inflammatory agents have produced inconsistent results. We reported that minocycline selectively improved negative symptoms when added to treatment as usual (TAU) for one year in early psychosis (PMID:22526685) and this selective benefit survived a meta-analysis of the few studies (PMID:27335660). A call by the Stanley Medical Research Institute (SMRI) enabled us to determine whether methotrexate with a different mechanism of anti-inflammatory action, might also improve negative or other symptoms. We used low dose methotrexate, an effective anti-inflammatory treatment for psoriasis and rheumatoid arthritis (RA). The mechanism of action is thought to involve inhibition of purine metabolism and T-cell function whereas its high dose anti-cancer effects are mediated by its anti-folate action.
Methods: The design and analysis plan were pre-registered (PMID: 25563714; NCT02074319). This was a double-blind placebo-controlled feasibility study of methotrexate (7.5mg weekly for 12 weeks) added to TAU in patients within 5 years of the onset of schizophrenia disorders. After screening, full clinical and safety assessments were carried out at randomization, 2, 4, 8 and 12 weeks. Cognitive assessments were carried out at randomisation and 12 weeks.
Efficacy assessments included Positive and Negative Syndrome Scales (PANSS), Clinical Global Impression (CGI), Global Assessment of Functioning Scale (GAF), EQ-5D quality of life scale and an insight scale. Cognitive assessments were: Stroop test, block design, verbal and category fluency, Coughlan visual and verbal list learning. Safety assessments included blood counts and side effects scales in keeping with methotrexate monitoring standards.
Statistics. Based on power calculation, we aimed to recruit 72 patients assuming a 10% drop-out giving 32 per group at 12 weeks. The intention to treat analysis determined group effects on 12 week clinical and cognitive outcomes co-varying for baseline measures with attention to possible biases from drop-outs. Generalised estimating equations (GEE) estimated the effect of treatment on longitudinal outcomes.
Results: 92 participants were randomised, 47 to placebo (PBO) and 45 to methotrexate (MTX) with respectively 39 and 37 completing 12 weeks of treatment. The 8 drop-outs in each group were due to loss of contact or co-operation. 2 refused medication in the MTX group. There were no serious adverse events or obvious side-effect differences.
There was no statistically significant effect of MTX on PANNS negative syndrome scores but the PANSS positive subscale improved more from baseline to 12 weeks on MTX [Mean (SD) 18.0(5.9) to 10.9(4.2)] than on placebo [15.7(6.4) to 12.3 (6.3)] giving a MTX/PBO treatment effect β -2.52; 95% CI (-4.65 to -0.39); $p = .023$. The corresponding treatment effect for the negative syndrome was β -0.39; 95% CI (-2.01 to 1.23); $p = 0.64$. The findings were confirmed by the GEE analysis. There was a significant treatment effect on GAF but not on CGI or quality of life. There were no significant effects of treatment on cognitive performance.
WCC reduced slightly in the MTX group over 12 weeks producing a significant treatment effect compared to PBO. However, subsequent causal mediation analysis did not indicate a mediating role in improving positive symptoms.
Conclusions: Methotrexate appears to exert a selective benefit on positive symptoms of schizophrenia early in the disorder. There were no effects on negative symptoms or cognitive performance. This is in contrast with a possible benefit of minocycline on negative symptoms rather than positive. Minocycline and MTX have a variety of actions on the immune system and they are both effective in some autoimmune disorders although MTX may be more effective in RA and psoriasis. Minocycline, has a number of CNS interactions which may be more relevant to negative symptoms. The efficacy of MTX may point to a role of immune mechanisms in positive symptoms. MTX reduced WCC to a similar extent to that observed in a recent study in patients with RA (PMID: 27335660). This suggests that patients were compliant with medication. However, this index of immune suppression did not appear to have a mediating role in improvement. This study shows that a definitive RCT of MTX in schizophrenia is worthwhile and feasible, and it provides a clear basis for sample size estimation.
Keywords: Schizophrenia, Methotrexate, Clinical Trial.
Disclosure: P1vital: Shareholder, Research Funding, Self.

M175. A Schizophrenia-Associated Missense Mutation in KAL9 Influences Dendritic Morphology Pathways in a Mouse Model

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Background: Kalirin (Kal) is a Rho GEF that is highly involved in regulation of the cytoskeleton within dendrites, a pathway that is the site of convergence of multiple genetic risk factors in schizophrenia. There are several isoforms of Kal that arise from differential splicing of its 66 exons. A missense mutation located within a region shared by the two longer Kal9 and Kal12 isoforms (P2255T, PTKAL9/12), has been associated with schizophrenia. When this mutation is overexpressed on the Kal9 background in vitro it results in increased activation of RhoA and reduced branching of basilar dendrites. We sought to determine the biological

effects of this mutation when expressed at the endogenous locus in a mouse model.
Methods: Using CRISPR/Cas9 genome editing, we introduced the PTKAL9/12 mutation at the endogenous gene locus in C57/BL6 mice. Absence of off-target effects as well as confirmatory sequence analysis was done with both PCR and Sanger sequencing. Cortical tissue homogenates were prepared from 4 wildtype and 4 homozygous PTKAL9/12 mutant mice. They were subject to either RNA extraction /qPCR, protein isolation/ western blotting, or trypsin digestion. The resulting peptides from digestion were enriched for phospho-peptides and analyzed by differential mass spectrometry. Peptide intensities were first calculated in Chorus and MaxQuant, select phospho-peptides were then manually evaluated in Skyline. Ingenuity Pathway Analysis (IPA) was used to perform functional enrichment of cellular processes most affected by changes in peptide phosphorylation in PTKAL9/12 mice.
Results: A homozygous male mouse free of any off-target effects was successfully generated via CRISPR/Cas9. Selective breeding produced multiple generations of mice with the PTKAL9/12 mutation. Transcript and protein levels of Kal isoforms in PTKAL9/12 cortical homogenate did not differ from WT. Mass spectrometry analysis of cortical homogenate phospho-peptide enrichments observed >4000 phospho-peptides. Of these, 589 were confidently quantified in all 8 mice. Phospho-peptides with evidence of altered levels in PTKAL9/12 mice were enriched for genes affecting neuronal morphology and microtubule assembly. MECP2, a gene known to be involved in intellectual disability and correlated with dendritic tree and spine deficits, was among the most significant phospho-peptides altered in PTKAL9/12 homogenates.
Conclusions: We have successfully created a mouse model of a rare schizophrenia-associated point mutation within KAL9/12. Confirming our in vitro observations of functional effects of this mutation, we have identified perturbed phosphorylation of proteins involved in neuronal morphology and microtubule dynamics, pathways known to be affected by kalirin. Additional analyses of pyramidal neuron morphology and electrophysiology are ongoing and will be presented. This model will facilitate developmental studies of the downstream signaling alterations due to PTKAL9/12. A better understanding of these changes in signaling will provide insight into upstream causes of the impaired dendritic morphology that has been observed in schizophrenia, and ultimately how this alters neuronal function.
Keywords: Schizophrenia, Dendritic Spines, Morphology, Phosphorylation.
Disclosure: Nothing to disclose.

M176. Efficacy of F17464, a New Preferential D3 Antagonist in a Placebo-Controlled Phase 2 Study of Patients With Acutely Exacerbated Schizophrenia

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Background: F17464 is a new highly potent preferential D3 antagonist, 5-HT1A partial agonist with 40x lower affinity for the dopamine receptor D2. F17464 has demonstrated antipsychotic-like activity in animal models of positive symptoms of schizophrenia and blocked completely MK-801-induced social interaction deficits showing potential efficacy against negative symptoms. F17464 had also promnesic actions in preclinical tests like reversal of scopolamine effect. Phase 1 studies performed in healthy volunteers characterised the PK profile and showed a good safety and tolerability profile. A PET-scan study in healthy volunteers after single oral administration determined a high D3 occupancy rate up to 22h after a single dose with negligible D2 receptor occupancy in the same experimental conditions.
Methods: This double-blind, parallel group, multicenter study included 144 patients with acute exacerbation of schizophrenia treated either with F17464 fixed dose 40 mg (20 mg bid) or placebo (randomization 1:1) for 6 weeks as antipsychotic monotherapy. The primary objective was to evaluate the efficacy of 40 mg/day of oral F17464 in comparison to placebo. The primary efficacy criterion was the PANSS total score change from baseline to Day 43 on the Full Anaysis Set. Inclusion criteria included a well-documented diagnosis of schizophrenia for a minimum of 1 year, and a recent acute exacerbation characterised by a PANSS total score at screening ≥ 70 and <120 with no significant change of the PANSS positive subscore between the screening and randomisation visits and no prominent negative symptoms; Clinical Global Impression of Severity (CGI-S) score ≥ 4. All patients had discontinued previous treatments within the week prior to administration of study medication. Following screening patients were maintained in hospital till the 3rd week of treatment. Patients who had CGI-I score of ≤ 3 after 3 or 4 weeks were then eligible for hospital discharge and could continue study participation as outpatients.
Results: A total of 144 patients were randomized to one of the two study treatments (72 in each group). Male patients represented 58.3% of the total population; mean (SD) age was 37.5 (11.4) years. The majority of patients (95.1%) experienced schizophrenia of the paranoid type. Mean (SD) time since schizophrenia diagnosis was 5.64 (5.31) years. Baseline PANSS total score (mean [SD] 89.6 [9.5]), and the sub-scores were similar between treatment groups.
63 patients (43.8%) discontinued the study treatment prematurely (38.9% in F17464 group and 48.6% in the placebo group). In both groups, the major reasons for premature treatment discontinuation were lack of efficacy or worsening of schizophrenia.
The primary analysis, on the change from baseline of PANSS total score to Day 43 on the FAS (LOCF), showed a statistically significant difference in favor of F17464 over placebo: the adjusted mean (SE) change was -13.5 (2.1) on F17464 and -7.8 (2.2) on placebo with a statistically significant treatment effect estimate -5.7 (2.7). Similar results were obtained on the per protocol dataset.
Secondary criteria analyses supported the primary analysis results: the effect on the PANSS positive factor, defined by Marder, was statistically significantly greater in the F17464 group than in the placebo group. The response rate to treatment defined as the PANSS total score reduction by 20% or 30% from baseline to end of treatment, was statistically higher in the F17464 group (47.2% and 25.0%) compared to the placebo group (30.6% and 13.9%).

A similar proportion of patients in the F17464 and placebo groups had at least one intake of any authorised adjunctive benzodiazepine (lorazepam or oxazepam) during study treatment (61.1% and 62.5%, respectively).
The overall incidence of treatment-emergent adverse events (TEAEs) was slightly higher in the F17464 group (70.8%) than in the placebo group (62.5%). The most frequently reported TEAEs (>5% of patients in either group) with a higher incidence on F17464 than on placebo (>2-patient difference) were worsening of schizophrenia, insomnia, agitation, akathisia, and increase in triglycerides. Most TEAEs were reported with a mild or moderate severity. There were no clinically-relevant hepatic, metabolic including no clinically relevant weight gain, or cardio-vascular disorders. No EPS was reported under F17464; AEs leading to definitive study drug discontinuation were lower in the F17464 group than the placebo group (23.6% vs. 31.9%, respectively).
Conclusions: This is the first compound with robust D3 receptor selectivity that demonstrates antipsychotic efficacy and indicates the value of the D3 receptor as a therapeutic target. The results of this phase 2 study also show the favorable safety profile of F17674 when compared to placebo. Future studies will examine the therapeutic dose range and comparative efficacy to other atypical antipsychotics.
Keywords: Schizophrenia, Antipsychotic, Clinical Trial.
Disclosure: Nothing to disclose.

M177. Xanomeline Plus Tropsium: A Novel Strategy to Enhance Pro-Muscarinic Efficacy and Mitigate Peripheral Side Effects

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Background: Muscarinic receptors, particularly the M1 and M4, have long been of interest to the field given the significant literature supporting their therapeutic potential for the treatment of psychosis and cognitive impairment. Xanomeline is a muscarinic agonist with preferential actions at the M1 and M4 receptors. It has shown antipsychotic efficacy and pro-cognitive effects in Alzheimer's disease (Bodick et al. Arch Neurol. 1997;54:465-473) and schizophrenia (Shekhar et al. Am J Psychiatry 2008; 165:1033–1039). Xanomeline's further clinical development has been hampered by classic muscarinic side effects including vomiting, nausea, diarrhea, salivation, and sweating. There is evidence that these side effects are produced by peripheral muscarinic receptors. We are developing a novel therapeutic that combines xanomeline with trospium chloride. Tropsium chloride is a peripheral muscarinic receptor antagonist that does not cross the blood brain barrier and its activity is thus restricted to peripheral tissues. We propose that this combination approach will mitigate xanomeline's peripheral side effects while preserving its potential efficacy for treating psychosis and cognitive deficits. To further explore the peripheral origin of xanomeline's side effects, we present comparative xanomeline side effect data from two Alzheimer's clinical trials: 1) unpublished data from a trial that assessed a transdermal xanomeline preparation and 2) published data from a trial of the oral preparation of xanomeline (Bodick et al, 1997) both completed by the originator of xanomeline. Lower rates of gastrointestinal (GI) side effects from the transdermal trial would provide support that GI tract muscarinic receptors rather than central receptors may be mediating these xanomeline's GI-related SEs. The xanomeline/tropsium combination is being testing in healthy volunteers to compare its tolerability to xanomeline + placebo with study readout anticipated in Q4 2016.
Methods: The oral formulation of xanomeline was tested in a Phase II, double-blind, randomized, placebo-controlled study in 343 mild-to-moderate Alzheimer's patients. Patients were assigned to 75 mg/d, 150 mg/d, 225 mg/day or placebo for 26 weeks. A transdermal formulation was assessed in a Phase II, double-blind, randomized, placebo-controlled study in 295 mild-to-moderate Alzheimer's disease patients. Patients were randomized to one of three dose arms: xanomeline 75 cm2, xanomeline 50 cm2, or placebo for 26 weeks. The 75 cm2 and 50 cm2 were chosen in part based on the similar maximum plasma concentration (Cmax) of the 225 mg/d and 150 mg/d dose levels of the oral formulation, both of which showed signs of efficacy in AD patients.
Results: Digestive system events were tracked using treatment-emergent signs and symptoms in both studies. One of the most problematic GI side effects in the oral AD study was vomiting, so we report here the overall rate of GI side effects and vomiting. In the oral study, the rate of patients reporting at least one GI TESS was 44.8%, 71.4%, and 89.7% in the placebo, 150 mg/d and 225 mg/d arms respectively. Vomiting was reported in 9.2%, 39.3%, and 42.5% across the dose arms. In the transdermal formulation study, the rate of patients reporting at least one GI TESS was 19.0%, 15.3%, and 19.6% in the placebo, 50 cm2, 75 cm2 arms respectively. Vomiting in the transdermal study was reported in 4.1%, 2.0%, and 3.0% across the dose arms.
Conclusions: In the transdermal study, the incidence rate of digestive system side effects was similar in the active and placebo arms. This is in contrast to the oral formulation of xanomeline where digestive system adverse events were present at rates far in excess of the placebo group and were a main driver of early discontinuation. This suggests that GI adverse events experiences with the oral formulation may have been due to high local gastrointestinal exposure associated with oral dosing, rather than by CNS activation of muscarinic receptors. This data suggests that the xanomeline plus tropsium chloride combination approach may be viable for reducing GI adverse events associated with xanomeline by blocking peripheral muscarinic receptor activation. A study comparing the tolerability of the xanomeline/trospium combination to xanomeline is expected to provide additional data to this hypothesis in late 2016.
Keywords: Muscarinic Acetylcholine Receptor, Schizophrenia, Alzheimer's Disease, Novel Therapeutics, Antipsychotic.
Disclosure: Karuna Pharmaceuticals: Employee, Self; Tal Medical: Shareholder, Self; Entrega: Member, Board of Directors, Self.

M178. Altered Transient Network Dynamics in Schizophrenia and Psychosis Spectrum Youth

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Background: Recent neuroimaging studies of what has been referred to as the "resting state" have revealed that human brain activity during this state and others is characterized by continuous, rapid shifts in activity across a large number of complex, varied networks, rather than a prolonged engagement of one or a small number of networks. Although there is much evidence indicating that schizophrenia and related psychotic disorders are associated with altered functioning of distributed brain networks, it is not yet known whether abnormalities in the spatial distribution or temporal characteristics of these transient "network states" are present in psychotic illness. Thus, in the current investigation, we used a novel method to identify the dynamic configurations of functional networks and quantify the occurrence frequency of these network activity patterns during a resting state in patients with schizophrenia or schizoaffective disorder. In addition, to test whether any changes associated with established psychotic illness are also observed in non-help-seeking youth with subclinical psychotic symptoms, data collected from college-aged adults with self-reported subthreshold psychotic experiences ("psychosis-spectrum youth") were also examined.
Methods: Resting state blood oxygen level dependent (BOLD) data (1-2 scans, 6 minutes and 12 seconds each in length) were collected from two cohorts of patients with DSM-IV diagnosed schizophrenia or schizoaffective disorder and demographically-matched controls (Dataset 1: 64 patients and 61 controls; Dataset 2: 35 patients and 39 controls), as well as a third cohort comprised of youth with elevated levels of subclinical psychotic symptoms and demographically-matched controls (Dataset 3: 22 psychosis-spectrum youth and 43 controls; these subjects had the highest and lowest scores, respectively, on a measure of psychotic experiences validated for use in the general population (the Peters Delusions Inventory) within a total cohort of 130 youth). Using machine learning technology, we developed a novel strategy to classify single-volume functional MR images into a finite number of clusters according to the spatial patterns of these images. This technology can reliably identify a set of unique activity patterns that can be considered specific "brain states". Nineteen transient states were identified in the data; different states appear with different probability. We computed the "occurrence frequency" of each state, i.e., the percentage of time each state occurred during the entire scan, and then compared that frequency between groups. Statistical tests conducted for the comparison between the two groups of Dataset 1 (the discovery sample) were corrected for multiple comparisons. Datasets 2 and 3 were used for replication/extension purposes ($p = .05$ uncorrected). In addition, control analyses were conducted to ensure that attentional effects did not account for group differences.
Results: In Dataset 1, three network states (primarily composed of the frontoparietal control network ($p = 1$ x 10-4), primary somatosensory-motor cortex ($p = 5$ x 10-7), and early visual areas and anterior insula ($p = 5$ x 10-6), respectively) were active less frequently and one network state (composed of default network and medial temporal lobe regions, $p = .01$) was active more frequently, in psychotic patients compared to controls. An identical pattern of results was observed in Dataset 2. In the psychosis-spectrum youth, a reduction in frequency of activity within visual/insula cortices was observed ($p = .005$) but no such reduction was observed in the other 3 network state frequency changes detected in the two patient samples. Lastly, within the full youth cohort ($n = 130$), the severity of subclinical psychotic experiences correlated with the reduction in frequency of the visual cortex state ($p = .004$).
Conclusions: These findings suggest that abnormal patterns in the moment-to-moment engagement of distinct brain networks over a time period of minutes may reflect a change in basic circuit function associated with psychosis. Also, evidence for diminished activity of a visual cortex network across clinical and subclinical psychosis phenotypes is consistent with models of psychosis emphasizing early disruptions of perceptual function. Based on this work, ongoing longitudinal studies can test for a putative progression of these changes (from early sensory to higher order cortices) over the course of illness.
Keywords: Schizophrenia, Functional MRI (fMRI), Resting State, Subclinical Psychosis.
Disclosure: Nothing to disclose.

M179. Levels of Glutamate and GABA in Initially Antipsychotic Naïve Patients With Schizophrenia and the Association to Treatment Outcome

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Background: Insufficient response to antipsychotics constitutes a major challenge in the treatment of patients with schizophrenia. Increased glutamatergic turnover has been found in the anterior cingulate cortex (ACC), left thalamus, and striatum in antipsychotic naïve patients with schizophrenia, and it has been proposed that glutamatergic modulating agents might have a beneficial effect in the early phase of the disease in subgroups of patients. Gamma-Aminobutyric acid (GABA) regulates glutamate and has been suggested as a therapeutic target in first episode schizophrenia as well. Interestingly, cross-sectional studies suggest that non-responder patients have glutamatergic but not dopaminergic disturbances, but it is unknown whether these changes are caused by the antipsychotic treatment or are biomarkers of a neurochemical distinct subtype of patients. In sum, longitudinal studies of brain glutamatergic and GABAergic metabolites in initially antipsychotic naïve patients are needed to determine if glutamatergic disturbances are most pronounced early in the disease, to what extent baseline levels of glutamate and GABA can predict treatment response, and whether non-responders are characterized by glutamatergic and GABAergic disturbances.

Methods: This is a longitudinal study in progress of 40 initially antipsychotic-naïve first episode schizophrenia patients and 40 matched healthy controls. Patients are treated with aripiprazole (monotherapy) (tool compound) for the first 1.5-month but are switched to another antipsychotic compound thereafter in case of inadequate response or unacceptable adverse effects. Participants are assessed at baseline and after 1.5 and 6 months with structural, functional, and neurochemical MRI, a neuropsychological test battery, level of function, and clinical outcome as measured with PANSS. Levels of glutamate in ACC and left thalamus, and levels of GABA in ACC are measured during MRI with proton magnetic resonance imaging (1H-MRS) and estimated with LC-Model and Gannet, respectively.

Results: To date 26 patients have been recruited, 19 completed 1.5 months follow-up, and 18 completed 6 months follow-up. Preliminary analysis reveals that levels of glutamate scaled to creatine (glu/Cr) in left thalamus are significantly higher in antipsychotic naïve patients with schizophrenia than matched healthy controls ($p = 0.043$). No baseline differences are found for glutamatergic metabolites or GABA in ACC. Analysis of follow-up data reveals a significant group*times interaction ($p = 0.0132$) of glu/Cr in left thalamus with initially increased glutamate in patients ($p = 0.04$) that decreases to a borderline significant level after 1.5 month ($p = 0.057$) and a significant level after 6 months treatment ($p = 0.005$)). No significant group*times interaction is seen in ACC, but levels of glutamate+glutamine scaled to creatine (Glx/Cr) decreases to a borderline significant level after 6 months treatment ($p = 0.059$). Analysis of the relation between glutamatergic and GABAergic metabolites at baseline and treatment outcome reveals a positive correlation between levels of GABA/Cr in ACC in the antipsychotic naïve state and reduction in PANSS positive after 1.5 months (rho = 0.68, $p = 0.01$) and 6 months (rho = 0.64, $p = 0.04$) as well as a positive correlation to PANSS total reduction after 1.5 months (rho = 0.70, $p = 0.007$). A negative correlation is seen between baseline levels of glu/Cr in left thalamus and reduction of PANSS total after 6 months (rho = 0.56, $p = 0.03$), and a borderline significant negative correlation with PANSS positive after 6 months (rho = 0.49, $p = 0.06$). Glx/Cr in thalamus at baseline is positively correlated with reduction in PANSS general after 1.5 months (rho = 0.46, $p = 0.047$) and Glu/Cr in ACC is positively correlated with PANSS negative reduction after 6 months (rho = 0.57, $p = 0.03$). The correlation between levels of glutamate, GABA, and cognitive measures at baseline will be presented at the meeting.

Conclusions: These preliminary data suggest abnormal glutamatergic neurotransmission in left thalamus of antipsychotic naïve patients with schizophrenia that is normalized during treatment. Patients and healthy controls have a different trajectory of glu/Cr in left thalamus and it is likely that antipsychotic treatment causes the decrease of glutamate in patients. High baseline levels of glu/Cr in thalamus might predict a poor response whereas high levels of GABA in ACC might predict a good response. Analysis of responders and non-responders after inclusion of the entire sample will reveal if the non-responder group is characterized by increased levels of glutamate in thalamus and ACC, and decreased levels of GABA in ACC from the beginning of the disease.

Keywords: Antipsychotic-Naïve First-Episode Schizophrenia, Glutamate, MRS, GABA.

Disclosure: Nothing to disclose.

M180. Advancing the Clinical Development of ITI-007 - Update on Efficacy for the Treatment of Schizophrenia

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Background: Individuals living with schizophrenia continue to be underserved by existing treatments. One percent of the world's population suffer from this serious mental illness and available medications fall short in providing patients with broad efficacy across the panoply of symptoms they experience.

ITI-007 is a first-in-class investigational agent in clinical development for the treatment of schizophrenia. ITI-007 60 mg has been shown to be effective in reducing symptoms of schizophrenia in two late-stage clinical trials at time of abstract submission. Further in both studies, ITI-007 was well-tolerated with favorable metabolic, motoric and cardiovascular profiles similar to placebo.

ITI-007 acts synergistically through serotonergic, dopaminergic and glutamatergic systems and represents a novel approach to the treatment of schizophrenia and other neuropsychiatric disorders. This centrally-acting small molecule is a potent 5-HT2A receptor antagonist, a mesolimbic/mesocortical dopamine phosphoprotein modulator (DPPM) with activity as a pre-synaptic partial agonist and post-synaptic antagonist at dopamine D2 receptors, a mesolimbic glutamate GluN2B receptor phosphoprotein modulator downstream from dopamine D1 receptor activation, and a serotonin reuptake inhibitor. This unique combined pharmacology has been predicted to translate clinically into a safe and well-tolerated treatment for schizophrenia and other neuropsychiatric and neurological disorders.

Methods: The ITI-007 program includes three randomized, double-blind, placebo-controlled clinical trials in patients with acute schizophrenia: ITI-007-005, ITI-007-301, and ITI-007-302.

In the Phase 2 trial ITI-007-005, 335 patients were randomized to receive one of four oral treatments once daily for 4 weeks: 60 mg ITI-007, 120 mg ITI-007, 4 mg risperidone (positive control) or placebo in a 1:1:1:1 ratio. In the first Phase 3 trial ITI-007-301, 450 patients were randomized to receive one of three oral treatments once daily for 4 weeks: 60 mg ITI-007, 40 mg ITI-007, or placebo in a 1:1:1 ratio. In the second Phase 3 trial ITI-007-302, 696 patients were randomized to receive one of four oral treatments once daily for 6 weeks: 60 mg ITI-007, 20 mg ITI-007, 4 mg risperidone (positive control) or placebo in a 1:1:1:1 ratio. In all three studies the primary efficacy endpoint was change from baseline in the Positive and Negative Syndrome Scale (PANSS) total score versus placebo at end of treatment. In the two Phase 3 trials, the key secondary endpoint was the Clinical

Global Impression Scale for Severity of illness (CGI-S). Other exploratory secondary endpoints were also included.
Results: In Phase 2, ITI-007-005, ITI-007 60 mg met the primary endpoint and demonstrated efficacy with statistically significant superiority over placebo at Day 28 as measured by the PANSS total score ($p = 0.017$).
In the first phase 3 study, ITI-007-301, ITI-007 60 mg also met the primary endpoint and demonstrated efficacy with statistically significant superiority over placebo at Day 28 as measured by the PANSS total score ($p = 0.022$). Moreover, ITI-007 60 mg, which requires no dose titration, showed significant efficacy as early as week 1 on both the PANSS total score and PANSS Positive Symptom subscale score, which was maintained at every time point throughout the study. ITI-007 60 mg also met the key secondary endpoint of statistically significant improvement on the CGI-S ($p = 0.003$).
In both completed trials, ITI-007 was well-tolerated with a favorable safety profile. [Please see companion abstract/poster for details on the safety analyses.]
Clinical conduct for the second Phase 3 trial, ITI-007-302, is ongoing and available data at the time of the congress will be presented.
Conclusions: ITI-007 represents a novel approach for the treatment of schizophrenia, as it demonstrates a unique pharmacology as well as a differentiating clinical profile. Data from the ongoing, late-stage schizophrenia program for ITI-007 continue to further characterize ITI-007's novel mechanism of action as well as the potential clinical benefits for patients, in terms of efficacy and safety.
Keywords: Antipsychotic, Schizophrenia, Phase III Trial.
Disclosure: Intra-Cellular Therapies, Inc.: Employee, Self.

M181. Antipsychotic Serum Concentrations in the "Neuroleptic Strategy Study" (NeSSy)

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Background: We have recently reported the results of the multicenter "Neuroleptic Strategy Study" (NeSSy), in which we compared the effects of first- (FGAs) versus second-generation antipsychotics on quality of life in schizophrenia (Gründer et al, Lancet Psychiatry 2016; 3: 717-729). We demonstrated that quality of life (as assessed with the SF-36) was statistically and clinically significantly more improved under treatment with SGA antipsychotics compared to FGAs. Here we report on relationships between doses and serum concentrations of the applied antipsychotics, which were systematically evaluated throughout the study, and clinical effects.
Methods: In the multicenter, randomized, double-blind NeSSy study, we recruited participants (aged 18–65 years) with schizophrenia (ICD-10: F20.X) who required treatment initiation or a change in treatment, from 14 psychiatric university hospitals and state hospitals in Germany. Double randomization allowed for restricted selection of a treatment within each antipsychotic drug group (FGA or SGA) for an individual patient: first, patients were assigned with a random number table to two of six possible drug pairs, each pair consisting of an FGA (haloperidol [3–6 mg] or flupentixol [6–12 mg]) given orally and an SGA (aripiprazole [10–20 mg], olanzapine [10–20 mg], or quetiapine [400–800 mg]) given orally, and the investigator then selected which pair was best suited to the patient; a second, double-blind random assignment allocated either the FGA or the SGA from the investigator chosen pair to the patient. Treatment duration was 24 weeks. Primary outcomes were change from baseline to week 24 in quality of life (SF-36) and clinical global impression (CGI-I), analyzed in all randomly assigned patients who received at least one dose of the study drug. The administered doses were adjusted according to clinical efficacy and side-effects resulting in a wide range of doses for each of the investigational drugs. Serum levels were determined after 4 weeks and at the end of treatment. Blood samples were taken from fasting patients in the morning. The serum concentrations were determined by high performance liquid chromatography tandem mass spectrometry (LC-MS/MS). During the course of study, the results of serum analysis were communicated to the study centers without naming the compound itself and only in relation to the therapeutic reference range ("below", "within" or "above") but not by numeric value. By this procedure, both patient safety and concealment of allocation was guaranteed.
Results: Between April 1, 2010, and May 31, 2013, 149 patients were randomly assigned, 69 to FGA treatment and 80 to SGA treatment. 136 patients received at least one dose of study drug (63 in the FGA group, 73 in the SGA group). A total of 145 serum samples were collected and analyzed during the study. 44 samples had to be excluded from the analysis, in most cases, because the measured values were below the limit of quantification, suggesting that the respective patients were non-compliant. The remaining 101 measurements in valid relation to trial treatment constitute the core data analyzed here. These samples were taken from 70 different patients, i.e. the mean number of samples per patient was 1.44. Mean and median serum concentrations of aripiprazole were 215 and 170 ng/mL (range 49-900 ng/mL), of flupentixol 4.7 ng/mL and 3.3 ng/mL (0.4-12 ng/mL), of haloperidol 2.2 ng/mL and 2.0 ng/mL (0.7-4.0 ng/mL), of olanzapine 38.5 ng/mL and 32.0 ng/mL (5.1-130 ng/mL, and of quetiapine 309 ng/mL and 370 ng/mL (16-630 ng/mL). If all compounds were taken together, regression analysis revealed a positive correlation between drug doses and serum concentrations ($p < 0.0042$), which was even more pronounced when dose per kg body weight was taken into account ($p < 0.0032$). This correlative relationship was also found for aripiprazole ($p < 0.0227$), flupentixol ($p < 0.0177$), and quetiapine ($p < 0.0385$), but not for haloperidol ($p < 0.0817$) and olanzapine ($p < 0.9864$). The same doses induced higher serum levels in females than in males and in non-smokers compared with smokers. 65.4% (SF36) and 70.4% (CGI), respectively, of the patients, who fulfilled the criteria for a clinically significant improvement with regard to the primary endpoints, presented with serum concentrations within the therapeutic reference ranges recommended by the AGNP Task Force Therapeutic Drug Monitoring (Hiemke et al, Pharmacopsychiatry 2011; 44: 195-235).
Conclusions: Doses of antipsychotic compounds used in this trial were clinically adjusted to improve the benefit/risk ratio of antipsychotic drug treatment. Two thirds of the obtained

mean and median serum levels in our trial were within the therapeutic reference ranges recommended by an international guideline on TDM of psychotropic drugs (Hiemke et al, 2011). This provides empirical support for therapeutic drug monitoring of antipsychotic treatment. However, to further validate the clinical usefulness of TDM for broader clinical application, prospective trials with a fixed-dose design are needed.

Keywords: Antipsychotics, Schizophrenia, Clinical Trials, Therapeutic Drug Monitoring, Serum Levels.

Disclosure: AstraZeneca: Supply of Study Drug, Self; Bristol-Myers Squibb: Supply of Study Drug, Self; Eli Lilly: Supply of Study Drug, Consultant, Speaker, Self; Lundbeck: Supply of Study Drug, Consultant, Self.

M182. Translational Imaging Studies Supporting the Development of F17464: A New Antipsychotic Drug With Preferential D3 Antagonist /5HT1A Partial Agonist Properties

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Background: F17464 is a new potent preferential dopamine D3 receptor antagonist, 5-HT1A partial agonist, with at least 40-time lower affinity for the D2 receptor. F17464 has demonstrated potent and dose-dependent antipsychotic-like activity in rodent models relevant of positive symptoms (inhibition of MK-801- and amphetamine-induced hyperactivity) and negative symptoms (inhibition of MK-801-induced social interaction deficits) of schizophrenia, suggesting potential clinical efficacy in several symptom domains of the disease. A recent double-blind, placebo-controlled, multicenter study that included 144 patients with acute exacerbation of schizophrenia demonstrated, for the first time, the antipsychotic properties of F17464 at 40 mg/day in patients treated for 6 weeks. The present study reports a summary of the translational imaging studies supporting the D3$>$ D2 mechanism of action of F17464.

Methods: Radioligand binding experiments and functional assays, [14C]F17464 dissociation kinetic experiments and dissociation of unlabelled F17464 delayed [3H]spiperone association were performed with human dopamine D3 and D2S receptors to establish dissociation kinetics for both receptors in vitro. In vivo radioligand binding with [3H] nemonapride and [3H]raclopride was performed in mice, to determine occupancy of D2 (mainly) receptors by F17464 in the brain. Positron emission tomography (PET) imaging was used in anesthetized non-human primates (NHPs) to characterize the in vivo occupancy of F17464, at D3 receptors. Imaging was performed in 3 rhesus monkeys, using the D3-preferring radiotracer [11C]-(+)-PHNO. An open-label study in healthy male subjects was carried out to characterize F17464 binding to D3and D2 receptors and the time course of receptor occupancy using PET imaging with [11C]-(+)-PHNO.

Results: Radioligand binding experiments indicate remarkably high affinity of F17464 at both the human dopamine D3 receptor and human serotonin 5-HT1A receptor and a unique D3 selectivity of 38 and 71 fold in vitro, when compared to D2S and D2L, respectively. Moreover, the preference in binding affinity of F17464 at D3 receptors when compared to D2 sites is also associated with different kinetic dissociation properties since F17464 dissociated from recombinant hD3 receptors more slowly than from hD2S sites (t1/2 of 110 min and 1.4 min, respectively). [3H] nemonapride and [3H]raclopride in vivo binding studies indicate D2 receptor occupancy by F17464 in the mouse brain, but at higher doses (ID50 of 15-24 mg/kg) than those necessary to obtain antipsychotic-like activities (ID50 of 0.09-0.28 mg/kg). In PET scan studies, the individual contributions of D3 and D2 receptor occupancy by F17464 were estimated by a regression model. In NHP PET studies F17464 administration induced a clear, dose- and concentration-dependent decrease in [11C]-(+)-PHNO binding to D3 receptors, consistent with dose- and concentration-dependent occupancy of D3 receptors by F17464. The study also demonstrated high potency and selectivity of F17464 for D3 compared to D2 receptors. All models predicted that D2 occupancy will be negligibly low at doses that attain 60% D3 occupancy. In healthy subjects, F17464 robustly and dose-dependently reduced [11C]-(+)-PHNO BPND in D3-rich brain regions such as the substantia nigra/ventral tegmental area, but not in the D2-dominated dorsal striatum. The regression model confirmed that F17464 preferentially occupies D3 receptors. Estimated D3 and D2 occupancies were 86 $\pm$ 9% and 15 $\pm$ 6%, respectively, at the highest dose tested and at the maximal occupancy time (6-9 hours post-dosing). Moreover, D3 occupancy level was already substantial 1 hour after dosing and remained remarkably high (60%) 22 hours after dosing, at a time when F17464 plasma concentrations were very low. D2 receptor occupancy was modest ($<$20%) under all conditions.

Conclusions: This is the first compound with robust selectivity for the D3 receptor that proves efficacy as antipsychotic indicating the therapeutic value of targeting preferentially the D3 receptor. The high affinity for the D3 receptor and the slow D3 receptor dissociation rate are key features of F17464 potentially relevant to support the selective and long lasting D3 receptor occupancy observed in PET studies in healthy subjects.

Keywords: D3 receptor, PET Imaging, non-Human primate, Healthy Subjects.

Disclosure: Pierre Fabre Medicament: Co-inventor of a Patent on F17464, Self.

M183. Deficits in Core Synaptic Signatures in a Human iPSC Model of Major Mental Disorders

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Background: Severe psychiatric illnesses, such as schizophrenia, are chronic and complicated neurological diseases

which affect a large portion of the world's population. One major hypothesis of the pathogenesis underlying schizophrenia is that functional deficits in synapse formation and synaptic transmission contribute to the mis-wiring of neural circuitry during childhood and adolescence, which precipitates clinical onset of the disease. While this hypothesis has gained indirect support from human post-mortem brain analyses and genetic studies, little is known about the pathophysiology of synapses in patient neurons and underlying molecular mechanisms due to the lack of experimental access to live human brain cells. A recent breakthrough in human disease research has emerged from cellular reprogramming technologies, which turn adult somatic cells, such as human skin fibroblasts into pluripotency, termed induced pluripotent stem cells (iPSCs). Since patient-derived iPSCs capture identical risk alleles as the donor individual and provide a renewable source of previously inaccessible, disease-relevant human cell types, iPSC technology has offered an unprecedented opportunity to recapitulate both normal and pathologic human development, thereby enabling a new approach for understanding human disease mechanisms and for drug discovery with higher predictability of their effects in humans. Here we investigate the synaptic mechanisms underlying major mental disorders with iPSCs derived from patients carrying a frame-shift mutation in the DISC1 (Disrupted-in-schizophrenia 1) gene, one of the best- supported susceptibility genes for major psychiatric disorders including schizophrenia, bipolar disorder and recurrent major depression.

Methods: Using an episomal non-integrating approach, we established iPSC lines from skin fibroblasts of four members of an American family (Pedigree H) in which a four base-pair frame-shift mutation of DISC1 co-segregated with psychiatric disorders, including two patients (one schizophrenia and one major depression) with the frame-shift DISC1 mutation and two unaffected members without the mutation. Importantly, to confirm the causal role of DISC1 mutation we generated different types of isogenic lines via genome editing, including one isogenic line correcting the mutation in patient iPSCs and two isogenic lines introducing the same mutation into the healthy controls. We further developed a differentiation protocol to generate human cortical excitatory neurons from iPSCs with high efficiency. With these disease-relevant cells, we examined the neural phenotypes at the molecular and cellular levels, including synapse formation by immunostaining with specific synaptic markers, synaptic transmission by electrophysiology, presynaptic vesicle releasing by live cell FM1-43 imaging, and gene expression profiles by RNA-seq and transcriptomic analysis. Furthermore, we developed a nonbiased iPSC-based high-throughput synaptic screen assay for candidate molecules that can ameliorate the disease relevant synaptic defects in patient neurons.

Results: Using this model system, we showed that mutant DISC1 causes a reduction of functional synapses and deficits in synaptic vesicle release in human cortical neurons. Mechanistically, mutant DISC1 depletes wild-type DISC1 as well as DISC1 interacting proteins, causing transcriptional dysregulation of many genes related to synapses and psychiatric disorders. Furthermore, the high-throughput synaptic screening has led to identifications of candidate compounds that can rescue the synaptic phenotypes in patient neurons.

Conclusions: Our study directly supports the synapse hypothesis for the etiopathology of psychiatric disorders and uncovers a novel mechanism through which the disease-relevant mutation affects synaptic functions via transcriptional dysregulation. Our study thus may lead to a better understanding of the role and mechanisms of major risk genes in modulating synaptic development and shed light onto the etiology and pathogenesis of schizophrenia and related major mental disorders. Importantly, our study may also facilitate the development of novel therapeutic treatments for psychiatric disorders based on rational design and hypothesis-driven investigation of relevant cellular pathophysiology.

Keywords: Induced Pluripotent Stem Cells (iPSCs), Mental Disorder, Schizophrenia, Synapses.

Disclosure: Nothing to disclose.

M184. Microtransplantation of Synaptic Membrane Reveals Functional Alterations in the Balance of Excitatory and Inhibitory Currents in Schizophrenia

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Background: Alterations of synaptic function in individuals with schizophrenia have been found in transcriptome, proteome, and genetic studies. Impaired synaptic neurotransmission in affected brain regions (e.g., dorsolateral prefrontal cortex; DLPFC) is thought to be involved in the core symptoms of schizophrenia. However, there are no quantitative measurements of synaptic function in the human DLPFC, therefore concrete and specific functional alterations glutamatergic (excitatory) and GABAergic (inhibitory) ion fluxes are lacking. We have begun to address this problem by directly measuring AMPA- and GABA receptor-mediated synaptic currents in postmortem brains from subjects with schizophrenia and unaffected controls. We demonstrate in our preliminary work that the function of synaptic receptors is maintained in postmortem brains and is significantly decreased in schizophrenia compared to controls.

Methods: We microtransplanted human synaptic membranes containing native AMPA and GABA receptors, from the postmortem DLPFC of 10 controls and 9 schizophrenia subjects, into Xenopus oocytes. These oocytes provide a living environment for the transplanted human receptors and allow us to record electrophysiological measurements of native ion currents. Ion currents through AMPA and GABA receptors were used to determine the functional excitatory to the inhibitory ratio (E/I) per subject. A proteomic analysis of the same synaptoneurosomal preparations was determined by label-free liquid chromatography-MS (LC-MS/MS) to characterize major synaptic elements with modulatory capacity on GABA and glutamate receptors. The E/I ratio

was also integrated with mRNA-Seq data in each subject to evaluate alterations in mRNA that code for excitatory and inhibitory postsynaptic density proteins.
Results: Functional responses of native GABA and AMPA receptors responses in SZ cases were reduced by 17% ($p > 0.05$) and 30%, respectively ($p = 0.01$ ANCOVA correcting for pH and RIN). The E/I ratio measured by electrophysiology was reduced in SZ by 24% ($p = 0.003$, ANOVA). Alterations in the E/I ratio measured by electrophysiology were significantly correlated with several transcriptomic and proteomic measurements. AMPA receptor subunits GRIA1, GRIA2 and GRIA3, and GABA receptor subunits alpha1, beta2, and gamma2 were identified by proteomics in all synaptoneurosomal membranes tested. None of the GABA or AMPA receptor subunits measured were statistically different between SZ and CTRL; however, the proteomic E/I ratio, measured as the sum of all GRIA subunits divided by the sum of all GABA subunits, exhibited a significant 13% reduction ($p < 0.03$) in line with our findings of reduced E/I ratio at the electrophysiological level. Correlation analysis with mRNA-Seq identified four splice variants of gephyrin, an inhibitory postsynaptic density protein that strongly correlated with GABA currents. These variants might be directly involved in the postsynaptic clustering of GABA receptors. Interestingly, correlations between GABA currents and gephyrin at the proteomic and transcriptomic level are disrupted in subjects with SZ.
Conclusions: The data suggest a potentially new functional measure of the endophenotype of synaptic alterations in persons with schizophrenia. Our data are consistent with prior studies of the DLPFC of persons with schizophrenia and suggest that inhibitory synaptic imbalance may underlie the hypofunction and decreased activation that has been consistently observed. AMPA and GABA receptors have crucial and complementary roles with the function of NMDA receptors. Our model explains an NMDA receptor hypofunction by enhanced inhibition and the potential shunt of NMDA receptor activation which may underlie the generation of psychoses in individuals with SZ. These preliminary results, if present in an independent replication, will generate a useful model for further pinpointing treatment of synaptic alterations in schizophrenia.
Keywords: Schizophrenia, Synaptic Aberrations, Postmortem Brain Tissue, Electrophysiology, AMPA receptors, GABA-A Receptors.
Disclosure: Nothing to disclose.

M185. A Population-Based Longitudinal Study of Symptoms and Signs Predicting Psychosis

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Background: Most studies attempting to predict psychotic disorder were set in specialized clinics, focusing on high-risk individuals. The aim of this study was to examine the rates of self-referral to mental health services in those later hospitalized for psychotic disorders, and to examine the symptoms of patients later hospitalized for psychotic disorders during the prodrome in a large population-based sample.
Methods: Archived data of clinical examinations of soldiers serving in the Israeli military (ages 18-21), were linked with data on later hospitalization in psychiatric hospitals. Symptoms were clustered into factors using categorical principal components analysis (PCA), which were then included in a Cox regression to predict future hospitalization for non-affective psychotic disorder (NAPD).
Results: Of the 115,070 adolescents assessed by mental health professionals and not diagnosed with a psychotic disorder at the time of examination, 283 (0.2%) were hospitalized for NAPD between 15-365 days after the index examination. Of all patients later hospitalized for NAPD, 15.3% sought or were referred to mental health services during their military service, with similar rates for those later hospitalized for other psychiatric disorders. Treatment seekers with at least one of the following symptoms: thought disorder, perceptual abnormalities, obsessive-compulsive behavior or impaired judgment were at substantially increased risk for hospitalization for NAPD 15-111 days after examination, (HR = 21.96, 95% CI = 15.05-32.02) and 112-365 days after examination (HR = 5.12, 95% CI = 2.73-9.60). Presence of at least one of the following symptoms: aggressive behavior, impulse control problems or emotional lability and also increased risk for NAPD (HR = 2.25,95% CI = 1.49-3.39 and HR = 2.27,95% CI = 1.43-3.62 for 15-111, and 112-365 days, respectively. However, the PPVs of these symptoms were very low (0.58%-2.79%).
Conclusions: In this population-based study, rate of referral to mental health services prior to first hospitalization for NAPD is low. This implies that attempts to identify future patients based on their prodromal symptoms will only address the minority of patients. Presence of psychotic symptoms and obsessive-compulsive behavior was associated with later hospitalization for NAPD. However, the PPVs of these symptoms were very low, indicating that these symptoms alone are not useful in predicting later hospitalization for NAPD in general psychiatry settings.
Keywords: Prodrome, Psychotic Disorders, Prediction.
Disclosure: Nothing to disclose.

M186. Bilateral Synchronous 1Hz rTMS of the Superior Temporal Gyri Diminishes Auditory Hallucinations and Alters Functional Connectivity in Patients With Intractable Voices

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Background: Auditory Hallucinations (AH) are among the most disruptive symptoms of psychotic disorders. There are data to suggest that 1Hz repetitive transcranial magnetic stimulation (rTMS) of the left Wernicke's area (WA) diminishes the severity of auditory hallucinations (AHs) in patients with schizophrenia. Many questions remain unanswered, including basic questions about stimulation laterality, parameters and mechanisms as well as clinical

questions about tolerability and efficacy. In this study, we examined the effect of bilateral synchronous 1Hz rTMS of the superior temporal gyri on AH severity and speech processing circuitry in patients with intractable voices.
Methods: 15 patients participated in this open-label study. All patients received daily 16-minute sessions of real 1Hz rTMS at 90% resting motor threshold to bilateral superior temporal gyri (Brodmann Areas 21 and 22 corresponding to WA and its right homologue) based on high-resolution structural magnetic resonance imaging (MRI) data. A daily total of 960 pulses were delivered in synchrony to each hemisphere over the course of 4 weeks or 20 sessions (up to 19,200 total pulses to each hemisphere). Clinical and neuropsychological assessments such as the Hallucination Change Score and the Auditory Hallucinations Rating Scale (AHRS) were performed at baseline and after each 5-session block of stimulation. Structural and functional MRI scanning was performed on each patient at the beginning and the end of the experimental protocol.
Results: A preliminary analysis suggests that bilateral synchronous 1Hz stimulation significantly reduced AH symptom severity and significantly improved digit-span task performance. These effects were uncorrelated. Uncorrected connectivity analyses suggest that TMS lead to increased functional connectivity between WA, midbrain, striatum and anterior insula as well as decreased connectivity between WA, anterior cingulate cortex and dorsolateral prefrontal cortex.
Conclusions: These preliminary findings suggest that modulating corticostriatal circuitry with bilateral synchronous 1Hz rTMS may diminish the severity of AHs. TMS is a powerful tool with which to map and modulate the circuitry underlying intractable voices. Further studies are needed to investigate Interventional Psychiatry strategies for psychotic disorders.
Keywords: Transcranial Magnetic Stimulation, Auditory Hallucinations, Functional Connectivity, Magnetic Resonance Imaging, Schizophrenia.
Disclosure: Nothing to disclose.

M187. Abnormal Responses to Lysergic Acid Diethylamide Require β-Arrestin Signaling

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Background: Recent experiments have shown that ligand binding can induce conformational changes in G protein-coupled receptors (GPCRs) that lead to differential signaling events. Ligand binding to GPCRs can activate, inhibit, or exert no effects on the G protein-dependent signaling pathway while exerting similar or diverse actions on a G protein-independent pathway through β-arrestin (βArr). The ability of a ligand to preferentially affect G protein-dependent or -independent signaling is termed functional selectivity. While this property has been established for a few GPCRs, none of the current ligands or drugs were developed with this characteristic in mind. Recently, we have shown that antipsychotic-like actions can be mediated through dopamine D2 receptor βArr2-mediated activity (Park et al, Neuropsychopharmacology 2016). To further examine the role of βArr2 in responses associated with schizophrenia, we have analyzed the effects of lysergic acid diethylamide (LSD) on behavior. LSD binds many different types of GPCRs and, at "recreational doses", it binds serotonin (5-HT) 1A, 2A, 2B, 2C, 5, and 6 receptors. The psychedelic effects of LSD in humans has been attributed primarily to agonism of the 5-HT2A receptor. The purpose of the present investigation was to determine whether disruption of the Arrb2 gene in mice could differentially affect their responses to LSD.
Methods: Adult male and female wild-type (WT) and βArr2-KO mice were injected with vehicle or 0.3 mg/kg LSD (i.p.) and assessed in tests for open field activity, repetitive behaviors, and prepulse inhibition (PPI).
Results: In the open field, no differences in baseline motor activities were observed between WT and βArr2-KO mice. After injection, LSD significantly stimulated locomotor activity in WT animals while this effect was blunted in the mutants. LSD also augmented rearing and stereotypical activities in WT animals, whereas in βArr2-KO mice these responses were not distinguished from the vehicle controls. Ethological analyses revealed that LSD increased repetitive and stereotyped responses (head-twitch, nose-pokes, retrograde walking, unsupported rearing, and grooming) in WT mice relative to those in βArr2-KO animals. PPI was strongly suppressed with LSD in WT controls, whereas it was unaffected in the mutant mice.
Conclusions: LSD is a well-known psychedelic drug that can induce abnormal behaviors in humans that include hallucinations. With mice we find that many LSD-mediated responses are blunted or abrogated when Arrb2 is deleted. Accordingly, these findings indicate that βArr2 signaling may mediate many of the aberrant responses that have been ascribed to this drug.
Keywords: Lysergic Acid Diethylamide, β-arrestin, G Protein Coupled Receptors, Mouse Model.
Disclosure: Rugen Holdings (Cayman) Limited: Research Support, Self; NIMH – U19MH82441: research support, Self.

M188. Reduced Nucleus Accumbens Density Associated With Dimensional Severity of Subclinical Negative Symptoms in Youth

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Background: Negative symptoms of schizophrenia such as amotivation have been linked to structural and functional abnormalities in brain reward circuitry, particularly the nucleus accumbens/ventral striatum. In at-risk populations prior to frank psychosis, subclinical negative symptoms are associated with increased risk of psychotic conversion, and predict impaired function independent of conversion. However, the neurobiology of negative symptomatology in adolescent populations has received little attention, and it is

unknown whether structural deficits in nucleus accumbens are associated with subclinical negative symptoms in youth. Here we examine unmodulated gray matter density, an MRI measure that is especially sensitive to individual differences, focusing on the accumbens in relation to a dimensional measure of subclinical negative symptoms.

Methods: The sample comprised 1,257 youth aged 11-23 years (mean 16.3 years) from the Philadelphia Neurodevelopmental Cohort (PNC) who had both clinical and structural 3T MR data. Of these, 422 were categorized as Psychosis Spectrum (PS) based on the presence of subthreshold psychotic symptoms. Dimensional sub-clinical positive and negative symptoms, as well as dimensional measures of other psychopathology domains (anxious-misery, externalizing, fear) were derived from factor analysis of a structured clinical interview (GOASSESS). Gray matter (GM) density was estimated with a custom preprocessing pipeline employing an unbiased, iterative tissue segmentation approach; modulated density (which reflects regional volume) was also examined for comparison. Region-of-interest (ROI) analysis focused on the bilateral nucleus accumbens, and voxelwise whole-brain analyses were also conducted.

Results: As hypothesized, bilateral accumbens GM density showed a significant inverse relationship to dimensional negative symptom severity across the full sample ($r = -0.09$, $p = 0.0008$), and within just the PS subsample ($r = -0.16$, $p = 0.0009$). This relationship remained significant after statistically controlling for severity of positive sub-psychotic symptoms as well as severity of mood/anxious-misery, behavioral/externalizing, and phobia/fear dimensions of psychopathology. The relationship with negative symptoms was also stronger than for any of these other dimensions tested individually (positive psychosis $r = -0.03$, $p = 0.2$; anxious-misery $r = +0.07$, $p = 0.01$; externalizing $r = -0.07$, $p = 0.02$; fear $r = 0.03$, $p = 0.3$). Although the GM density measure correlated strongly with GM volume in the accumbens ($r = 0.73$), the correlation with negative symptoms was significantly stronger for density than volume ($r = -0.04$, $p = 0.2$). A whole-brain voxelwise analysis showed that the relationship of negative symptoms to density was regionally selective – a cluster centered on bilateral accumbens and extending into olfactory cortex was the most significant, with other significant correlations found in sensorimotor cortex and temporal pole.

Conclusions: Prior neuroimaging studies in the PNC and other at-risk samples have supported the continuum view of psychosis by identifying structural and functional abnormalities similar to those found in frank schizophrenia. Here we extend this evidence to structural deficits in a key brain reward region, and demonstrate a selective relationship to subthreshold negative symptoms. In this population-based sample of youth, these structural abnormalities were not attributable to effects of chronic illness or medications. Although the effect size we observe is small, statistically robust findings in this very large sample provide reliable evidence for reduced accumbens density as a neurobiological correlate of negative symptoms. Given likely heterogeneity, currently undetected subgroups may show even greater effect sizes, and multivariate imaging analyses may reveal complex patterns with greater predictive power. Such imaging markers may enhance the specificity and predictive value of early-onset negative symptoms that may otherwise be relatively non-specific. Ultimately, this and other biomarkers can be integrated with clinical, cognitive, and genetic data to develop robust predictors of prognosis, and contribute to efforts at early identification and treatment.

Keywords: Magnetic Resonance Imaging, Gray Matter Density, Psychosis Risk, Negative Symptoms, Adolescence.

Disclosure: Nothing to disclose.

M189. Widespread Heritable Brain Circuitry Associated With Critical Cognitive Domains Among Non-Psychotic Relatives in Multiplex Schizophrenia Families

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Background: Heritability of schizophrenia (SZ) is about 70%. However, the mechanisms through which heritability increases SZ risk and cognitive impairments are poorly understood. Since SZ may be a "disconnection syndrome", genetic factors may also affect brain circuitry that, in turn, impact neurocognitive and psychopathological manifestations. Diffusion Tensor Imaging (DTI) is a non-invasive in vivo neuroimaging method that characterizes anisotropy of water diffusion (fractional anisotropy, FA) that can be used to examine anatomical brain circuitry. Prior studies report impaired connectivity in both the anatomical and functional networks between SZ and first-degree relatives of SZ. We examined heritability of brain circuits and the association of heritable networks with psychopathology and cognitive performance among relatives of SZ patients who are past the highest risk period for SZ and unrelated controls enrolled at Pittsburgh and Philadelphia.

Methods: The Diagnostic Interview for Genetic Studies (DIGS 2.0), the Family Interview for Genetic Studies (FIGS), medical records data and consensus meeting reviews were used for psychiatric evaluation and confirmation of diagnoses of a unique sample of 175 multigenerational non-psychotic relatives (mean age, 42.83 ± 18.06 years) of 23 multiplex schizophrenia families and 240 unrelated controls (40.20 ± 16.34 years) (total $n = 438$). Inter-site reliability was maintained at $\kappa > 0.9$. The Penn Computerized Neurocognitive Battery (CNB) evaluated 8 domains of neurocognition with accuracy and speed of processing as outcome variables. We examined DTI data collected on identical Siemens Tim Trio 3T MRI systems. Imaging parameters were ($b = 1000$ s/mm2, slices = 64, thickness = 2.4 mm, TE = 90ms, TR = 6300 ms). Four regularly interspersed B0 reference images were also acquired. by scanning phantoms and test subjects. The inter-scanner reliabilities were tracked using phantoms with no significant differences. DTI data were processed using FSL 4.1 and DTI Studio to investigate altered FA of 20 white matter regions. Polygenic inheritance (h2r) was examined using the Sequential Oligogenic Linkage Analysis Routines (SOLAR). Controls were included since they could contribute to the estimation of the population mean of the phenotype and the covariate effects so that the non-representative family members could be evaluated in the

context of the general population. Group differences in FA were examined using MANCOVA controlling for age, sex, site and mother's education followed by Bonferroni-corrected univariate tests. For the heritability of tracts, 40 tests (20 tracts*2 sides), and for partial correlation with cognitive performance, 153 tests (17 tests*9 tracts showing significant heritability) were conducted. Using the Bonferroni method, a critical α of 0.00125 for heritability and 0.0003 for partial correlation tests were set. Corrected p values are reported.

Results: Significant total additive genetic heritability was observed in all 3 major types of tracts, namely the association, commissural and projection fibers. After correcting for multiple tests, significant heritability was observed in the right fornix ($h2r = 0.42$, $p = 0.026$) (association fibers), the bilateral splenium (left, $h2r = 0.69$, $p = 0.01$; right, $h2r = 0.70$, $p = 0.001$) and body of the corpus callosum (left, $h2r = 0.54$, $p = 0.01$; right, $h2r = 0.49$, $p = 0.003$) (commissural fibers), bilateral superior corona radiata (left, $h2r = 0.62$, $p = 0.00001$; right, $h2r = 0.37$, $p = 0.03$), right anterior corona radiata ($h2r = 0.57$, $p = 0.01$) and the left posterior corona radiata ($h2r = 0.63$, $p = 0.0001$) (projection fibers). FA of all these tracts were significantly decreased among SZ subjects compared to controls and relatives but not between controls and relatives. When relatives were divided into those with and without lifetime non-psychotic psychiatric diagnosis, the FA of relatives did not differ from controls. Cognitive impairments (mainly in processing times) were noted in SZ patients compared to controls and relatives; relatives being intermediate between SZ and controls. The right fornix FA positively correlated with immediate and delayed face memory accuracy, negatively with response time for immediate and delayed word memory, and emotion recognition. The FA of body of corpus callosum correlated negatively with response times for delayed face memory, spatial processing and executive functions and positively with accuracy of visual memory. Both splenium and the body of the corpus callosum FA negatively correlated with response time for executive functions. The right anterior corona radiata FA correlated negatively with response time for executive functions (partial r 0.19-0.24; all $p < 0.01$ corrected). Posterior and superior corona radiata FA did not correlate with cognitive performance.

Conclusions: Our data show significant heritability of all 3 major types of white matter tracts among relatives of SZ patients. Observed tracts appear to be involved in large-scale integration of information as supported by multiple-test corrected correlation with memory (verbal memory), complex cognition (executive functions) and social cognition (emotion recognition, face memory and visual memory) deficits. Correlations were predominantly observed for the reaction time compared to the accuracy. Previous meta-analyses consistently show impaired processing speed to be central feature of cognitive deficit in SZ. Cognitive remediation therapies show largest effect sizes on improved processing speed. Absence of FA differences between relatives with or without psychiatric diagnoses and controls may be because relatives are past the highest risk for SZ.

Keywords: Heritability, Schizophrenia, Neurocognition.

Disclosure: Nothing to disclose.

M190. In Vivo Imaging Alterations in Endocannabinoid Metabolism in Schizophrenia

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Background: The endocannabinoid (eCB) system mediates brain responses to cannabis and stress, two factors that increase the risk for onset and relapse of psychosis. Fatty Acid Amide Hydrolase (FAAH) is primarily responsible for setting brain levels of anandamide (AEA), one of two major eCBs in the brain. Stress responses and cerebrospinal fluid (CSF) AEA levels are altered in psychosis, suggesting a role for FAAH in schizophrenia (SCZ). Together, previous data suggest a role of eCBs and specifically anandamide (AEA) in SCZ. However, the role of FAAH, AEA's primary catabolic enzyme, has never been investigated in-vivo in brain in SCZ patients. Importantly, how in-vivo FAAH levels relate to behavior and cognition has never been previously explored in living humans.

Methods: We used Positron Emission Tomography (PET) with the novel and selective FAAH ligand, [11C]CURB to map FAAH in the brains of (mostly) untreated individuals with SCZ and matched healthy volunteers (HV) using high resolution research tomograhy (HRRT) and arterial input function to quantify FAAH with the validated outcome measure λk3 . [11C]CURB λk3 was analyzed through an analysis of variance (ANOVA) with [11C]CURB λk3 in striatum and DLPFC as our outcome measures and group diagnosis as predictor.

Results: We imaged 12 SCZ patients (age 26.3 ± 5.5 years) and 15 HV (age 25.5 ± 5.9 years) with the HRRT. ~25% of our SCZ patients were taking either antipsychotics and/or antidepressant medication at the time of scanning. Mean duration of untreated psychosis (DUP) was 34.1 months (range 1-106 months).

There were significant effects of group on [11C]CURB λk3, such that binding was lower in SCZ than in HV in the striatum (19%; $F = 15.71$, $p = .001$) and DLPFC (18%; $F = 11.13$, $p = .003$), consistent with the reported elevated cerebrospinal fliud (CSF) AEA levels.

In addition, we provide the first in-vivo evidence of a relationship between, duration of untreated psychosis (DUP), life stressors and FAAH in SCZ. Longer DUP was significantly associated with higher [11C]CURB binding ($r = .620$, $p = .032$). Controlling for DUP, higher [11C]CURB binding in striatum was associated with greater PANSS total score ($r = .660$, $p = .027$). Similarly, higher [11C]CURB binding in DLPFC was associated with poorer cognitive performance ($r = -.650$, $p = .030$), particularly on the attention subscale ($r = -.885$, $p = .0003$). Further, individuals reporting a greater number of recent stressful life events (RLE) or reported higher levels of chronic stress (TICS) exhibited higher [11C]CURB binding in DLPFC (life events: $r = .712$, $p = .014$; chronic stress: $r = .618$, $p = .032$).

Conclusions: These preliminary data reflect the first investigation of FAAH in vivo in brain in psychosis, suggesting lower FAAH in SCZ and a primordial role in stress, DUP and cognition. These data recapitulate findings from CB1R PET studies in SCZ in that the greatest reductions of [11C]CURB binding were observed in

untreated first episode of psychosis individuals, relative to more chronic SCZ patients pointing to the need to study patients in their FEP and critically, the clinical high risk (CHR) state that precedes psychosis.

Keywords: Endocannabinoids, Early Psychosis, Neurochemistry.

Disclosure: Nothing to disclose.

M191. DNA Methylation as a Mechanism for Altered Dendritic Spine Density in the Superior Temporal Gyrus of Individuals With Schizophrenia

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Background: Reduced dendritic spine density (DSD) in cortical layer 3 is among the most consistently observed findings in postmortem studies of individuals with schizophrenia (SZ), affecting multiple brain regions including the superior temporal gyrus (STG). Understanding the molecular mechanisms of this SZ intermediate phenotype are incompletely understood. DNA methylation (DNAm), a regulator of gene transcription, is a strong candidate for such a mechanism given that it has been implicated in many other contexts characterized by changes in dendritic spine complement. Here, we explore the potential contribution of DNAm alterations to reduced dendritic spine density in the STG of individuals with SZ.

Methods: The Illumina Infinium HumanMethylation450 Beadchip Array was used to quantify genome-wide DNAm in the STG of postmortem brains from 17 individuals with SCZ and 17 age-matched individuals without DSM-IV psychopathology in which DSD had previously been measured. Linear regression was used to model the relationship between DNAm and DSD.

Results: DNAm sites in many genes were found to be related to DSD and many of those DNAm sites exhibited a diagnosis-dependent relationship. Among the genes containing DSD-associated DNAm sites was the brain-specific angiogenesis inhibitor 1-associated protein 2 (BAIAP2), a known regulator of DSD and a SZ risk gene. Multiple BAIAP2 DNAm sites associated strongly with DSD and, for most, the correlation was diagnosis-dependent, thus suggesting a role for BAIAP2 DNAm in SZ DSD reductions in the STG.

Conclusions: DNAm may be a mechanism for SZ-related reductions in DSD, perhaps through its effects on transcription of genes implicated in actin dynamics and post-synaptic signaling like BAIAP2. Future studies will seek to confirm these relationships between DNAm and dendritic spine density and understand the effects of DNAm on gene transcription using targeted genomic approaches as well as explore the role of DNAm in DSD in an in vitro model system.

Keywords: DNA Methylation, Dendritic Spines, Schizophrenia.

Disclosure: Nothing to disclose.

M192. Relationship Between Toxoplasma Gondii Infection and Acoustic Startle in an Inner City Population

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Background: Toxoplasma gondii (TOXO) is a neuroinvasive protozoan parasite that induces the formation of persistent cysts in mammalian brains. The parasite tends to remain relatively quiescent in immunocompetent adult hosts, but can cause subtle behavioral changes both in rodents and humans. Chronic infection is strongly associated with a heightened risk for schizophrenia. We previously reported the association of TOXO with slowed acoustic startle latency, an index of neural processing speed, in a sample of schizophrenia and healthy control subjects. The alterations in neurobiology with TOXO latent infection may not be specific to schizophrenia. Therefore, we examined TOXO in relation to acoustic startle in an urban, predominately African American, population with mixed psychiatric diagnoses, and healthy controls.

Methods: The Grady Trauma Project (GTP) is a study of an impoverished highly traumatized urban minority population. Our dataset included 364 subjects who provided demographic information such as age, sex, race, employment status, education and income, diagnostic and symptom assessment, a blood sample, and startle testing. TOXO seropositivity was determined with an ELISA assay for TOXO-specific IgG, and a discrete titer was calculated based on standard cut-points as an indicator of seropositivity.

Psychophysiological data was collected using Biopac MP150 with electromyographic (EMG) recording of the eyeblink component of the startle response via electrodes placed over the orbicularis oculi muscle. Startle magnitude is the peak magnitude of the response; latency is the time from the startling stimulus until the peak magnitude of the eyeblink.

Results: Overall, TOXO seropositivity was found in 13.46% of the study participants. We found a reduced likelihood of TOXO seropositivity in those with major depression compared to psychiatrically healthy controls after adjusting for demographic factors (OR = 0.23; 95% CI 0.09-0.61). Similarly, a reduced likelihood of TOXO seropositivity was seen in subjects with posttraumatic stress disorder (PTSD) compared to controls after demographic adjustment (OR = 0.39; 95% CI 0.16-0.91). TOXO was not associated with schizophrenia in this sample.

TOXO, before adjusting for any covariates, was positively associated with acoustic startle magnitude ($t = 2.87$, $p = 0.014$), with a mean startle magnitude significantly higher for those with TOXO, as compared to those without. In regression analyses, TOXO seropositivity remained significantly and independently associated with higher startle magnitude when stepping in demographic variables, and dichotomous variables for presence vs. absence of psychiatric and substance use diagnoses ($p = 0.004$ to 0.015; $\beta = 0.197$ to 0.216). TOXO also remained significant in a sensitivity

analysis using a self-reported smoking variable and an alcohol use variable. TOXO serointensity (i.e. titer as a continuous variable) was significantly associated with higher startle magnitude when stepping in psychiatric covariates.

TOXO showed no association with startle latency ($t = 0.49$, $p = 0.63$) in an unadjusted model, nor was TOXO associated with latency in models that included demographic factors. After stepping in individual psychiatric disorders, we found a significant association of latency with a diagnosis of PTSD, such that participants with PTSD had longer startle latency but no increase in ASR amplitude. However, there was no significant association of latency with other psychiatric diagnoses.

Conclusions: We found a robust association between chronic TOXO infection and higher acoustic startle magnitude, even after adjusting for demographic factors and the presence of psychiatric diagnoses. Few studies have investigated the effects of any infection on acoustic startle in humans. Considering the proposed ability of chronic TOXO infection to influence brain function, our study using the acoustic startle response as an index of neurocircuitry function provides a new approach to examine the relationship between TOXO and psychiatric illness. The mechanism by which TOXO infection is associated with high startle magnitude is not known, but possible mechanisms include high TOXO cyst burden, parasite recrudescence, or molecular mimicry of a host epitope by TOXO. The TOXO genome contains two genes that code for tyrosine hydroxylase, leading to enhanced synthesis of dopamine, which in turn can increase startle magnitude. The activation of inflammatory cytokines in response to TOXO could trigger production of glucocorticoids through release of corticotropin releasing hormone, which is known to affect startle magnitude. Future studies will focus on the neurobiology underlying the effects of chronic TOXO infection as a potential inroad to the development of novel treatment targets for psychiatric disease.

Keywords: Infection, Acoustic Startle, PTSD, Immune Markers, Cytokines, Synapses, Schizophrenia, Autism.

Disclosure: Supported by the NIMH (MH071537 and MH096764 to KJR, and R01MH092512 to BDP), the VA Merit Review Program (ED), and the National Center for Advancing Translational Sciences (NIMH UL1TR000454).

M193. Blunted Prefrontal Dopamine Release in an NMDA Receptor Hypofunction Mouse Model

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Background: The dopamine hypothesis of schizophrenia is one major schizophrenia hypothesis. A considerable body of evidence from in vivo PET imaging studies demonstrates an increase in psychostimulant-induced dopamine release in the striatum of patients with schizophrenia (Laruelle, 2014). A recent PET imaging study also showed a deficit in amphetamine-induced dopamine release in the dorsolateral prefrontal cortex (DLPFC) in schizophrenia (Slifstein et al, 2015). This "dual dysregulation" of dopamine alteration cannot simply be explained by an increase in dopamine neuron activity in schizophrenia, because, in contrast to rodents, primate dopamine neurons embedded in the ventral tegmental area, substantia nigra, and retrorubral field, all projects to the cortex as well as striatum (Williams and Goldman-Rakic, 1998).

Convergence of both clinical and preclinical data suggest that dysfunction of dopamine system in schizophrenia may be following to a deficit in NMDA receptor (NMDAR) function. For example, uncompetitive NMDAR antagonist ketamine has been shown to an increase in amphetamine-induced striatal dopamine release in humans (Kegeles et al, 2000). In rats, infusion of competitive NMDAR antagonist CPP to mPFC increases the extracellular dopamine and its metabolites DOPAC and HVA in the nucleus accumbens (NAc) as well as motor activity. Furthermore, simultaneous injection of GABAA receptor agonist muscimol into mPFC attenuated the increase in the dopamine in the amygdala and hippocampus (Del Arco et al, 2011). These results suggested that CPP infusion evokes cortical disinhibition by the NMDAR blockade at the synapses on the GABAergic neurons, which may result in subcortical dopamine hyperactivity. However, a direct assessment to test this hypothesis has not been explored. Furthermore, no studies have been addressed the impact of NMDAR blockade on dopamine alteration in PFC of human or animals.

Methods: We used two distinct NMDAR hypofunction mouse models, in which conditional GluN1 (NR1) deletion is introduced in a subset (~50%) of cortical and hippocampal GABA neurons in early postnatal development (gender mixed, 12-18 weeks old) or in adulthood (gender mixed, 16-24 weeks old), respectively (Belforte et al, 2010). Notably, over 80-90% of parvalbumin (PV)-containing GABA neurons in the mPFC are GluN1-ablated, while 5-10% of PV local neurons are affected in the striatum. We first examined psychostimulant-induced locomotor activity before and after i.p. injection of amphetamine (2.5 mg/kg) or methamphetamine (1.5 mg/kg). Next, we measured the tissue dopamine and its metabolites by tissue HPLC using postnatal KO mutant mice. Finally, we measured the extracellular dopamine from accumbens and prefrontal cortex by in vivo awake microdialysis techniques. Two days after the surgery of implantation the cannula into the prefrontal anterior cingulate/prelimbic or the NAc lateral shell of the left brain hemisphere, baseline samples were collected every 20 min for 60 min. The mice were then injected with amphetamine (2.5 mg/kg, i.p.) and additional samples were collected every 20 min for the next 120 min. Dialysis samples were analyzed for dopamine by HPLC. Data are given as mean ± SEM. Statistical comparisons have been performed with the unpaired Student's t-test for tissue HPLC and repeated ANOVA for behavior and microdialysis.

Results: Both amphetamine and methamphetamine showed an increase in the horizontal locomotor activities in the open field. However, the postnatal GluN1 KO mutant mice show much higher activity in response to the both stimulants compared to the controls ($F(1, 16) = 1.793$ and $P = 0.01$ for amphetamine, $F(1,13) = 4.93$ and $p = 1.74E\text{-}11$ for methamphetamine, repeated ANOVA). Tissue HPLC analysis revealed a slight increase in mutant DOPAC level

($p < 0.046$, $n = 8$ for controls, $n = 7$ for mutants, t test), while no difference was detected in dopamine and HVA levels. In iv vivo brain microdialysis, there were no differences in the baseline dopamine levels in mPFC ($p = 0.51$, t-test) and NAc ($p = 0.56$, t-test) before the drug treatment. However, amphetamine-induced dopamine release increased 21.5-fold from the baseline in the NAc lateral shell of the postnatal KO mice, whereas only 5-fold increase in the floxed control mice (genotype result, F (1, 10) = 9.4, $p = 2.3E-06$, $n = 7$ for controls, $n = 5$ for mutants, repeated ANOVA). In a separate cohort, no obvious amphetamine-induced dopamine increase was detected from the baseline level in mutant mPFC, whereas 4.7-fold increase was observed in the control mPFC (genotype result, F (1, 16) = 2.7, $p < 0.039$, $n = 9$ for controls and mutants, repeated ANOVA). In adult GluN1 KO mutant mice, while there was no difference in baseline dopamine level in mPFC compared to the controls ($p = 0.56$, t-test), the levels of amphetamine-induced dopamine increase in mPFC were comparable between the two (genotype result, F (1, 11) = 0.50, $p = 0.73$, $n = 8$ for controls, $n = 5$ for mutants, repeated ANOVA).

Conclusions: GABAergic NMDAR hypofunction occurring in cortical and hippocampal GABA neurons during early postnatal development is crucial for later emergence of dual dopamine dysregulation in both striatum and mPFC. In particular, blunted prefrontal dopamine release in response to psychostimulant recapitulated the phenotype reported in the in vivo PET imaging study with patients with schizophrenia (Slifstein et al, 2015), further suggesting a role of GABAergic NMDAR hypofunction in schizophrenia pathophysiology.

Keywords: Dopamine, NMDA Receptor, GABA Neuron, mPFC, Accumbens.

Disclosure: Nothing to disclose.

M194. A Perceptual Inference Mechanism for Hallucinations Linked to Striatal Dopamine

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Background: Increased dopamine release in the striatum may be the best characterized neurobiological substrate of psychosis in schizophrenia; however, how striatal dopamine excess leads to auditory hallucinations, a cardinal symptom of psychosis, remains unclear. Bayesian models of perception and inference are becoming an increasingly popular framework for understanding the computational mechanisms underlying psychotic symptoms, including hallucinations and delusions. These models postulate that the brain represents context-dependent expectancies as well as the uncertainty associated with these expectancies to shape subjective perception and decision making. Here we tested whether the influence of uncertainty on subjective perception could represent a cognitive mechanism underlying hallucinations in schizophrenia and whether it relates to inter-individual variability in striatal dopamine release capacity.

Methods: We employed a novel auditory task, the Variable Context Tone Reproduction (VCTR) task, in which participants listened to pure tones (1000 Hz) and reproduced the duration of 700 ms target tones preceded by 2-4 context tones. While the duration of target tones did not vary, series of context tones preceding the reproduced target tone differed in both mean tone duration (context mean: long [1080 ms], intermediate [700 ms], or short [543 ms]) and variance in tone duration across the tones within a context train (context variance: high, low). We first tested this task in a validation cohort of 30 healthy individuals to establish its ability to induce shifts in subjective perception that were degraded by contextual variance (i.e., its ability to induce an effect of contextual uncertainty on perception). We then used this task in a separate cohort of 16 unmedicated patients with schizophrenia and 17 matched healthy controls, most of whom received PET imaging scans using the radiotracer [11C]raclopride. Both the VCTR task and PET scans were acquired before and after amphetamine administration. The percent change in striatal binding potential from pre- to post-amphetamine (ΔBPND) provided an index of striatal dopamine release capacity. Intensity of hallucinations and other symptoms were measured using the Positive and Negative Symptom Scale (PANSS).

Results: The mean duration of context tones altered subjective perception of target tones ($p < 10\text{-}9$), an effect that was degraded by introducing variability in context tone duration (all $p < 0.01$). Our primary measure of interest, the influence of contextual variance on shifts in subjective perception (the uncertainty effect, measured as the interaction of context mean by context variance) did not differ between schizophrenia patients and healthy controls. However, in patients, the severity of auditory hallucinations correlated to the uncertainty effect even when controlling for other symptom types (Spearman's $\rho = 0.70$, $p = 0.008$) indicating that subjects with more severe hallucinations tended to perceive target tones as closer to the context tones in conditions of higher uncertainty (i.e., hallucinators tended to exhibit an assimilation illusion under uncertainty). We next tested whether stimulating dopamine release via an amphetamine challenge would induce such an assimilation-under-uncertainty pattern and found that this was the case for subjects who did not already show such a pattern in the pre-amphetamine condition ($\beta = 0.04$, $p = 0.003$). Finally, we found that assimilation under uncertainty, the perceptual pattern specifically associated with hallucinations, was associated with greater striatal dopamine release capacity across all subjects (Spearman's $\rho = 0.57$, $p = 0.014$) and that patients with greater striatal dopamine release had more severe hallucinations (Spearman's $\rho = 0.71$, $p = 0.022$).

Conclusions: To the best of our knowledge, this is the first report providing direct evidence in favor of Bayesian models of psychosis that emphasize the role of uncertainty and its control via neuromodulators such as dopamine. Consistent with such models, our findings suggest that auditory hallucinations may result from a tendency to perceive stimuli as closer to (top-down) prior expectations (i.e., from perceiving what is expected, known as an assimilation effect) and particularly so in conditions where the expectations are uncertain (assimilation under uncertainty). Furthermore, we found that the assimilation-under-uncertainty pattern associated with hallucination severity depended on dopamine: it was exaggerated by amphetamine administration and correlated with dopamine release capacity in the striatum. Although not incompatible with the role of glutamate in

perception, these findings suggest a specific computational mechanism that may be disrupted by striatal dopamine dysfunction leading to abnormal integration of expectations into perceptual experiences and ultimately resulting in hallucinations.
Keywords: Perception, Schizophrenia, Auditory Hallucinations, Dopamine, Bayesian Inference.
Disclosure: Nothing to disclose.

M195. Use of a GABA-Optimized MEGA-PRESS Sequence for Glutamate Measurement

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Background: Magnetic resonance spectroscopy (MRS) is the application of radio frequency pulses to tissue in a strong magnetic field to detect and measure metabolites of interest. Applied to the central nervous system, 1H-MRS is an invaluable method to investigate in vivo neurochemical profiles in brain tissue in specific regions. Glutamate and γ-amino butyric acid (GABA) are two MRS-visible molecules of particular interest due both to their function as the primary excitatory and inhibitory neurotransmitters, as well as to their prominent roles in current models of neurotransmitter derangements in neuropsychiatric illnesses. Thus, it would be ideal to be able to measure both neurotransmitters simultaneously from the same brain region. Because GABA is present at lower concentration than several other molecules whose resonances overlap that of GABA, the GABA signal cannot be measured by the conventional MRS sequences typically used to measure glutamate. To address this problem, GABA-optimized, J-difference spectral editing sequences have been developed. The most widely used is the MEGA-PRESS sequence, which is comprised of interleaved acquisitions employing editing (on-resonance) alternating with non-editing (off-resonance) MEGA pulses. By subtracting the off- from the on-resonance spectrum, a "difference" spectrum is produced in which a GABA peak can be isolated for more accurate measurement. However, it has not been established whether glutamate can be accurately measured from in vivo spectra acquired with a GABA-optimized MEGA-PRESS sequence on clinical scanners. Glutamate resonances co-edit with GABA when using the most common GABA-optimized MEGA-PRESS sequence and can be measured in the difference spectra. In addition, the off-resonance MEGA-PRESS spectra are highly similar to conventional PRESS spectra and offer an alternate means of measuring glutamate with curve-fitting algorithms like LCModel. The purpose of the current study is to compare the LCModel estimates of glutamate values from GABA-optimized MEGA-PRESS off-resonance and difference spectra to the LCModel estimates of glutamate values derived from a standard PRESS sequence. Schubert et al, (2004) suggest that a PRESS sequence with a TE of 80 msec may be optimal for obtaining a measurement of glutamate uncontaminated by glutamine and other nearby resonances. Thus, we use a PRESS sequence with TE = 80 as our gold standard for glutamate in this report.

Methods: MRS data were obtained from a 16 cc voxel in the left DLPFC in 18 healthy volunteers with a Siemens TIM-Trio scanner using two different pulse sequences: 1) PRESS (TE/TR = 80/1500, 160 averages, total scan time = 4 minutes) and 2) GABA-optimized MEGA-PRESS (TE/TR = 68/1500, 288 averages, total scan time = 14.4 minutes, edit pulse BW = 45 Hz, on-resonance = 1.9 ppm, off resonance = 7.5 ppm). Glutamate values were estimated using LCModel from the PRESS spectra (P80), the off-resonance MEGA-PRESS spectra (MP-O), and the MP difference spectra (MP-D). Glutamate values were normalized to creatine, except for the MP-D spectra, where they were normalized to N-acetyl aspartate (NAA). We used coefficients of variation (COV) across the 18 healthy volunteers to estimate variance due to measurement error for the glutamate values obtained from each method. We used correlations to compare the two MP methods to the P80 method, our gold standard.
Results: COVs for glutamate were similarly low for P80 and MP-O spectra (6.6% & 6.1%), and much higher for the MP-D difference spectra (12.5%). Glutamate values from P80 and MP-O spectra were highly correlated (r = .82), while glutamate values from the MP-D spectra were uncorrelated with either P80 or MP-O values (r = -.29 & r = -.28). This pattern of results was unchanged when glutamate values from all three types of spectra were normalized to creatine or to NAA, or when LCModel estimates of glutamate + glutamine (Glx) were used instead of glutamate.
Conclusions: LCModel estimates of glutamate derived from GABA-optimized MEGA-PRESS off-resonance spectra closely approximate those derived from conventional PRESS spectra using TE = 80, in terms of relative concentrations. Both methods have similarly low variance attributable to measurement error. In contrast, LCModel estimates of glutamate derived from MEGA-PRESS difference spectra are more variable and do not provide useful information about glutamate content. Thus, by using the off-resonance spectra for measuring glutamate, it is feasible to use a single MEGA-PRESS scan sequence for both glutamate and GABA. This allows for simultaneous measurements of both metabolites, without extending total scanning time by including a conventional PRESS scan.
Keywords: 1H MRS, GABA, Glutamate, Neurotransmitters.
Disclosure: Nothing to disclose.

M196. Reproducible Results in Human Neuronal-Like Cells Transdifferentiated From Blood Circulating Monocytes

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Background: Despite progress, our understanding of psychiatric and neurological illnesses at a cellular level remains poor, due in part, to the inability to access neurons directly from patients. The paradigms currently available such as Induced Pluripotent Stem Cells (iPS) and Olfactory Neuroepithelial Cells are beginning to bring much needed questions to the field. But significant work remains, including the

search for a practical and less invasive method to obtain neuronal-like cells with the capacity to deliver reproducible results.

Methods: We have developed a model to transdifferentiate blood circulating monocytes into neuronal-like cells in only twenty days by combining different growth factors, antioxidants and conditioned media. This model provides a window into the neurodevelopment of adult individuals and only requires a standard blood sample. Unlike other models such as iPS cells, the genome is not altered with viral insertions which can become a confounder in illnesses with a strong but still misunderstood genetic component such as schizophrenia and autism.

Results: We have transdifferentiated monocytes into neuronal-like cells from over 50 individuals and established that transdifferentiated neuronal-like cells resemble human neurons early in development, express several neuronal markers and present spontaneous action potentials as well as postsynaptic inhibitory and excitatory currents. During differentiation, these cells undergo similar structural stages to those present in neurons while developing from rounded neuroblasts. We have also determined that when these neuronal-like cells are exposed to either dopamine or colchicine, they respond similarly to neurons by retracting their neuronal arborizations. In addition, we recently obtained blood samples from healthy individuals at two different points in time and tested differentiation rates, neurite arborizations and expression of dopamine receptors. These neuronal-like cells deliver reproducible results in all the examined areas.

Conclusions: Monocytes can be consistently transdifferentiated into neuronal-like cells that resemble human neurons during early brain development. Moreover, transdifferentiation of monocytes obtained from two blood samples from the same individual but separated by weeks and even months delivers consistent results. These data collected from several individuals suggest that our model can be used to compare cells from patients and controls without the potential confounding factor of differences between samples from the same person.

Keywords: Stem Cells, Disease Model, Neurons, Neruopil, Neurodevelopment.

Disclosure: Nothing to disclose.

M197. Bioenergetic Uncoupling of Astrocytes and Neurons May Underlie Cognitive Deficits in Schizophrenia

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Background: Pharmacologic, genetic and theoretical considerations have firmly established schizophrenia as a disorder of synapses. Converging evidence from human schizophrenia studies and animal models of "broken" synapses suggest developing a brain with defective synapses yields metabolic abnormalities. This may be due to failure of astrocytes to develop or maintain metabolic coupling with neurons. Neurotransmission relies heavily on this metabolic coupling. Glycogen rich glial cells rapidly synthesize pyruvate from glucose, convert pyruvate to lactate via lactate dehydrogenase (LDH), and transport lactate via monocarboxylate transporters (MCTs) to neurons for energetic use (the astrocyte-neuron "lactate shuttle"). Working memory performance, long term potentiation, and long term memory formation in rodents are impaired following disruption of the MCTs and bioenergetic coupling. This suggests bioenergetic coupling through the lactate shuttle and neurotransmission are tightly coupled to cognitive function, and these pathways could be important pathophysiological substrates in schizophrenia. We hypothesize there are defects in the lactate shuttle which may contribute to cognitive deficits in this illness. We utilized human postmortem substrate as well as the NR1 N-methyl-D-aspartate receptor (NMDAR) subunit knockdown (KD) model of schizophrenia.

Methods: Commercially available assays were used to assess the activity of LDH (Sigma-Aldrich, MAK066) in postmortem samples. Each sample was assayed with and without a specific inhibitor (in triplicate) and normalized to protein loaded into the assay. We probed for differences in protein expression using western blot analysis.

In NR1 knockdown animals, we affinity purified PSD-95 protein complexes from the frontal cortex of NR1 and WT mice ($n = 3$ per group) and ran each sample through our liquid chromatography-tandem mass spectrometry (LC-MS/MS) protocol in singlicate. We performed pathway analysis with the EnRICHr suite of bioinformatic tools and compared wildtype to NR1 KD PSD-95 interactomes using the top 20 differentially expressed proteins. Finally, we performed a reverse transcription polymerase chain reaction (qPCR) pilot study on 5 genes important in the lactate shuttle system (MCT1, MCT2, MCT4, GLUT1, and GLUT3).

Results: In postmortem tissue, we found a significant decrease in LDH enzyme activity in the dorsolateral prefrontal cortex (DLPFC) in schizophrenia ($n = 16$ per group). LDH activity was unchanged in the frontal cortex of chronically treated antipsychotic rats ($n = 10$ per group) compared to vehicle. We also measured LDH activity in a set of rodent brains simulating 3 postmortem interval (PMI) time points and did not find any appreciable changes in LDH activity out to 24 hours PMI. We did not find any changes in LDH, LDHA, LDHB, HXK, or MCT1 protein in schizophrenia ($n = 20$ per group). In our pathway analysis study, wildtype mice show increased signaling through pathways relevant for synaptic plasticity (as expected), while the NR1 KD analyses yielded several metabolic pathways with direct links to the lactate shuttle. We found a 63% decrease in astrocytic MCT4 and 60% decrease in neuronal GLUT3 transcripts in NR1 mice when compared to wildtype ($n = 5$ per group).

Conclusions: LDH interconverts pyruvate and lactate and is a key enzyme in both mitochondrial respiration and glycolysis. Our data suggest that LDH enzyme capacity, rather than expression, is defective in schizophrenia. Disruption of glycolytic capacity could diminish the ability of astrocytes to supply neurons with energetic substrates, which can directly impact cognitive tasks such as working memory. These results do not appear to be due to a medication or PMI effect. Similar to metabolic deficits seen in schizophrenia, we found the NR1 KD model to have region-level metabolic abnormalities including altered

glycolytic pathways and decreased lactate shuttle transcripts. The top differentiated pathway from our proteomic analysis indicates a shift towards gluconeogenesis in the NR1 KD mouse. Lactate and pyruvate are common substrates for this energy demanding metabolic pathway, and an increase in lactate/pyruvate shunting into mitochondrial respiration and gluconeogenesis could result in diminished lactate supply for neuronal consumption. This is also supported by our transcript studies showing a substantial decrease in the astrocytic transporter MCT4 moving lactate from astrocytes into the extracellular space for neuronal uptake. This suggests biochemical changes directly affecting the capacity to produce and transport lactate from astrocytes to neurons and may contribute to pathophysiology of schizophrenia.

Keywords: Postmortem, NMDA Receptor Knockdown, Lactate Shuttle, Metabolic Defect.

Disclosure: Nothing to disclose.

M198. Structure and Function of the Executive Network Across the Psychosis Spectrum

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Background: Executive function deficits are well established in individuals with schizophrenia, and due to their presence across illness phases and in unaffected relatives, are often considered to be an endophenotype for the disorder. These deficits are difficult to treat, and have been associated with poorer long term outcomes. However, beyond schizophrenia, the presence of executive dysfunction in individuals across the broader range of psychotic disorders has not been as frequently investigated. Here, we sought to examine the neural basis of executive function deficits across a sample of youth with a range of psychotic spectrum disorders.

Methods: 51 young individuals with diagnoses across the psychosis spectrum (schizophrenia, schizoaffective, psychosis NOS, major depressive disorder with psychosis, bipolar with psychosis), and 53 age matched unaffected controls participated in the Multimodal Evaluation of Neurodevelopmental Disorders (MEND) study at Zucker Hillside Hospital, which included multimodal neuroimaging (task based functional MRI, resting state fMRI, diffusion tensor imaging (DTI)), cognitive testing, and diagnostic clinical interviews. Participant ages ranged from 14-23, with the aim of assessing functional and structural differences present during the time period most commonly associated with illness onset.

Results: Even in a group that included a wide range of psychotic disorders, we found disruptions in the executive network across neuroimaging modalities. Behaviorally, the patient group showed impaired working memory performance as measured by the MATRICS Battery, and across all patients the degree of impairment correlated with the level of positive symptoms on the Brief Psychiatric Rating Scale (BPRS). We then explored the neural circuitry that supports executive functions. First, in our task based fMRI analysis, we found that during a working memory task, even when matched for performance, patients showed altered activation in the executive network relative to controls. Secondly, in the resting state fMRI analysis, we found an alteration in patients in the functional connectivity between the executive network and another large scale intrinsic brain network, the salience network. Finally, in the DTI analysis of structural connectivity, the superior longitudinal fasciculus (SLF) the main fronto-parietal connection showed decreased white matter integrity in the patient group.

Conclusions: We found evidence for disruptions in the structure and function of the executive network in young individuals with early stage psychotic spectrum disorders. Furthermore, across individuals with a range of symptom severity and illness types, working memory deficits scaled with level of psychotic symptoms, potentially indicating a specificity for psychosis. Thus, executive network function and working memory performance should be assessed in individuals not only with schizophrenia, but across the psychosis spectrum, and may be important targets for intervention.

Keywords: Schizophrenia, Development, Executive Function, Neuroimaging.

Disclosure: Nothing to disclose.

M199. Whole Brain Spectroscopic Imaging of Glutamate in Antipsychotic-Naïve and Minimally-Treated First-Episode Psychosis

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Background: Proton magnetic resonance spectroscopy (H-MRS) single-voxel studies of schizophrenia generally report increases in glutamatergic compounds (glutamate, glutamine or glutamate+glutamine -Glx) in different gray and white matter brain regions. However, the spatial distribution of these abnormalities has not been examined. We used echo planar spectroscopic imaging (EPSI), a 3-dimensional H-MRS that facilitates high-resolution volumetric (multisection) acquisition at short time-to-echo (TE), to examine Glx and N-acetyl-aspartate compounds (NAAc, a marker of neuronal viability), in psychotic subjects before and following treatment with antipsychotic medication.

Methods: Twenty-seven subjects seeking treatment for their first episode of psychosis and seventeen healthy control subjects were studied at baseline. Nineteen schizophrenia/schizophreniform and 8 bipolar subjects were studied. Seventeen patients were re-imaged following antipsychotic treatment. We acquired EPSI at 3 Tesla with a 32 channel coil with the following parameters: TR/TE = 1551/17.6 msec; spatial array of 50 x 50 x 18, FOV of 280 x 280 x 180 mm3 (corresponding to a nominal voxel size of 5.6 x 5.6 x 10 mm3); flip angle of 71°; complex points = 512; bandwidth of 1024 Hz; and number of signals acquired, one. Water suppression with frequency-selective saturation pulses and inversion-recovery nulling of lipid signal was performed with an inversion time of 198 milliseconds (total duration = 26 min). Glx and NAAc were fitted with MIDAS.

Results: At baseline, Glx was increased in the psychotic group in one cluster encompassing the Rolandic operculum and the superior temporal gyrus on the right hemisphere

(FDR alpha = 0.05, for a cluster of 125 2x2x2mm voxels). No differences in NAAc survived FDR correction. Finally, there were no significant changes in Glx or NAAc in the psychotic patients following treatment.
Conclusions: Increases in glutamatergic metabolism are apparent early in the course of psychosis in the intersection of the central sulcus with the superior temporal gyrus in the right hemisphere. However, there is no evidence of neuronal damage at this stage of psychosis. This is consistent with our recent 2-dimensional H-MRS study showing persistently increased cortical Glx with NAAc changes later in the course of schizophrenia.
Keywords: Glutamate, First Episode Schizophrenia, Bipolar, N-acetylaspartate.
Disclosure: Otsuka: Speaker, Self.

M200. Intact Emotion Processing in the Context of Reduced Prefrontal Control in Individuals With Schizophrenia

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Background: Anhedonia, or the reduced capacity to experience pleasure, is a well-documented feature of schizophrenia. Despite this, recent work has shown that emotional stimuli can elicit similar responses in limbic and paralimbic networks for schizophrenia patients and healthy comparison subjects (Ursu et al, 2011). Consequently, the neural correlates of anhedonia may instead be more specifically associated with a disruption of prefrontally-mediated anticipation and maintenance of emotional experience.
Methods: To further investigate this relationship, recent onset individuals with schizophrenia ($n = 28$) and healthy controls ($n = 36$) participated in a fMRI paradigm similar to Heerey & Gold (2007) in which participants make button presses based on the emotions they experience. Participants first view an affective target picture, which they are instructed to experience as deeply as possible because they subsequently have the opportunity to indicate whether they want to see the target image again. The target picture is followed by a neutral or affective distractor image. Finally, a response phase is presented where the individual can either indicate a "want" response if they want to see the target image again, a "no want" response to not see the target again, or a "no care" response if the individual does not have strong feelings about the target image. The goal of this task is to maintain the emotion generated by the target stimulus, continue to maintain this emotion during the distractor stimulus, and subsequently respond at the end of the trial to indicate the desire to see the target item again. For the purposes of these preliminary analyses, evaluation of the distractor phase of the task is the primary focus. All data was collected on a 3T Siemens Trio scanner and processed using SPM8. Group random effects analyses for the emotional versus neutral distractor contrast were height thresholded at $p < .001$ and FWE cluster corrected at $p < .05$. DLPFC and amygdala regions of interest were derived a priori from the WFU PickAtlas.
Results: Behaviorally, individuals with schizophrenia showed similar patterns of responding, indicating comparable interest in viewing emotional stimuli. However, individuals with schizophrenia made significantly more "unexpected" responses, for example, giving a "want" button press to an unpleasant target stimulus or a "no want" button press to a pleasant target stimulus. When comparing patients and controls on brain activity during the emotional distractor versus the neutral distractor, both groups showed comparable ability to modulate limbic and prefrontal activity based on the affective nature of the stimulus. However, analyses of brain activity while viewing distractor images versus fixation showed significantly less activity throughout limbic and cognitive control regions in individuals with schizophrenia compared to controls.
Conclusions: These findings highlight a pattern of generally intact emotion processing in individuals with schizophrenia, in the context of unusual patterns of responding. The higher rate of "unexpected" responses may indicate an increased propensity to distraction during emotion maintenance in individuals with schizophrenia. However, while the schizophrenia group showed less prefrontal and amygdala activity in general, the ability to increase prefrontal and amygdala activity in the context of emotional versus neutral distraction was not different from the control group. These data support an intact network for emotion processing but a general impairment in prefrontal cognitive control circuitry in schizophrenia.
Keywords: Emotion Processing, Functional MRI (fMRI), Schizophrenia, Anhedonia.
Disclosure: Nothing to disclose.

M201. The Limited Resource Model of Auditory Refractoriness in the Rhesus Monkey and its Link to Auditory Deficits in Schizophrenia

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Background: Individuals with schizophrenia (SZ) show decreased performance in simple auditory tasks that have been linked to impaired encoding of information into perceptual short-term memory (pSTM). In addition, SZ show abnormal auditory refractoriness, a non-invasive electroencephalographic (EEG) measure believed to reflect the formation and gradual decay of a basic pSTM trace. Auditory refractoriness refers to the finding of smaller EEG responses to tones preceded by shorter periods of silence. To date its physiological mechanisms remain incompletely understood, thus limiting the insights gained from findings of abnormal refractoriness in SZ. Furthermore, the functional properties and the content of the pSTM trace encoded via refractoriness have never been studied in detail thus preventing direct links between the altered physiological biomarker and the behavioral deficit.
To address these open questions, we developed a physiologically inspired model, the limited resource model of

refractoriness, that quantifies duration, selectivity and capacity of the pSTM trace encoded via refractoriness. Further, we made detailed measurements of auditory refractoriness in the rhesus macaque across the systems-, circuit- and single-cell level using a novel paradigm that avoids confounds from high-level processes related to stimulus predictability. Finally, we fit the model to the data from all three levels of observation to provide a detailed description of what information is encoded.

Methods: We measured auditory refractoriness in the rhesus macaque (macaca mulatta), one of the most relevant animal models of auditory function and dysfunction in SZ due to important perceptual, functional and (micro-)structural similarities to the human. At the systems level, we used grids of up to 32 chronically implanted cranial EEG electrodes. At the circuit level, we recorded laminar local field potential- (LFP) and current-source density- (CSD) profiles using multi-contact linear electrode arrays in the superior temporal plane. At the neuron-level we extracted extracellular single- (SUA) and multi-unit spiking activity (MUA) from the same electrode arrays. Animals passively listened to sounds whose identity and timing was random thus preventing animals from forming valid predictions about upcoming sounds. Stimulus onset asynchrony ranged between 0.2 and 12.8 sec to encompass the clinically relevant time-scale. All methods were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

Results: The limited resource model views refractoriness as the expenditure and gradual recovery of a limited physiological resource that determines generator excitability. In the model, recovery time-constant τ maps onto the duration of the memory trace, and expended fraction U determines both encoding strength and content of the trace. At the systems level, model fits indicate that processing of each tone expends ~65% of the available resource. This relatively high level of expenditure in combination with the relatively slow rate of recovery (time-constants between 0.5 and 2 seconds) leads to situations in which preceding tones are 'overwritten' because little resource is left to encode new tones. This creates a non-linearity that biases the content of the pSTM trace towards the most recent tone ("single-item memory"). Ongoing experiments quantify the expended fraction and other properties at the circuit- and single-cell level. Our preliminary data shows that time-constants for LFP/CSD and MUA/SUA are similar to the ones identified for EEG. In several cases, expended fraction at the circuit level is lower than at the systems level. Hence, the content of the trace at this level is less biased towards the last item and instead weighs recent tones according to temporal proximity ("average item memory"). At the neuron level, we have identified several units that match the high expended fraction observed in the EEG data. Data presented at the meeting will include a more complete sampling of activity across several auditory cortex regions in the superior temporal plane.

Conclusions: The observed time-constants and expended fractions are largely consistent with the notion that auditory refractoriness of cranial EEG recordings is caused by short-term depression of synapses in auditory cortex. Ongoing experiments are further testing this hypothesis and the related notion that altered refractoriness in SZ may result from altered short-term synaptic plasticity. On the pre-synaptic side, a number of processes contribute to short-term synaptic plasticity such as vesicle recycling and release probability. Release probability is regulated by multiple proteins, some of which, e.g., synaptophysin and voltage-gated calcium channels, are altered in SZ. If the link between refractoriness and synaptic plasticity is confirmed by ongoing experiments, we speculate that vesicle release probability may be a target for novel pharmacological interventions to ameliorate pSTM deficits in SZ.

Keywords: Auditory Deficits in Schizophrenia, Auditory Short-Term Memory, Short-Term Synaptic Depression, Non-Human Primate, Electroencephalography.

Disclosure: Nothing to disclose.

M202. Schizophrenia With History of Alcohol Use Disorder Suffers a Worse Course of Psychosis: Results From NIMH Catie Study Analyses

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Background: Patients with schizophrenia are known to have difficulty with reward processing. History of alcohol use disorder (AUD) in schizophrenia may identify a phenotype representing a heightened deficit in reward processing. Therefore, these patients with comorbid AUD may suffer a worse course of schizophrenia. Prospective studies characterizing the long-term course of schizophrenia complicated by AUD are lacking.

Objective: To compare the course of schizophrenia in patients with or without a history of AUD over a period of 18-months.

Methods: This study examined time to exacerbation of schizophrenia in outpatients who were enrolled in the first phase of the National Institute of Mental Health (NIMH) Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study ($n = 1432$)1. This first phase of CATIE prospectively followed this cohort for up to 18 months or until antipsychotic treatment was discontinued. Patients were categorized into those with a history of AUD (Schiz + AUD) in the past 5 years and those without AUD (Schiz only). Further, a subset of patients identified as having controlled symptoms at baseline defined as Positive and Negative Symptom Scale (PANSS) score between 50 and 90 were also categorized into Schiz + AUD or Schiz only groups. Subjects with non-AUD drug use disorders were excluded from both data sets. The primary outcome for this analysis was time from randomization to the first event of exacerbation. Exacerbation was defined as an event meeting any of the following criteria during phase 1 of CATIE: hospitalization, worsening in PANSS, clinically significant aggression or suicidal/homicidal ideations, use of rescue medication, emergency room visit, discontinuation due to lack of efficacy, and arrest or incarceration.

Results: Of the 1262 participants without non-AUD substance disorder history, 303 had Schiz + AUD and 959

had Schiz only. Time to exacerbation of schizophrenia was significantly shorter in patients with Schiz + AUD (median: 3.8 vs. 6.1 months, $p<0.001$, hazard ratio of 1.45 [95% CI: 1.23, 1.70]). In the subset of patients with PANSS 50-90, 129 participants had Schiz + AUD and 437 had Schiz only. Time to exacerbation of schizophrenia was also significantly shorter in this cohort of patients as well (median: 6.0 vs. 8.1 months, $p=0.019$, hazard ratio of 1.36 [95% CI: 1.05 to 1.75]).

Conclusions: Patients with schizophrenia complicated by AUD suffered a worse course of psychosis in the CATIE phase 1 cohort. Additional outcomes and more effective interventions need to be examined in this important, yet understudied population.

Keywords: Schizophrenia, Alcohol use Disorder, Psychiatric Comorbidity.

Disclosure: Alkermes, Bristol-Myers Squibb, Forum, Gerson Lehrman Group, IntraCellular Therapies, Janssen/J&J, Lundbeck, Medavante, Medscape, Otsuka, Pfizer, ProPhase, Sunovion, Supernus, Takeda, and Teva: Income source & Equity of $10,000 per year or greater, Self.

M203. Altered Gradients of Glutamate and GABA Transcripts in a Distributed Cortical Circuit in Schizophrenia

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Background: Visuospatial working memory (vsWM), which is impaired in schizophrenia, requires information transfer from primary (V1) and association (V2) visual cortices in the occipital lobe to posterior parietal (PPC) and dorsolateral prefrontal (DLPFC) cortices via excitatory pyramidal neurons in layer 3. In primate cortex, the layer 3 excitatory, glutamate-containing neurons in V1 have a lower density of dendritic spines, less complex dendritic arborization, and smaller soma sizes compared with the homologous layer 3 pyramidal neurons in the DLPFC. The activity of layer 3 excitatory neurons is shaped by local inhibitory neurons. The relative number of these GABA-containing neurons, and the expression of GABAA receptor α1 subunit mRNA, are higher in V1 than DLPFC. These findings suggest that caudal and rostral cortical regions may differ in the relative amounts of glutamate and GABA inputs. Interestingly, recent transcriptome studies reported the presence of rostro-caudal gradients of mRNA expression in the primate neocortex. Whether these gradients are present in cortical layer 3 for glutamate and GABA system transcripts in postmortem human tissue and if they are preserved in schizophrenia have not been studied. Therefore, we sought to answer the following questions: 1) Are gradients in expression present across regions of the vsWM network? 2) Are these gradients altered in schizophrenia? 3) Is any disease effect conserved across regions?

Methods: Using laser microdissection, tissue samples of layer 3 were obtained from V1, V2, PPC and DLPFC from 20 matched pairs of schizophrenia and unaffected comparison subjects. Quantitative PCR was used to measure mRNA levels of functionally analogous transcripts of the following glutamate and GABA system: glutamate (GLS1) and GABA (GAD67) synthesizing enzymes; vesicular glutamate (vGLUT1) and GABA (vGAT) transporters; synaptic glutamate (EAAT2) and GABA (GAT1) transporters; NMDA receptor subunit (GRIN1); AMPA receptor subunit (GRIA2); and GABAA receptor subunit (GABRG2) in all samples.

Results: To assess the presence of mRNA expression gradients, we measured the levels of these glutamate and GABA transcripts in unaffected comparison subjects. Transcript levels for the glutamate markers, EAAT2 ($F3,56=19.2$, $p<0.001$), vGLUT1 ($F3,56=57.1$, $p<0.001$) and GRIA2 ($F3,56=59.3$, $p<0.001$), showed a caudal-to-rostral gradient, with lowest expression in visual cortices and highest in DLPFC. In contrast, the GABA transcripts, GAD67 ($F3,56=4.5$, $p<0.007$), vGAT ($F3,56=11.8$, $p<0.001$) and GABRG2 ($F3,56=34.1$, $p<0.001$), showed the opposite gradient with highest expression in visual cortices and lowest in DLPFC. To determine if these regional gradients were altered in schizophrenia, we generated normalized composite measures for the five glutamate and four GABA transcripts studied. These measures confirmed the presence of opposite regional gradients for glutamate ($F3,114=14.5$, $p<0.001$) and GABA ($F3,114=5.4$, $p=0.002$) system transcripts in unaffected comparison subjects. In contrast, in the subjects with schizophrenia, the regional gradient for glutamate transcripts was diminished ($F3,114=1.3$, $p=0.28$), whereas the regional gradient for GABA transcripts was enhanced ($F3,114=15.8$, $p<0.001$). That is, in the schizophrenia subjects both the glutamate and GABA system transcript levels were higher in V1 and lower in the DLPFC relative to the unaffected comparison subjects. Since the regional gradients of glutamate and GABA system transcripts were differentially altered in schizophrenia, we examined whether there was a disease effect on transcript levels that was conserved across regions. Levels of vGLUT1 mRNA were significantly lower in schizophrenia in all regions studied (V1: -18%, $p<0.01$; V2: -14%, $p<0.05$; PPC: -14%, $p<0.05$; DLPFC: -22%, $p<0.01$). Levels of EAAT2 mRNA were significantly higher in visual cortices (V1: +286%, $p<0.001$; V2: +258%, $p<0.001$), but not in DLPFC or PPC. All other glutamate and GABA system transcripts studied did not show an effect of illness that was conserved across regions. We also examined whether the difference between glutamate and GABA composite measures within a region was significantly different in schizophrenia subjects and found no significant effect of diagnosis on transcript expression ($F1,114=0.3$, $p=0.56$).

Conclusions: In layer 3, glutamate and GABA system transcripts exhibit opposite expression gradients across a vsWM cortical network, suggesting that molecular regulation of excitatory-inhibitory balance differs across cortical regions. Altered expression of some of these transcripts in schizophrenia may disrupt normal regional patterns of excitatory-inhibitory balance (i.e., markers of excitatory-inhibitory balance are downregulated in the DLPFC and upregulated in V1), contributing to the neural substrate for vsWM deficits in the illness.

Keywords: Schizophrenia, Glutamate GABA, Visuospatial Working Memory, mRNA Expression Gradients.

Disclosure: Nothing to disclose.

M204. New Techniques for Oxidative Stress Measurement: Potential Biomarker and Outcome Measure in Schizophrenia

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Background: Growing evidence suggests that inflammation, oxidative stress, and redox dysregulation may play a role in the pathophysiology of schizophrenia. Evidence shows that people with schizophrenia have lower levels of serum antioxidants such as glutathione and increased levels of defense enzymes, while their blood cells can have increased signs of oxidative damage such as lipid peroxidation. Interest in studying oxidative stress is increasing and some have suggested it may serve as a biomarker for the disease and also serve as a target or outcome measure for medication treatment response. However, the molecular mechanisms of oxidative stress and optimal ways to measure oxidative stress have yet to be determined. Many report levels of individual measurements in the blood as a proxy for oxidative stress; however, an alternative approach to detect oxidative stress is to adapt antioxidant capacity assays to provide a single global comprehensive measure of oxidative stress.
Methods: In our study we developed a technique to use a redox mediator to probe serum samples for chemical information relevant to oxidative stress. Specifically, we used an iridium (Ir) salt (K2IrCl6) to probe serum for reducing activities that can transfer electrons to iridium and thus generate detectable optical and electrochemical signals. We compared the reducing capacity in terms of trolox equivalents (a standard used for antioxidant reducing assays) between our assay and a commercial Copper (Cu) reducing assay in detecting the reducing contribution from low molecular weight components of serum such as ascorbic acid (AA), glutathione (GSH), and uric acid (UA). We then examined differences in mean serum measurements of 10 people diagnosed with schizophrenia and compared these to 5 healthy control subjects using both the Cu and Ir assays. We characterized the diagnostic performance using a Receiver Operating Characteristic (ROC) curve analysis to determine if the measurements could discern the schizophrenia group from the healthy controls. Lastly, we characterized psychiatric symptoms in schizophrenia, using the Brief Psychiatric Rating Scale (BPRS), and examined these in relation to global oxidative stress.
Results: Our data shows that the Ir-reducing assay can detect various biological reductants in serum and is especially sensitive to glutathione (GSH) compared to alternative existing assays. With the Ir-reducing assay we find that people with schizophrenia have a significantly lower average reducing capacity compared to healthy controls ($p < 0.05$). In contrast, the standard Cu-reducing assay shows no differences in oxidative stress between schizophrenia and healthy controls, likely due to its low sensitivity to thiols (i.e., glutathione). Furthermore, the calculated area under the ROC curve (AUC) values for the Ir-reducing capacity assay (with electrochemical detection) for serum was determined to be 0.92 (95% confidence interval (CI): 0.76–1.08; $p = 0.01$), which compares to the value of 0.6 for the Cu-reducing serum assay (95% CI: 0.26–0.94; $p = 0.54$). Within the schizophrenia group, we find a significant negative correlation in reducing capacity with BPRS positive symptoms ($r = -0.64$, $p = 0.048$) and anxiety/depressive symptoms ($r = -0.74$, $p = 0.015$).
Conclusions: Our results show a more sensitive approach to oxidative stress analysis, particularly for the inclusion of glutathione compared to commercially available measures. While it is a small preliminary sample, we also show that oxidative stress measured by this global measure may distinguish a schizophrenia group from healthy controls as a possible biomarker of the illness. Lastly, symptom severity, particularly positive and anxiety/depressive symptoms are higher in those with lower oxidative stress suggesting this as a target or outcome measure for future trials to improve oxidative stress parameters in schizophrenia.
Keywords: Schizophrenia, Oxidative Stress, Biomarker.
Disclosure: Nothing to disclose.

M205. Dysbindin-1 Knockout Mice Have Altered Signaling Downstream of the NMDA Receptor That is Associated With Impaired Fear Learning

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Background: Genetic and biochemical evidence suggests that Dystrobrevin-binding protein 1 (DTNBP1 or dysbindin-1) contributes to the pathophysiology of schizophrenia. Several studies have found genetic association between dysbindin-1 and schizophrenia. DTNBP1 haplotypes have been associated with a higher level of negative and cognitive symptoms in schizophrenia. Patients with schizophrenia exhibit reduced dysbindin-1 expression in the dorsolateral prefrontal cortex and hippocampus. Dysbindin-1 is expressed in axon terminals of glutamatergic pyramidal neurons, where it regulates glutamatergic transmission. Mutant mice that lack dysbindin-1 (dys-/-) have impaired postsynaptic NDMAR function in the prefrontal cortex (PFC) that is associated with cognitive deficits, a hallmark symptom of schizophrenia. These mice also show impairments in long-term potentiation, as well as imbalanced excitatory and inhibitory transmission. However, the cellular and molecular mechanisms underlying these changes are not well characterized.
Methods: Using dys-/- mice and their wild-type (WT) littermates, tissue from PFC, amygdala, and hippocampus (HIPP) were collected for western blot and high-performance liquid chromatographic analysis of amino acids. A trace-fear conditioning paradigm was used to explore learning and memory performance in dys-/- mice. Dysbindin protein levels were measured by Western blot in human amygdala tissue from controls and subjects with schizophrenia.
Results: We found that dys-/- mice exhibited reduced levels of freezing (impaired memory) compared to WT mice during contextual retrieval (24hr after training), while there was no difference in contextual extinction between groups.

Biochemically, we found that protein levels of phospho-Ca2+/calmodulin- dependent protein kinase II α (pCaMKIIα) and activity-regulated cytoskeleton-associated protein (Arc) were down-regulated in the PFC, amygdala and HIPP of dys-/- mice, whereas Akt signaling and serine racemase expression did not differ between groups. Additionally, the glutamate/glutamine ratio was decreased in the PFC and amygdala of dys-/- mice, with no change in the L-serine/D-serine ratio. We are currently examining the protein expression of dysbindin-1 in human amygdala tissue from controls and subjects with schizophrenia.

Conclusions: Our results demonstrate that elimination of dysbindin-1 in mice impairs fear memory and contextual retrieval in a trace-fear conditioning paradigm. This abnormality in learning and memory is likely due, at least in part, to a disruption in pCaMKIIα signaling and Arc expression, in brain regions important for fear memory. Furthermore, the changes in the ratio of glutamate to glutamine in dys-/- mice could be contributing to the alterations in glutamatergic transmission. Taken together, these results show that dysbindin-1 regulates pathways downstream of the NMDAR, which are essential for synaptic plasticity and learning.

Keywords: Schizophrenia, Dysbindin, pCaMKIIa, Glutamate.

Disclosure: This work was supported by NIMH ROOMH099252 to DTB.

M206. Replication of Significant Improvement in Auditory Verbal Hallucinations After 5 Days of Double-Blind, Sham Controlled Inhibitory (Cathodal) tDCS in Schizophrenia Patients Treatment Resistant to Antipsychotics

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Background: Approximately 70% of schizophrenia (Sz) patients experience auditory verbal hallucinations (AVH) as a significant presenting symptom. As opposed to older theories, which linked persistent AVH to psychodynamic aspects of Sz or to abnormal dopaminergic drive, more recent theories focus on persistent hyperactivity of auditory language regions located in left superior temporal gyrus (STG). This hyperactivity, in turn, may be related to impaired bottom-up and top-down, glutamatergic control over local inhibitory processes within auditory cortex. The localization of AVH to resting hyperactivity of left STG, suggests that anatomically based-, rather than pharmacologically based-, treatment approaches may be of particular benefit.

An alternate technique for modulation of brain function, termed transcranial direct current stimulation (tDCS) has been developed. In tDCS, extremely low (<2 mA) currents are applied to the scalp from devices powered by 9V "transistor" type batteries. Despite the low currents involved, significant modulation of underlying brain function can be observed. Because direct currents are used, the polarity of stimulation significantly modulates the effect. Anodal stimulation applied over a specific brain region, induces an enhancement in underlying activity, while cathodal stimulation induces an inhibition.

The present project follows up on a recently published study (Brunelin 2012) which showed highly significant, 31% reduction in AVH severity in a group of 15 Sz who received active tDCS stimulation vs. 15 treated with sham (Cohen's $d = 1.58$, $p < 0.001$).

Methods: Participants were randomized in a 1:1 ratio to 20 minute treatments of inhibitory (cathodal) active vs. sham tDCS per day over 5 consecutive days, following the procedures established by Brunelin 2012. Criteria were age between 18 and 55 with a SCID diagnosis of Sz or schizoaffective disorder, right handed, mean Auditory Hallucination Rating Scale (AHRS) item score >2 (moderate), and on stable dose of antipsychotic medication for at least 1 month.

The primary outcome was the AHRS assessed at baseline, following the final treatment, and at 1- and 3-month follow-up time-points. A subsample underwent fMRI and MRS assessments performed prior to and following treatment will evaluate the degree to which improvement of AVH is associated with normalization of AVH-related physiological abnormalities.

Results: 82 subjects were enrolled (active: 42 and Sham: 40) across the two sites. Primary analysis was conducted in the 78% who were outpatients (active: 33 and Sham: 31). There were no demographic or behavioral scale differences between active and sham groups. Baseline AHRS and PANSS total scores were similar between groups, and suggestive of moderate baseline symptoms.

Among outpatients, significant between group differences were seen for total AHRS ($F1,55 = 4.2$, $p = 0.046$, $d = 0.53$) and PANSS rated hallucinations ($F1,53 = 5.4$, $p = 0.025$, $d = 0.54$) in analysis controlling for baseline symptoms. AHRS improvement was most pronounced for the AHRS loudness item. 27% of those assigned to active treatment improved in the total AHRS through one month, as opposed to 8% of those assigned to sham. No significant between group differences in AHRS were seen for inpatients.

In general, tDCS treatment was well tolerated, with 98.7% completed the 5-day treatment. One patient in the active group was withdrawn for a non-serious adverse event after 3 days of treatment, but completed the 1-week follow-up. 85% subjects completed 3 months of treatment. MRI analysis is ongoing, and will be presented at the meeting.

Conclusions: The present report represents the largest study of tDCS for auditory hallucinations. We replicate previous reports and demonstrate significant improvement in AVH in Sz patient's treatment resistant to antipsychotics. Ongoing MRI analysis will inform future design.

Keywords: Schizophrenia; Technology, Auditory hallucinations, Brain Stimulation.

Disclosure: Nothing to disclose.

M207. A Comparative Study of Accuracy of Self-Assessment in Bipolar and Schizophrenia Patients Focusing on Employment and Living Status

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Background: While self-assessments are frequently used as outcome measures in the evaluation of interventions for severe mental illnesses, there is considerable evidence across different illnesses that these reports can be inaccurate. The purpose of this study is to understand the relationship between self-assessment of disability relative to objective functional achievement as defined by everyday milestones in patients with bipolar (BP) and schizophrenia spectrum disorders (SCZ). To our knowledge, there has been no previous study that directly compares these measures between patients with schizophrenia and bipolar disorder.
Methods: The sample consisted of participants from the Suffolk County Mental Health Project. Participants were entered after their first psychotic episode and were followed for 20 years. Inclusion criteria were age 15-60 years of age, residence in Suffolk County NY, and psychosis. Subjects were excluded if they had a psychiatric hospitalization more than 6 months before the index admission. We examined 20-year follow-up data from 146 participants who were diagnosed with schizophrenia spectrum disorders and 87 individuals who received a diagnosis of bipolar disorder. We compared self-assessments 12 item version of the World Health Organization Disability Scale (WHODAS) with objectively determined interviewer-ascertained outcome data. For outcomes we selected two measures: independence in residence (without external support; no group homes or supported settings) and gainful employment (defined as competitive part time or full time employment in a real world, compensated setting).
Results: Compared to schizophrenic patients, patients with bipolar disorder evaluated themselves as globally more capable and they were much more likely to have achieved current milestones (Work:SCZ 25%; BP 52%; Independence: SCZ 43%; BP 85%). In both schizophrenia and bipolar groups there were minimal differences in self-assessments between those individuals who lived independently and those who did not, with no significant differences in total scores across groups. Further, SCZ and BP individuals who were not living independently rated themselves as generally "minimally" disabled, stating that they had 8 and 6 "ill days" per month respectively, while individuals with independent residence rated themselves exactly the same. Accuracy of self-assessment of work capability was substantially better than self-assessment of the ability to perform everyday activities, in that unemployed patients with SCZ and BP both rated themselves as more globally disabled than those who were employed.
Conclusions: Patients with bipolar disorder and schizophrenia manifested similar patterns of mis-estimation in their functioning, with patients living in supported settings seeing themselves as minimally disabled. Work functioning appears to be an easier domain in which to self-assess functioning in individuals with these conditions and it may be that individuals with no experience in residential independence have no basis by which to judge their functioning. Motivation to engage in therapeutic activities may be related to the ability to understand current limitations. Thus, treatment success, both pharmacological and psychosocial may require initial interventions aimed at realistic assessment of everyday functioning.
Keywords: Schizophrenia, Bipolar, Functional Outcome, Disability.
Disclosure: Nothing to disclose.

M208. Comprehensive Assessment of Auditory Perception and its Relation to Impaired Emotion Recognition in Schizophrenia

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Background: Basic perceptual processes are impaired in schizophrenia and may explain key social deficits such as difficulty identifying emotions in other people. Auditory perceptual deficits, such as impairment in pitch processing, may be closely linked to the neural circuits relevant for the development of psychotic symptoms and thus may be useful predictors of psychosis onset and promising therapeutic targets. However, previous work in this area has focused on a relatively narrow assessment of auditory deficits and there is a lack of information regarding the relationship between basic auditory processing deficits and impairments in emotional processing and cognition.
Methods: We have assessed 87 patients with schizophrenia and 73 healthy controls between the ages of 18 and 60 on a comprehensive battery of tasks spanning the four empirically derived domains of auditory function. We explored group differences across the battery of basic auditory tasks using between-groups t-tests. We also explored the relationship between basic auditory processing and auditory emotion recognition within the patient group using correlational analysis.
Results: We observed significant group differences in the ability to identify emotion in vocal samples and in several basic auditory skills, including tests from all 4 domains of auditory function (loudness & duration, amplitude modulation, familiar sounds, and pitch & time). Performance on all of the basic auditory tests correlated with auditory emotion recognition at the $p < .01$ level in the patient group, with 9 out of 13 tests correlating with emotion recognition at $r = .40$ or greater. Partial correlational analysis suggested that after controlling for cognition, the basic auditory skills that were most robustly correlated with emotion recognition were sinusoidal amplitude modulation detection at 60 Hz. ($pr = -.43$, $p < .001$), formant discrimination ($pr = .38$, $p < .05$) and syllable recognition ($pr = .37$, $p < .05$).
Conclusions: Previous research has indicated a correlation between basic pitch processing deficits and impairments in auditory emotion recognition in individuals with schizophrenia. Our results build upon this finding and suggest that patients exhibit broad deficits in basic auditory processing that are correlated with impaired recognition of emotion in

voice. Of particular note, tests that require frequency processing in the range at which the auditory system is able to phase lock to the stimulus waveform or envelope periodicity were amongst the most impaired auditory skills in the patients and the most highly correlated with emotion recognition. These results suggest that impaired phase-locking in auditory brain structures in individuals with schizophrenia may be associated with difficulty identifying emotion from the vocal properties of speech.
Keywords: Perception, Cognition, Schizophrenia, Audition.
Disclosure: Nothing to disclose.

M209. Representativeness of Patients With Schizophrenia Participating in Clinical Pet Studies: A Systematic Review

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Background: While neuroimaning studies have provided insightful data on the association between dopamine D2 receptor blockade with antipsychotic drugs and clinical effects, selection bias remains a serious concern when those data are interpreted. The selection bias seems especially relevant for functional brain imaging work, including positron emission tomography (PET) studies, since patients with severe psychopathology, for instance, may not be fully cooperative to stay still in the scanner for a certain period of time which often exceeds 60 minutes in these investigations. However, to the best of our knowledge, there has been no systematic review to shed light on representativeness of patients with schizophrenia participating in clinical PET studies.
Methods: PET studies that measured dopamine D2 receptor blockade with antipsychotic drugs were identified. The following terms were adopted in a systematic literature search of PubMED and EMBASE (last search: July 1, 2016): (schizophreni* OR schizoaffective OR psychosis OR psychoses OR psychotic) AND (PET or "positron emission tomography") AND dopamine*. Limits were set for "humans" and "English". Cross-referencing the bibliography of reports and review articles that resulted from this search was also performed. Unpublished trials were searched, using Clinical Trials.gov (http://clinicaltrials.gov/); the search terms were applied in the following function: (schizophreni* OR schizoaffective OR psychosis OR psychoses OR psychotic) AND (PET or "positron emission tomography") AND dopamine. All of the titles or abstracts were independently reviewed by two of the authors (S.K. and H.U.).
PET studies involving any radiotracer, and using any modeling method, were included if they (a) measured the availability of dopamine D2 receptors in patients with schizophrenia or related psychoses, (b) estimated D2 receptor occupancy levels with antipsychotic drugs by comparison of receptor binding in drug-treated subjects with healthy controls or drug-free patients with schizophrenia, (c) included introduction of antipsychotic treatment or antipsychotic regimen change in a systematic, prospective manner, and (d) included selection criteria of study subjects. In the event of several publications generated from the same group of investigators that were clearly based on overlapping samples of subjects, publications with the largest sample size were included.
Inclusion and exclusion criteria (e.g. age, sex, illness severity, stabilization period, illness stage) were extracted. Variables relating to participants (e.g. age, sex, race, duration of illness, assessment scores of psychopathology, side effects, cognitive function, and social functioning, educational background, marriage status, and employment status), interventions, study design, and geographical areas and years of study conduction were also extracted. Information regarding the sources of funding was collected. All data were extracted independently by two of us (S. K. and H.U.).
Results: From the initial list of 1953 hits, 24 published and 2 unpublished studies were identified. Second and first generation antipsychotics were examined in 19 (70.8%) and 5 (29.2%) studies, respectively. The most frequently used ligand was [11C]raclopride ($n=22$) followed by [18F] fallypride ($n=2$), [11C]-(+)-PHNO ($n=1$), and [18F] fluoro-ethyl-spiperone ($n=1$). Age limit was included in 11 studies (42.3%); one study solely included geriatric patients (i.e. 50 years of age or older) while others targeted younger adults. 9 (34.6%), 5 (19.2%), and 3 (11.5%) studies specifically included clinically stable patients, patients with severe psychopathology, and antipsychotic-free or -naive patients, respectively. Nineteen (73.1%) and 18 (69.2%) studies excluded patients with physical comorbidity and substance abuse, respectively. As a result, the mean age of subjects ranged from 23 to 42 years when one study targeted geriatric patients (mean age = 60) was excluded. Positive and Negative Syndrome Scale (PANSS) was used in 13 studies; mean PANSS total score ranged from 54 to 95. Clinical Global Impression - Severity (CGI-S) was assessed in 13 studies; mean CGI-S score was below 4 (moderately ill) in 7 studies (53.8%), 4 or more but less than 5 (markedly ill) in 5 studies (38.5%), and 5 or more in 1 study (7.7%). Only 2 studies were double-blind randomized controlled trials, and no comparison active-drug or placebo arm was employed in 23 studies (88.5%). Blind assessment of symptomatology was performed in only 5 studies (19.2%). Seventeen studies were funded by pharmaceutical companies.
Conclusions: In general, subjects participating in clinical PET studies were relatively young, presented with mild symptomatology, and did not suffer from either substance abuse or physical comorbidity, suggesting that the sample may not be entirely representative of patients with schizophrenia encountered in the real-world clinical settings. These characteristics will need to be taken into account when interpreting the results and translating the findings into the actual clinic. Moreover, while the methodology of PET seems rigorous, study designs of clinical PET studies as clinical trials could be improved to achieve more precise evaluation of efficacy and side effects of target drugs in relation to dopamine D2 receptor occupancy.
Keywords: Schizophrenia, PET, Clinical Trial Design, Clinical Trial Methodology, Psychopharmacology.
Disclosure: Nothing to disclose.

M210. Abnormalities in the Copper Metabolite, CTR1, in the Postmortem Substantia Nigra in Schizophrenia

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Background: Genome-wide association studies have identified a number of genetic loci associated with risk for schizophrenia (SZ), yet the functional implications of these genetic variants are not clear. The dystrobrevin binding protein 1 (DTNBP1) gene, which encodes the dysbindin protein, is among the top candidate risk genes for SZ. Dysbindin is downregulated in the cortex and hippocampus of schizophrenia subjects. Dysbindin is involved in many functions; however, it is poorly understood which of its downstream targets, altered by its loss or downregulation, play a role in SZ pathology and symptoms. One function of dysbindin that has not been explored in SZ is its ability to modulate copper. Copper is required for proper monoamine metabolism, mitochondrial activity, and myelination. Dysbindin knockout mouse models result in a significant decrease in the copper transporters ATP7A and CTR1; both are critical components of the copper regulatory network. In fact, experimental manipulations that decrease copper levels in brain produce demyelination, increases in dopamine, and behavioral impairments reminiscent of some SZ symptoms. In spite of compelling evidence for a role of dysbindin in SZ, no one has linked a decrease in dysbindin function with abnormal copper homoeostasis in SZ brain. Taken together, alterations in dysbindin and subsequent downstream alterations of copper could result in SZ-like pathology including impaired white matter integrity, excess dopamine, grey matter loss, and altered mitochondrial activity, and yet we are the first laboratory to propose investigating these alterations together.
Methods: The current study used Western blot analysis to compare protein levels of dysbindin and copper transporter CTR1 in postmortem substantia nigra (SN) in schizophrenia subjects ($n = 13$) and matched controls ($n = 12$). Controls and patients were matched for age (50 vs 42 yrs), sex (9M/3F vs. 10M/3F), race 9C/3AA vs 8C/5AA), PMI (15.3 vs. 14.2 hrs), pH (6.6 vs 6.6) and years frozen. As a preliminary analysis, the schizophrenia group was subdivided by 1) treatment status: off- ($n = 4$) or on-medication ($n = 9$); or 2) treatment response: treatment resistant ($n = 5$) or treatment responsive ($n = 4$).
Results: The combined schizophrenia group exhibited significantly decreased CTR1 protein levels (a decrease of 42.6%, $p = 0.0003$) when compared to matched controls. When subdivided by medication status, this decrease was observed in both on-medication (a decrease of 32.9%, $p = 0.033$) and off-medication subjects (a decrease of 52.9%, $p = 0.002$) when compared to controls. No difference was found between on or off-medication subgroups. Decreased CTR1 protein levels were also observed in responsive schizophrenia subjects in comparison to controls (a decrease of 47.1%, $p = 0.007$) when subdivided by treatment response. Protein levels of dysbindin were not significantly different for any of the analyses. No significant correlations were observed between CTR1 and dysbindin in the whole sample, normal control, or schizophrenia subjects. However, schizophrenia subjects exhibited a slightly negative correlation ($r = -0.343$, $p = 0.211$), whereas control subjects exhibited a slightly positive correlation ($r = 0.398$, $p = 0.225$). Upon comparison of correlation coefficients, a trending difference was observed between schizophrenia subjects and controls ($p = .087$).
Conclusions: These data show a robust downregulation of the copper transporter, CTR1, in the substantia nigra in schizophrenia. At least for this enzyme and this brain region, the decrease in CTR1 is not dependent on dysbindin protein levels or medication status in schizophrenia. Future studies can include investigation of dysbindin and copper transporters in known regions with altered dysbindin to examine the relationship among dysbindin, copper, and medication status. Furthermore, upstream modulators of copper enzymes other than dysbindin can be examined to establish the mechanism leading to a decrease in copper enzymes. The significance of a decrease in CTR1 in the brain in SZ is that copper may not be cleared from the blood, leading to increases in blood copper levels (which have been reported in SZ) and a decrease of copper in neurons and glial cells.
Keywords: Copper Metabolism, Dysbindin, Pathology.
Disclosure: Nothing to disclose.

M211. Adult Stress Exposure Blunts Dopamine System Hyperresponsivity in Mam Rats

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Background: Stress is a major risk factor for the development of both schizophrenia and depression (Schmitt et. al, 2014). Depression and schizophrenia are comorbid disorders, suggesting a relationship between the two disorders that affects the risk and severity for both disorders (Buckley et. al, 2009). Although both disorders are characterized by dopamine (DA) system malfunction (Grace, 2016), depression is linked to DA system hyporesponsivity (Belujon and Grace, 2014; Chang and Grace, 2014) whereas schizophrenia is distinguished by DA system hyperrresponsivity (Lodge and Grace, 2007; Grace, 2015). For example, rats exposed to chronic mild stress (CMS), a widely used animal model of stress-induced neurobehavioral alterations relevant to depression, exhibit a reduction in the proportion of spontaneously active DA neurons (i.e. population activity) in the ventral tegmental area (VTA) and increased immobility in the forced swim test (FST). In a neurodevelopmental model of schizophrenia in which rats are treated prenatally with the mitotoxin methylazoxymethanol acetate (MAM) on gestational day (GD) 17, adult rats exhibit increases in DA neuron population activity that correlate with enhanced locomotor responses to amphetamine. However, the comorbid effects of MAM and CMS have yet to be explored and could provide insight into shared mechanisms of disease. To this end, we combined the MAM neurodevelopmental model with adult CMS exposure, used immobility in the FST and amphetamine-induced hyperlocomotion (AIH) as schizophrenia and depression-related endophenotypes, and performed extracellular recordings of VTA DA neurons.

Methods: Saline and MAM-treated offspring were assigned to standard housing or 4 weeks of chronic mild stress around postnatal day (PND) 65-75. Animals were tested in the forced swim test (FST) and for locomotor activity in response to an acute amphetamine (0.5mg/kg i.p.) injection within two weeks (i.e. 1 test per week, counterbalanced). These tests were selected because of their wide use in preclinical depression and schizophrenia research, respectively. Single-unit recordings of VTA DA neurons were conducted within a week after the last behavioral test using 3 parameters: number of spontaneously active DA cells (i.e. population activity), firing rate and firing pattern.
Results: MAM animals subjected to behavioral testing (AIH, FST) exhibited a blunted amphetamine-induced locomotor response compared to SAL animals exposed to the same tests. However, MAM animals exhibited comparable levels of FST immobility behavior to controls. CMS exposure reduced amphetamine-induced locomotor activity and increased immobility behavior in both SAL and MAM animals, with a tendency of these effects to be exacerbated in MAM-CMS animals. Moreover, both types of stress exposure (i.e. FST, CMS) induced a persistent reduction in DA system activity, as indexed by the number of spontaneously active VTA DA neurons in MAM animals that was absent in stress naïve (i.e. no behavioral testing) MAM rats. Post-FST, MAM animals had a similar number of spontaneously active DA neurons compared with SAL animals. The same was true for CMS-exposed animals post-FST, in which both groups (i.e. SAL, MAM) exhibited a dramatic reduction in population activity compared to standard housed groups tested in the FST. This reduction was associated with specific track locations across the medio-lateral extent of the VTA. No effects of infant treatment (SAL vs MAM) or adult housing (CON vs CMS) were found for other parameters of DA system activity.
Conclusions: Collectively, our findings suggest that both acute (i.e. FST) and prolonged (i.e. CMS) stressors have powerful and long-lasting effects on DA system responsivity in MAM rats, which is consistent with prior reports of abnormal corticosterone regulation in these animals (Zimmerman et. al 2013). These effects were observable across multiple levels (i.e. behavioral, electrophysiological) and associated with a blunted hyperdopaminergic phenotype. Furthermore, these data suggest that the extent of DA system down-regulation is dependent on the nature and duration of the stressors.
Keywords: Acute and Chronic Stress, Schizophrenia, Dopamine, Ventral Tegmental Area (VTA).
Disclosure: Nothing to disclose.

M212. Neural Correlates of Tolcapone Enhanced Cognitive Control in COMT-Genotyped Healthy Adults

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Background: Cognitive control (CC) is a higher cognitive process, mediated by the prefrontal cortex (PFC), that broadly impacts goal or context representation and maintenance, attention allocation and stimulus-response mapping, and is essential for decision-making and daily functioning. Impaired CC is thought to contribute significantly to functional impairment in chronic psychotic disorders, including schizophrenia (SZ). Antipsychotic (AP) medications are less effective in treating these deficits and pro-cognitive drug trials have so far yielded negative results. Tolcapone, a reversible catechol O-methyl transferase (COMT) enzyme inhibitor, FDA approved to treat Parkinson's disease, significantly improved performance on PFC-based working memory and executive function tasks in healthy subjects (HS) carrying the Val/Val genotype of the COMT gene (SNP rs4680). However, tolcapone's effects on the cognitive control are unclear, and the neural targets of tolcapone's pro-CC effects are unknown. We used an electroencephalogram (EEG)-based CC task – a reverse-translated 5-choice continuous performance test (5C-CPT) - to identify the neural targets underlying tolcapone's effect on CC in COMT-genotyped healthy adults.
Methods: Carefully screened, medically and psychiatrically healthy 18-35 yo adults ($n = 10$ Met/Met and $n = 17$ Val/Val), completed two test days separated by one week. On each test day, subjects received either tolcapone 200 mg or placebo (PBO) p.o. in a double blind, randomized, counterbalanced, within-subject cross-over design. Effects of tolcapone on 5C-CPT performance and ERP measures were analyzed using repeated measures ANOVA. Biomarkers predicting tolcapone's neurocognitive sensitivity were assessed, including SNP rs4680, demographic variables and baseline cognitive performance.
Results: Overall, 200 mg dose of tolcapone was well tolerated, and biologically active noted by significant elevation of systolic blood pressure at 210 minutes post-pill ingestion ($p < 0.05$), and transient elevation of liver function test during post-study FU visit (alanine transaminase (ALT) and total bilirubin $p < 0.05$). Tolcapone significantly improved within-session 5C-CPT performance measured by d' scores ($F(2,50) = 4.2$, $p < 0.05$) and enhanced frontal P 200 amplitude during non-target trials ($F(9,225) = 2.1$, $p < 0.05$) in individuals with low baseline d' scores, but had the opposite effect in the high baseline d' score group. Tolcapone-enhanced frontal P2 amplitude was correlated with tolcapone-reduced false alarm rate ($R = 0.4$, $p < 0.05$; i.e. enhanced P2 amplitude associated with reduced false alarms). Tolcapone enhanced CC and frontal activation was independent of COMT genotype status.
Conclusions: Among healthy adults, tolcapone (200 mg) enhanced cognitive control, as measured by d' scores, and activated frontal electrodes during the response selection stage (enhanced frontal P2 amplitude) of correctly responded non-target trials in poor baseline performers. Moreover, tolcapone enhanced activation of frontal electrodes was associated with reduced false alarm rate during non-target trials. Although an electrical point source was not computed, these findings suggest that tolcapone's CC effects have a frontal locus of bioactivity, and that tolcapone effects on frontal activation are associated with enhanced response selection and cognitive control. These findings provide a strong rationale for assessing tolcapone effects on CC in SZ patients.
Keywords: Tolcapone, Cognitive Control, Event-Related Potential.

Disclosure: This work was supported by NIMH R01-MH059803 and R01-MH094320; Behavioral & Brain Research Fund; APF/Kempf Award; 1 KL2 TR001444-01; and VA San Diego Healthcare System; VISN-22 Mental Illness Research, Education, and Clinical Center.

M213. Anterior Cingulate Cortex Glutamatergic Response to Heat Pain in Schizophrenia

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Background: Regulation of stress response involves top-down mechanisms involving the frontal-limbic glutamatergic system. We hypothesized that schizophrenia patients may have abnormal reactivity of glutamate within the dorsal anterior cingulate cortex (dACC), a key region involved in perception of and reaction to stress.
Methods: We developed a somatic stress paradigm involving pseudorandom application of painfully hot stimuli to the forearm of participants undergoing functional proton magnetic resonance spectroscopy at 3T to measure glutamate and glutamine in the dACC. This paradigm was tested in a sample of 21 healthy controls and 23 patients with schizophrenia.
Results: Glutamate levels significantly decreased following exposure to thermal pain ($F = 4.97$, $p = .009$), while ratio of glutamine to glutamate significantly increased ($F = 3.53$, $p = .035$). However, patients exhibited a trend towards initial increase in glutamate levels that was significantly different from controls, after controlling for age, gender and heat pain tolerance ($\beta = .286$, $p = .042$; partial $\eta 2 = .102$). Furthermore, in patients the acute glutamate response was positively correlated with childhood trauma ($r = .414$, $p = .050$) and inversely correlated with working memory ($r = -.494$, $p = .023$).
Conclusions: The preliminary results from this somatic stress paradigm indicate a possible abnormal glutamatergic response to stress in schizophrenia patients. The clinical correlates of this glutamate response support the hypothesis that environmental risk factors for schizophrenia may sensitize schizophrenia patients to stress via alterations of the glutamatergic system.
Keywords: Glutamate, Acute Stress, Pain, Schizophrenia.
Disclosure: Nothing to disclose.

M214. A Combined Proton 7T Magnetic Resonance Spectroscopy and Magnetoencephalography Study in First Episode Schizophrenia

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Background: Schizophrenia (SZ) is an illness whose heterogeneity has impeded understanding the underlying pathophysiology. Here we used two complementary brain imaging techniques, magnetoencephalography (MEG) and magnetic resonance spectroscopy (MRS at 7T), to explore the neurophysical patterns of variation in SZ across these two modalities in the same subjects.
Methods: 20 minimally treated first-episode SZ and 20 healthy controls (HC) matched for age, gender, and family socio-economic status were recruited. Neurometabolite levels were obtained from the bilateral dorsal anterior cingulate cortex using 7T proton MRS with an ultra-short echo time (5ms) STEAM sequence. The MRS scan took approximately 6 minutes. MEG was performed in a 4D systems 148 channel magnetometer. There were three recording runs, each lasting 5 minutes. All runs were made with the subjects resting quietly on their backs with their eyes closed. MEG data was sampled at 1 kHz, and filtered from 0.1 to 300 Hz. Epochs with clear artifacts (such as swallowing) were identified by eye and excluded from the analysis. Independent components was used to identify other artifacts (such as cardiac and blink artifacts) and remove them from the data. The first MEG run was passive resting state. The second MEG run had 500 msec duration tone pulses of 20 Hz and 40 Hz presented in pseudorandom order (pre-attentive). The third MEG run was exactly the same as the first run, except that the subjects were instructed to quietly count the number of discrete tone bursts (attentive). The resting state data was analyzed by performing Fourier transforms on 2-second Hamming windowed data segments across the entire 5-minute period and averaging. The auditory evoked MEG signals were analyzed by extracting equivalent dipoles for the left and right auditory cortices and averaging the time series locked to stimulus onset time.
Results: The auditory evoked MEG response was on average very similar between SZ and HC, at least under these conditions. While there was a trend for the evoked MEG response to the 40 Hz tone burst to have greater power in the HC compared to the SZ, this was not significant for either the pre-attentive or attentive conditions ($P > 0.05$). However, the resting state MEG exhibited significantly elevated theta-band activity in SZ relative to HC (t-test, $P < 0.05$). Individual 7T MRS metabolites did not significantly separate out HC from SZ in this cohort, but using independent components, one pattern of co-variation across the 7T MRS metabolites (SZ tended to have high GABA, low NAA, and high creatine relative to HC) did significantly separate the SZ from the HC (t-test, $P < 0.05$). The MRS and MEG metrics that separated HC from SZ were uncorrelated with each other ($r2 = 0.107$).
Conclusions: The lack of correlation between the MRS and MEG components that significantly separated SZ from HC suggests that these may reflect independent pathological mechanisms. Further multi-modal studies may help to provide insight into the heterogeneity of SZ.
Keywords: MEG, 1H MRS, Evoked Response Potential, Resting state, Auditory Cortex.
Disclosure: Nothing to disclose.

M215. Abnormal Superior Longitudinal Fasciculus Fiber Orientation in Recent-Onset Psychosis

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Background: Over a decade of diffusion tensor imaging (DTI) studies have identified brain white matter abnormalities in the neurobiology of schizophrenia and associated psychotic disorders. In particular, abnormalities in the superior longitudinal fasciculus (SLF) have been identified among individuals at risk for developing psychotic disorders in the early stages of psychosis, and strongly predict deficits in neuropsychological and social/role functioning, making this tract a potentially important target for intervention. The most widely reported measure from DTI studies is fractional anisotropy (FA), a putative measure of white matter microstructure. Although standard DTI performs well in regions consisting of a single fiber orientation, this approach cannot resolve fiber tracts aligned along different axes and thus, a critical unresolved question in the field concerns the potential role of aberrant crossing fibers in the neurobiology of psychosis. The field has therefore moved toward the use of techniques that can resolve crossing-fibers such as diffusion spectrum imaging (DSI). The main drawback of DSI, however, is the long scan time making it difficult to implement in humans. Accelerated acquisition of DSI may be accomplished, however, by leveraging the sparsity of diffusion space data in a suitable transform domain. Using compressed-sensing (CS), the missing diffusion data points in a Cartesian q-space pattern can be reconstructed from the acquired randomly under-sampled data. Our goal in the current study was to investigate fiber orientation complexity within the superior longitudinal fasciculus using an accelerated DSI acquisition to reduce scan time by a factor of four in patients with recent onset psychosis and healthy volunteers.
Methods: Thirty-four (24M/10F) patients with recent-onset psychosis (mean age = 23.6 years; SD = 5.2) were recruited from the inpatient service at The Zucker Hillside Hospital in New York. Mean age at first psychotic symptoms was 21.5 (SD = 5.6). Twenty-three (11M/12F) healthy volunteers (mean age = 26.3 years; SD = 7.1) were included in this study.
MR imaging exams were conducted on a 3T whole-body MRI system (GE Healthcare, Waukesha, WI USA). We performed a 26-minute compressed sensing (CS) DSI scan (T2-weighted image + 127 diffusion directions, bmax = 6,000 sec/mm2, FOV = 24 cm, 128x128 matrix, slice thickness = 3 mm, TR/TE = 12 sec/125-134 msec, 27-31 slices). To accelerate DSI acquisition (from 105-minutes to 26-minutes), four-fold-accelerated compressed sensing was applied (reducing 514 diffusion samples to 127). The CS-DSI data were reconstructed using total variation and wavelets as sparsifying transforms. Using a region-of-interest (ROI) seed-generation template ROIs were identified for the right and left SLF and manually optimized for visualization using Trackvis software. The orientation distribution functions of CS-DSI was performed using a small angle-threshold of 38 degrees, which allowed false-positives to be significantly reduced (compared to 55-70 degrees typically used in DTI). Dependent measures included FA and an orientation distribution function (ODF) based metric, multi-directional anisotropy (MDA), which is analytically equivalent to FA in single-direction diffusivity, but superior to FA in its sensitivity to the underlying anisotropy of multi-directional diffusivity. The number of fiber orientations, N, was defined as the total number of peaks found on the ODF, which was generated for 181 discretized directions on a hemisphere, equivalent to an approximate 10-degree tessellation angle.
Results: Repeated measures ANCOVA revealed significant interactions of group with hemisphere for FA, MDA and the number of fiber orientations within the SLF. Investigation of asymmetry indices [(right – left) / (right + left)] revealed significant group differences in all 3 measures. Independent groups t-tests indicated that patients had significantly lower FA and MDA in the left hemisphere compared to healthy volunteers in the absence of group differences in the right hemisphere. Paired t-tests indicated significant asymmetry (L > R) in both FA and MDA as well as the number of fiber orientations (R > L) in healthy volunteers, and that these asymmetries were either absent or reversed in patients. FA asymmetry correlated significantly and inversely with fiber orientation asymmetry with significant effects observed in both patients and healthy volunteers. Analysis of right and left hemispheres separately revealed that lower FA correlated significantly with a greater number of fiber orientations across both groups with effects being more robust among healthy volunteers compared to patients.
Conclusions: Our findings provide in-vivo data identifying a relationship between FA, a putative measure of white matter microstructure, and number of fiber orientations in healthy humans. This effect was less robust among patients with recent-onset psychosis compared to healthy volunteers, however, suggesting that other factors may be influencing findings of lower FA within the SLF that have typically been observed in patients. The finding of abnormal asymmetry in fiber orientation in patients compared to healthy volunteers is consistent with an aberrant neurodevelopmental process in the pathophysiology of psychosis. We further demonstrate that a measure of multi-directional diffusivity (i.e., MDA), which has been demonstrated to be superior to FA in its sensitivity to the underlying anisotropy in regions of crossing fibers, can be used to distinguish patients from healthy volunteers.
Keywords: Diffusion Spectrum Imaging, Psychosis, White Matter, Superior Longitudinal Fasciculus.
Disclosure: Nothing to disclose.

M216. Examining 28-Day Abstinence on P300 in Cannabis Dependent Patients With Schizophrenia Compared to Non-Psychiatric Controls

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Background: Cannabis is the most commonly used illicit drug in schizophrenia that significantly exacerbates their

symptoms including working memory deficits. P300 is an event-related component that is elicited during working memory performance and has been shown to modulate with working memory load. Given that cannabis modulates working memory performance, it is possible that P300 may be modulated with cannabis use in schizophrenia. In this study, P300 was evaluated during the N-Back task pre and post 28-day abstinence period in cannabis-dependent patients with schizophrenia and controls.

Methods: Fifteen patients (mean age 30.93 (+/-) 9.31 years) and 15 non-psychiatric controls (mean age 28.94 (+/-) 6.39 years) male cannabis-dependent performed the verbal N-Back task administered at the 1- and 3-Back working memory loads. P300 was measured from the fronto-central electrodes for correct responses to targets using EEG. Abstinence was reinforced using contingency management.

Results: In patients with schizophrenia, P300 amplitude was highest in the 1-Back compared to the 3-Back working memory load at baseline. P300 was negatively related severity of symptoms indexed by the PANSS Total Score ($r = .945$; $p = 0.015$). In contrast, lower P300 amplitude was observed in the 1-Back compared to the 3-Back in controls. Following abstinence (50% quit rate in completers), P300 amplitude increased in the 1-Back and 3-Back as well as the difference between these 2 conditions. In contrast, there was a small decrease in P300 with abstinence in controls.

Conclusions: While preliminary, P300 may represent a neurophysiological marker of cannabis state induced effects among patients with schizophrenia. Moreover, these findings may suggest that P300 may provide us a better understanding of the pathophysiology of schizophrenia with comorbid cannabis use disorders that may lead to treatment innovation.

Keywords: Cannabis Dependence, Schizophrenia, Event Related Potentials.

Disclosure: Supported in part by NARSAD Young Investigator Grant to Dr. Barr and a Canadian Institute of Health Research (CIHR) operating grant MOP#115145 to Dr. George.

M217. Electroconvulsive Therapy for Clozapine-Resistant Schizophrenia: Cognitive Effects

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Background: Clozapine is the only medication shown to be effective in the treatment of antipsychotic-resistant schizophrenia. Nonetheless, up to 70% of patients fail to benefit from or partially respond to this drug.

We reported earlier on the efficacy of electroconvulsive therapy (ECT) as an augmentation strategy for clozapine-resistant schizophrenia. Patients on a stable dose of clozapine with serum levels > 250 meq/ml for at least 8 weeks, with persistent psychotic symptoms (> 12 in the Brief Psychiatric Rating Scale-Psychotic Symptoms BPRS-PS) and no mood symptoms were included in the acute phase of the study. Patients were randomized to receive 8 weeks of ECT in addition to clozapine or to continue with clozapine treatment. Patients in the pharmacotherapy arm, who did not respond after 8 weeks, crossed-over to ECT and received the combination treatment for 8 weeks. Using as response criterion 40% reduction in the BPRS-PS, we reported acute response rates of 50% in the single-blind phase of the study and 48% in the cross-over phase of the study.

We report here the cognitive effects of the augmentation strategy for those patients who responded to the acute phase and participated in up to 6-month maintenance ECT follow-up.

Methods: Nineteen patients who met response criteria were offered maintenance ECT for up to 6 months. Psychopathology and neurocognitive evaluations performed at baseline, end of acute phase and end of the maintenance phase or exit from the study.

Results: Fourteen patients agreed to participate in the maintenance phase.

For these patients the mean BPRS-PS was 17.1 (+ 6.94) before the acute course of ECT. Their mean BPRS-PS at maintenance baseline was 7.69 (+ 3.66) and at the end of the study 8.2 (+ 4.32). The mean mini-mental state exam (MMSE) was 20.69 (sd 6.03) at baseline, 25.73 (sd 10.3) and 22.66 at the end of maintenance phase. The modified MMSE was 33.69 (sd 14.36), 42.86 (sd 10.9) and 44.44 (sd 7.29) at the three respective time points. The mean times for Trails A were 65.57 (sd 54.95), 71.13 (63.03) and 52.14 (sd 20.55) sec for the 3 time points. For trails B the mean times were 177.86 (sd 96.68), 184.49 (sd 45.52) and 146.92 (sd 74.58) respectively. The mean AVLT scores were 6.46 (sd 3.30), 6.85 (sd 2.34) and 8.87 (2.29). The mean Cowat scores were 25.90 (sd 13.22), 18.14 (sd 8.31) and 28.37 (sd 10.33).

Conclusions: These data show that our patients started treatment at a level of relatively low cognitive function (global, memory, speed and verbal fluency) which became slightly more compromised at the end of the acute course. However, at the end of maintenance ECT cognitive performance recovered and improved in all domains even above baseline,

Maintenance ECT plus clozapine seems to be protective against relapse for at least 6 months and offers the opportunity for cognitive improvement beyond the levels before the acute ECT.

Keywords: ECT, Schizophrenia, Clozapine, Medication Resistance.

Disclosure: Nothing to disclose.

M218. Efficacy and Safety of MIN-101: A New Drug for the Treatment of Negative Symptoms in Schizophrenia

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Background: To compare the efficacy, safety, and tolerability of MIN-101, a compound with high affinities for sigma 2 and 5-HT2A receptors, to placebo in treating negative symptoms in patients with stable symptoms of schizophrenia.

Methods: This multi-national Phase 2b trial enrolled 244 patients diagnosed with schizophrenia who were

symptomatically stable for ≥ 3 months prior to entering the trial and had baseline scores ≥ 20 on the 3-factors negative subscale of the PANSS. Patients were randomized to daily monotherapy with MIN 101 32 mg, MIN-101 64 mg, or placebo in a 1:1:1 ratio. The primary endpoint was the PANSS negative symptom score based on the 5-factors (pentagonal) model. Secondary outcomes were the rest of the PANSS scores, CGI, the Brief Negative Symptoms Scale (BNSS), the Brief Assessment of Cognition in Schizophrenia (BACS), the Calgary Depression Scale for Schizophrenia (CDSS), and the Personal and Social Performance (PSP) scale. Safety parameters included treatment-emergent adverse events (TEAE), clinical laboratory, vital signs, electrocardiograms, Sheehan-suicidality tracking scale (S-STS), and the Abnormal Involuntary Movement Scale (AIMS). The Mixed-Effect Model Repeated Measure (MMRM) was used for analyzing the efficacy data.

Results: Statistically significant and dose dependent reduction in the primary endpoint score was demonstrated for MIN-101 32 mg and 64 mg compared to placebo ($p \leq 0.022$; effect size (ES) 0.45 .and ≤ 0.003; ES 0.58, respectively). The ES was particularly high (ES = 1.3) in the younger patients. The validity of effects on the primary endpoint was supported by similar effects on most of the secondary measurements including: PANSS 3-factors negative symptoms subscale, PANSS total score, CGI, CDSS, and PSP. Items in the PANSS measuring depressed mood and anhedonia as well as the total score of CDSS benefited from MIN-101. There were no statistically significant differences in PANSS positive subscale scores between MIN 101 and placebo.

No weight gain or clinically significant changes in vital signs, prolactin levels, routine laboratory values, metabolic indices and extrapyramidal symptom scores (EPS) were observed. One patient on 64 mg MIN-101 was discontinued from the trial based on a-priori established QT interval prolongation criteria and a second one following an episode of syncope. The three treatment groups were balanced on all demographic and illness-related baseline characteristics.

Conclusions: MIN-101 at dosages of 32 and 64 mg/day demonstrated statistically significant efficacy of medium ES in reducing negative symptoms and good tolerability in stable schizophrenia patients. Since positive symptoms and EPS did not change, the improvement in negative symptoms was not secondary to improvement in positive symptoms or EPS, suggesting that MIN-101 might be the first specific treatment to have a direct effect on negative symptoms. Furthermore, since the phenomena called negative symptoms is not exclusively manifested in schizophrenia, but also in other brain disorders it is possible that MIN-101 could benefit negative symptoms-like manifestations beyond schizophrenia.

Keywords: Schizophrenia, Negative Symptoms, Treatment, Sigma 2, 5HT2.

Disclosure: Minerva Neuroscience: Consultant's Fee and Stock, Self.

M219. Elevations in Central Noradrenaline are Associated With Sleep Impairments in Veterans With PTSD and/or a History of Repetitive Blast mTBI

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Background: The efficacy of the α1 noradrenaline receptor antagonist prazosin for treating PTSD symptoms in general and the sleep-related symptoms of PTSD in particular has strengthened the hypothesis that increased central noradrenergic activity may mediate the expression of these symptoms. Furthermore, the higher-than expected rate of comorbidity of PTSD and persistent sequelae of blast-related mild traumatic brain injury (bmTBI), along with the prominence of sleep disturbance in this population, has raised the question of whether this mechanism may be common between the two disorders.

To test the idea that increased release of central noradrenaline might be a common mediating factor for these symptoms, we measured cerebral spinal fluid (CSF) levels of noradrenaline in a population of combat Veterans with different degrees of exposure to bmTBI, and assessed the relationship of CSF noradrenaline both to bmTBI exposure and to current symptoms of PTSD, looking at both overall symptom severity and at sleep disruption in particular.

Methods: As part of an ongoing longitudinal study of bmTBI, 59 combat Veterans (40 with a history of bmTBI and 19 without; of the total, 28 met criteria for PTSD and 31 did not) underwent lumbar puncture, and the concentration of noradrenaline was quantified using HPLC. PTSD symptoms were evaluated using the Clinician Administered PTSD Scale (CAPS), which provided both diagnosis of PTSD and, for those who had reported experiencing a criterion A qualifying event ($N = 42$), a graded quantification of both overall symptoms and sleep symptoms. Exposure to blast explosions and lifetime history of mTBI was assessed using a structured interview. All analyses were carried out as linear regression models adjusting for age, and all p-values are reported as two-tailed.

Results: There was no statistically significant difference in mean CSF noradrenaline levels (NA) in those with vs without a history of bmTBI, or in those with or without a diagnosis of PTSD. Among those with a qualifying criterion A event, allowing analysis using CAPS scores, there was a trend towards increased CSF NA with increasing total CAPS score ($p = .06$), but the degree of collinearity between PTSD status and bmTBI exposure did not allow reliable separation of the extent to which this effect may have been due to bmTBI history, PTSD status, or both.

In contrast, sleep disruption as captured by CAPS scores was strongly associated with increased CSF NA levels. Although when analyzed separately both the CAPS item addressing nightmares (B2) and the item addressing general sleep disruption (D1) were strongly associated with increased CSF NA at $p < .001$ and $p < .02$, respectively, when combined in one model, only B2 was still associated with increased CSF NA with a regression coefficient of 14 ($p = .002$), while D1 was no longer associated ($\beta = 3$, $p = .46$). The relationship between B2 and CSF NA was also preserved when a history of bmTBI was added into the model, as well ($\beta = 14$, $p = .005$).

Conclusions: In a Veteran population containing individuals with high rates of bmTBI and PTSD, CSF NA was strongly associated with the presence and severity of nightmares, even when adjusting for age and a history of bmTBI exposure. Increased CSF NA was also associated with general sleep disruption. These findings support the hypothesis that increased central release of NA contributes to post-traumatic nightmares.
Keywords: Sleep Disturbance, PTSD, Noradrenaline, TBI.
Disclosure: Nothing to disclose.

M220. VTA Dopaminergic Neurons Regulate Ethologically Relevant Sleep-Wake Behaviors

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Background: Motivated behaviors are critically dependent upon arousal and the capacity to organize periods of sleep and wakefulness in response to specific environmental and homeostatic conditions is essential for survival. Nonetheless, little is known about the neuronal mechanisms that coordinate motivational processes with sleep–wake regulation. VTA dopaminergic neurons are central regulators of motivational processes, yet their role in the generation and maintenance of wakefulness is unclear.
Methods: To determine the causal role of VTA dopaminergic neurons in sleep-wake regulation, we recorded neuronal activity using fiber photometry in freely behaving mice and combined behavioral, chemogenetic and optogenetic manipulations together with polysomnographic recordings.
Results: We demonstrate that VTA dopaminergic neurons are necessary for arousal and that their chemogenetic inhibition suppresses wakefulness, even in the face of ethologically relevant salient stimuli. Nevertheless, before inducing sleep, chemogenetic inhibition of VTA dopaminergic neurons promotes goal-directed and sleep-related nesting behavior. Optogenetic stimulation, in contrast, initiates and maintains wakefulness and suppresses sleep and sleep-related nesting behavior. We further demonstrate that different projections of VTA dopaminergic neurons differentially modulate arousal.
Conclusions: Collectively, our findings reveal a fundamental role for VTA dopaminergic circuitry in the maintenance of the awake state and in the regulation of a hard-wired ethologically relevant behavior that is conducive to sleep and may be selectively targeted to treat arousal disorders.
Keywords: Dopamine, Sleep, Wakefulness, VTA, Nest-Building.
Disclosure: Nothing to disclose.

M221. A Functional Homology Between ADHD and Acute Sleep Deprivation: Preliminary Results From an ALE Meta-Analysis of fMRI-Monitored Executive Functioning

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Background: Sleep disruption is a common symptom reported in those with attention deficit hyperactivity disorder (ADHD). Moreover, inattention and impulsivity as described in ADHD are markedly similar to those experienced in healthy individuals subjected to acute sleep loss. Less understood, however, is whether the behavioral deficits present in ADHD and those observed following sleep deprivation share a common neural mechanism. While no study to date has combined experimental manipulation of sleep and functional magnetic resonance imaging (fMRI) in ADHD, studies have used fMRI to study either sleep deprivation or ADHD, independently. To advance what is known about the shared neurocircuitry underlying sleep loss and ADHD, we conducted the first analysis of likelihood estimation (ALE) coordinate-based meta-analysis of fMRI studies of executive function in sleep deprivation and ADHD.
Methods: As in prior studies, we used the gold-standard GingerALE software to conduct a coordinate-based meta-analysis of fMRI studies of sleep deprivation and of ADHD. First, we conducted a literature search for fMRI studies of sleep deprivation and ADHD in PubMed and PsychInfo, from which we honed our preliminary analyses to task-based fMRI studies of executive function. A total of 63 experiments were analyzed representing data from 2939 scanned participants: 47 experiments comparing ADHD to healthy controls (HCs), and 16 experiments contrasting acute total sleep deprivation compared with rested conditions in HCs. For each article, peak fMRI coordinates were extracted for contrasts of interest (i.e., "ADHD < HC", "ADHD > HC", "Sleep Deprivation < Rested", "Sleep Deprivation > Rested") and normalized to a single stereotaxic atlas-space (Talairach) for comparison. We first implemented ALE analyses (thresholded at $p < 0.005$) for the ADHD and sleep deprivation literatures separately. These initial estimates were then forwarded to a conjunction analysis to identify brain regions mutually hypo- or hyper-activated in both ADHD and sleep deprivation. Additional contrast analyses investigated where in the brain hypo- or hyper-activations may differ between ADHD and sleep deprivation.
Results: The ALE-conjunction analysis revealed 6 regions within the central executive function network where decreased activation was present in both those with ADHD and following acute sleep deprivation: the dorsal medial anterior cingulate cortex (overlapping with paracingulate and medial prefrontal cortices), the left inferior parietal lobule, the left middle occipital gyrus, the left precentral gyrus, the left superior parietal lobule, and the right inferior frontal gyrus. In contrast, our conjunction analysis found hyper-activation in both ADHD and sleep loss were observed in the posterior cingulate cortex, a key node of the resting default-mode network. In addition, ALE-contrast analyses identified exaggerated hypo-activations in ADHD within the left dorsolateral prefrontal cortex, right anterior insula, and the left temporoparietal junction. No regions were hyper-activated to a greater degree in ADHD, compared to sleep loss. In contrast, significantly greater deactivations in sleep deprivation were identified within the right inferior occipital gyrus, the bilateral angular gyrus, the middle cingulate gyrus together with significantly greater hyper-activations in the bilateral thalamus and the left globus pallidus.
Conclusions: Our study indicates that ADHD and acute sleep deprivation may share, in part, a common neural signature: hypo-activation of executive-function-regulating neuroanatomy in favor of hyper-activation of the resting

default-mode-network. Such a shift in brain networks may explain the lapses in attention and inhibition common to both conditions. Taken together with the common occurrence of sleep difficulties in ADHD, these data highlight the need for greater research uniting these often disparate contexts. Simultaneous assessment of sleep and functional neuroanatomy in those with ADHD, both at baseline and after sleep loss, may begin to disentangle these complex inter-twined clinical phenomena. Moreover, this analysis indicates which neuroanatomy may be shared, rather than distinct, between sleep loss and ADHD, thus identifying target regions for future experiments examining the intersection of these conditions.

Keywords: ADHD, Sleep, Functional MRI (fMRI).

Disclosure: Nothing to disclose.

M222. Ventral Medial Prefrontal Cortex Theta Burst Stimulation Decreases Salience Network Activity in Both Alcohol Users and Cocaine Users

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Background: Preclinical research has established that attenuating activity in the ventral striatum can decrease cocaine self-administration. Clinical neuroscientists are now attempting to translate those data to a neural circuit-based intervention for substance dependence, through non-invasive brain stimulation (NIBS). The goal of this study was to determine if continuous theta burst stimulation (cTBS; an attenuating form of NIBS) to the medial prefrontal cortex (mPFC) could reliably decrease frontal-striatal connectivity in cocaine-dependent and alcohol-dependent individuals.

Methods: Fifty participants were enrolled in this single-blind, sham-controlled, crossover study (25 cocaine dependent, 25 alcohol dependent). Frontal-striatal connectivity was measured with multimodal interleaved TMS/BOLD imaging immediately before and after 6 sessions of cTBS (110% resting motor threshold, FP1 location, 6 sessions/day). The effects of real versus sham cTBS on BOLD response to single pulses of TMS were measured for both groups (full factorial, scalp-to-cortex distance covariate, false discovery rate-corrected clusters reported, $p < 0.05$).

Results: Among cocaine users, real cTBS induced a significant decrease in bilateral BOLD response in mPFC, medial temporal gyrus, and precentral gyrus. Relative to sham, real cTBS induced a significant decrease in BOLD response in anterior cingulate cortex, mPFC, and temporal pole. Among alcohol users, real cTBS induced a significant decrease in BOLD response in left mPFC and middle temporal gyrus. Relative to sham, real cTBS induced a significant decrease in BOLD response in left mPFC, temporal pole, and parahippocampal gyrus. There was no significant effect of sham cTBS on BOLD response in any brain region. The brain response, as measured by BOLD activity, to real versus sham cTBS was not significantly different between cocaine and alcohol users.

Conclusions: These data suggest that 6 sessions of mPFC cTBS delivered in a single day reliably decreases BOLD response to single pulses of TMS in the mPFC, anterior insula, and parahippocampal gyrus—all regions monosynaptically connected to one another, which regulate salience and limbic tone. The reliability of this pattern across cocaine- and alcohol-dependent individuals suggests that this may be an effective treatment target for multiple substance-dependent populations postulated to have dysregulated connectivity between infralimbic and cortical brain regions. Effects of TBS on cognitive control and reward responsivity in substance abusing populations needs to be evaluated.

Keywords: Brain Stimulation, Addiction, Alcohol, Orbitofrontal, Transcranial Magnetic Stimulation.

Disclosure: Nothing to disclose.

M223. Selective BDNF-TrkB-PLCγ1 Signaling Conveys Anti-Addictive Properties That are Dissociated From Trkb Effects on Cocaine-Induced Dendritic Spine Density

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Background: Chronic cocaine induces dendritic spine growth in accumbens shell (NACsh) neurons, but this has not been shown to directly enhance addictive behavior. Spine formation is functionally linked to BDNF-TrkB signaling in other brain regions, but whether BDNF-TrkB signaling can alter cocaine-induced spines is unknown. We tested whether pathway-specific TrkB signaling can modulate spine formation induced by chronic cocaine self-administration (CSA) and compared effects with the modulation of CSA behavior.

Methods: Four novel Herpes Simplex Virus (HSV) vectors, all bicistronic for GFP, were constructed for this study. 1) HSV-TrkB-WT overexpresses the wild-type TrkB receptor, 2) HSV-TrkB-KD is a kinase-dead dominant negative mutant, 3) HSV-TrkB-515/SHC is a mutant that selectively blocks Src homology 2 domain-containing protein (SHC) docking complexes, but preserves TrkB signaling via phospholipase Cγ-1 (PLC), 4) HSV-TrkB-816/PLC selectively blocks signaling through PLC, while preserving TrkB signaling via SHC. HSV-GFP serves as a negative control. Follow-up experiments used HSV-PLCγ1, a previously characterized vector that overexpresses PLCγ-1 protein. All vectors were characterized with HEK293 cells and western blotting. Rats with bilateral NACsh cannulae trained for fixed ratio CSA 3h/day for 3-4 weeks, then CSA dose-response was assessed before, during, and after transient HSV-mediated expression of TrkB mutants, HSV-GFP, or HSV-PLCγ1. A second HSV infusion was performed at least 2 weeks later to assess motivation for cocaine on a progressive ratio (PR) reinforcement schedule. Separate cohorts engaged in cocaine or saline SA for 3 weeks, and dendritic spine density was assessed 3d after HSV infusions and 1d after CSA. Spine densities were quantified in GFP-labeled neurons by confocal microscopy and Volocity 3D analysis. All animal studies were approved by the UTSW Institutional Animal Care and Use Committee and were

conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.
Results: Western blots showed that TrkB-WT leads to increased levels of the pERK and pPLC pathways while TrkB-KD blocked both TrkB signaling pathways. The 816/PLC mutant blocks all TrkB-induced pPLC activity but maintains TrkB-induced pERK signaling, while the 515/SHC mutant has full pPLC activation but muted pERK signaling. During CSA, both the 816/PLC and KD TrkB vectors caused a transient leftward shift in the dose threshold necessary to maintain CSA compared with GFP controls, and increased breakpoints on the PR task. These results indicate increased sensitivity and motivation for cocaine reinforcement with loss of endogenous TrkB-PLC signaling. In contrast, WT and 515/SHC TrkB vectors did not alter CSA behaviors. CSA increased distal dendritic spine density in GFP-expressing NACsh neurons compared to saline. However, the WT, 515/SHC and 816/PLC TrkB vectors all reversed cocaine-induced spine changes without affecting baseline levels, whereas the dominant negative TrkB-KD vector failed to alter cocaine-induced spines. These findings indicate that gain of TrkB function (either through SHC or PLC signaling) reverses cocaine-induced increases in dendritic spine density. Follow-up experiments using HSV-PLCγ1 demonstrated that activating PLC signaling decreases cocaine reinforcements during DR and decreased breakpoints on the PR task, confirming that PLC signaling has anti-addictive properties. However, HSV-PLCγ1 also reversed cocaine-induced spine density, similar to the 816/PLC loss of function TrkB mutant. Since both the 816/PLC TrkB mutant and PLCγ expression enhance BDNF-induced ERK phosphorylation, BDNF-ERK signaling may ultimately mediate the reversal of cocaine-induced dendritic spine formation.
Conclusions: BDNF-TrkB activity after CSA triggers neuroplasticity that reverses the expression of dendritic spines, while loss of TrkB-PLC signaling enhances cocaine reinforcement, and these morphological and behavioral effects are entirely dissociable. Also, selective signaling through PLCγ-1 is a novel anti-addictive pathway that reverses both the structural and behavioral effects of cocaine-induced neuroplasticity.
Keywords: BDNF, Cocaine Addiction, TrkB, Dendritic Spines, Self-Administration.
Disclosure: Nothing to disclose.

M224. White Matter Integrity in Alcohol Use Disorder Domains

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Background: Many brain imaging studies have demonstrated reductions in gray and white matter volumes associated with alcohol use disorders (AUD). Fewer researchers have used diffusion tensor imaging (DTI) to examine white matter integrity in AUD.
Alcohol dependent patients (AD) are known to have neurocognitive deficits in decision-making, particularly in decisions related to emotionally-motivated behavior. It is widely believed that these types of deficits are related to frontal-limbic dysfunction. Previous DTI studies have shown abnormalities in cortico-limbic fiber degradation through fiber tracking (Pfefferbaum et al. 2009; Chanraud et al. 2009). To our knowledge no studies have investigated the white matter alterations in the addiction cycle, described by three stages: Binge/Intoxication, Withdrawal/Negative Affect, and Preoccupation/Anticipation as defined in Koob and Volkow (2016). In this study we investigated white matter integrity between AD and healthy controls (HC) with respect to the three stages of the addiction cycle.
Methods: Whole brain 80 direction DWI data was acquired using a 3T scanner of 22 non-smoking healthy controls (nsHC), 20 non-smoking alcohol dependent patients (nsAD), and 22 smoking alcohol dependent patients (sAD). All DWI data were preprocessed in TORTOISE v.2.5.1 (Pierpaoli et al, ISMRM abstract, 2010). Preprocessing involved registration-based correction for motion, eddy current distortion correction, and EPI distortion correction. These volumes were visually inspected for artifacts that were not adequately suppressed, and such volumes were removed from analysis.
Diffusion tensors were then estimated in AFNI's FATCAT v.16.1.11 at each voxel. Regions of interest were derived from Freesurfer parcellations that were aligned in each subject's DWI native space, selected a priori based on regions implicated in the stages of the addiction cycle. Each of these ROIs was seeded and a probabilistic tractography algorithm was performed in which the scalar values across the white matter tracts were calculated and uncertainty parameters (the modeling of the possible fiber directions from each seed) were estimated. DTI was used to measure Axial Diffusivity (AD), Radial Diffusivity (RD), Fractional Anisotropy (FA), Mean Diffusivity (MD), and number of tracts (nT).
Connectivity matrices based on the white matter ROI tracts were derived and used for ANCOVA analysis comparing nsAD, sAD and nsHC, controlling for years of education. Age and sex did not differ among the three groups.
Results: Preliminary results show significant differences in white matter integrity between AD patients (combined nsAD and sAD) and HC participants. We did not observe any differences between sAD and nsAD patients or between HC and nsAD. However, we detected significant differences between sAD patients and nsHC participants.
In the Binge/Intoxication circuitry we found increased radial diffusivity between the left nucleus accumbens and left caudate in sAD compared to nsHC. In the same circuit we found a decrease in the number of tracts between the right putamen and right thalamus. In the Withdrawal/ Negative Affect circuit we found significant differences in the number of tracts between the nucleus accumbens and the amygdala in all three groups with HC having the highest number of tracts and sAD having the fewest number of tracts. Finally, in the Preoccupation/Anticipation circuit there was progressively decreased axial diffusivity from nsHC to nsAD to sAD between vmPFC and caudate and between mPFC and nucleus accumbens.
Conclusions: Our results demonstrate the alterations in structural connectivity between the regions involved in the neurocircuitries of the addiction cycle. These alterations might be contributing to inefficient functional connectivity in reward system and executive functions in individuals with

alcohol use disorders. Obviously, it is not clear from this study whether any of this finding is a predisposition to addiction, an effect of addiction, or both. In general, we found that smoking exacerbates the effect of alcohol dependence on white matter integrity. Our findings suggest that there may be an interaction between smoking and non-smoking in AUD on white matter integrity.
Keywords: Alcohol use Disorders, Diffusion Tensor Imaging, Neural Circuits.
Disclosure: Nothing to disclose.

M225. Very Rapid Onset Cannabis Dependence Risk in Relation to Co-Occurring use of Other Psychoactive Drugs

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Background: Epidemiological estimates for lifetime cumulative incidence indicate that for every 9-11 who start using cannabis, one becomes a case of the cannabis dependence syndrome (CDS) – i.e., roughly 9%-11%. More recent estimates clarify that CDS risk might be much lower among 'cannabis only' users, due in part to the fact that most 'cannabis only' users try the drug 1-2 times and never again. We turned to Hill functional analysis in order to study CDS risk soon after 1st cannabis use, estimated across strata defined by the number of recent days of cannabis use with a possibility for persistence of cannabis use beyond a few trials (i.e., a potentially higher risk subgroup).
Methods: United States National Surveys on Drug Use and Health (NSDUH), 2004-2014, sampled and assessed more than 500,000 participants, yielding a nationally representative probability sample of 13,874 newly incident cannabis users, with CDS assessment no more than 12 months since 1st use. For this analysis, we focused on the subgroup of 4,934 subjects with persistence of cannabis use into the 30 days prior to assessment. For this subgroup, we used Hill functions to estimate CDS risk across strata defined by cannabis-using days during the 30 days prior to assessment, and by history of using other psychoactive drug compounds.
Results: Our preliminary results show that among 'cannabis only' users ($n = 1,811$) the probability of developing CDS rises from about 1% (95% bootstrap confidence interval, CI: 0, 2) for occasional users to about 9% (95% CI: 4.5, 23) for daily users. However, estimated CDS risk for daily users is greater when cannabis plus ethanol (but no other drugs) have been used ($n = 1,753$): 63% (95% CI: 47, 84); here, use on same day is not required. Our presentation will show additional Hill function estimates for other cannabis and drug combinations (e.g., cannabis and tobacco, cannabis and alcohol and tobacco).
Conclusions: Notwithstanding NSDUH self-report methods and other limitations, the main finding is that probability of developing cannabis dependence is greater when cannabis use co-occurs with other psychoactive drug use. CDS probability is relatively low for 'cannabis only' users even when 'trial' users are excluded. These epidemiological estimates are consistent with a re-appraisal of cannabis dependence risk for 'cannabis only' users.
Keywords: Cannabis Dependence, Polydrug Use, Statistical Methods.
Disclosure: Nothing to disclose.

M226. Adolescent Cannabinoid Exposure Persistently Alters Natural Reward Learning and Motivation

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Background: Adolescence is a critical period in cognitive and emotional development, during which synaptic pruning reshapes circuit connectivity in a long-term, perhaps permanent fashion. Endocannabinoids are involved in this process, and exposure to exogenous cannababinoid receptor (CBR) agonists like WIN55, 212,2 (WIN) during adolescence causes long-lasting deficits in memory and social interaction, and increases in reward seeking. This implies that adolescent cannabinoid exposure (ACE) alters the developmental trajectory of neural circuit maturation in a manner that causes enduring changes in cognition and reward.
Methods: Here, we exposed adolescent male rats to 14 daily injections of WIN (1.2mg/kg) from PD 30-44 (early adolescence), and examined effects of this ACE protocol on adult learning and motivation for a food reward, and responses to novelty. After 14-day washout (PD60, young adulthood), rats underwent Pavlovian conditioned approach (autoshaping, or sign/goal tracking), palatable food intake (novel and familiar foods, intake after acute hunger or satiety), and response to novelty tests (locomotion in a novel environment, novelty preference). Finally, we examined endocannabinoid levels after 24hr acute food restriction (or no restriction) in dissected nucleus accumbens, lateral hypothalamus, and prefrontal cortex using liquid chromatography/mass spectroscopy.
Results: Prior WIN ACE increased approach of food cues in the sign/goal tracking paradigm, increased binge-like palatable food intake, and increased preference for a novel environment. ACE also disrupted homeostatic regulation of hunger, since acute food deprivation failed to appropriately increase sucrose intake in ACE animals. Satiety decreased sucrose intake similarly in both groups, and locomotor response to a novel environment was also unaffected. Biochemical analyses point toward region-specific increases in AEA, but not 2-AG levels following acute food restriction.
Conclusions: These results show that ACE can persistently enhance natural reward seeking long into adulthood, resulting in a behavioral phenotype reflecting increased vulnerability to motivational effects of reward cues, and potentially development of compulsive drug or natural reward seeking. These behavioral effects are likely due in part to persistently dysregulated endocannabinoid signaling mechanisms and functional connectivity within mesolimbic reward circuits, a possibility we are currently exploring further.
Keywords: Adolescence, Endocannabinoids, Sign-Tracking, Anandamide, Nucleus Accumbens.
Disclosure: Nothing to disclose.

M227. Characterization of Psychosocial Stress-Induced Cocaine Seeking Using a Novel Model of Relapse in Rats

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Background: A prominent feature of cocaine abuse is the high frequency of relapse events that occur even following prolonged periods of abstinence. Stress-induced relapse is frequently modeled in experimental animals using reinstatement procedures which employ physical (e.g. footshock) or pharmacological (e.g. yohimbine) stressors that lack face and translational validity. Social defeat stress has been proposed as an ethologically-valid psychosocial stressor in rodents and has been argued to more closely model the forms of psychosocial stress experienced by drug abusers that promote craving and relapse. We therefore sought to develop and characterize a novel preclinical model of stress-induced cocaine relapse in rats using social defeat stress.
Methods: Adult male Long-Evans rats were trained to self-administer cocaine (0.5 mg/kg/infusion) under a continuous schedule of reinforcement in daily 2 hr sessions for 20 days. On days 11, 14, 17, and 20, subjects were removed from the chamber immediately following the session and either subjected to social defeat stress using a conventional resident-intruder procedure or placed into an empty cage. Discrete environmental stimuli (odorous and tactile) present within the operant chamber on these days signaled the impending social defeat stress or empty cage exposure. Extinction training then began on day 21, during which responses no longer resulted in cocaine infusions. Once responding was extinguished, rats were reexposed to the discrete cues that previously signaled impending social defeat stress or empty cage exposure and allowed to lever-press under extinction conditions. Immediately following the reinstatement test session, animals were transcardially perfused and brains were removed and processed for c-Fos immunohistochemistry. All protocols and procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Emory University Institutional Animal Care and Use Committee.
Results: Compared to their non-stressed counterparts, animals exposed to psychosocial stress-predictive cues exhibited significantly greater reinstatement of cocaine seeking that was paralleled by a significant rise in plasma corticosterone levels. Ethographic analyses of social defeat encounters revealed that the average amount of time the rats had engaged in "active" coping behaviors during prior social defeat episodes was positively associated with the magnitude of cocaine-seeking behavior when reexposed to cues signaling impending social defeat. Additionally, preliminary findings from c-Fos immunohistochemical analyses indicate the possible recruitment of a hypothalamic-midbrain defensive circuit during psychosocial stress-induced drug seeking.
Conclusions: These studies describe a novel model for psychosocial stress-induced relapse of cocaine use in rodents. Our initial findings suggest that 1) distinct coping behaviors might predict individual propensity to exhibit drug-seeking behavior in response to perceived impending psychosocial stress, and 2) psychosocial stress-induced drug seeking may involve the activation of a conserved defensive brain circuit that has not previously been associated with substance abuse disorders. We plan to continue these experiments with a systematic comparison of the brain activation patterns during psychosocial stress-induced reinstatement and reinstatement produced by non-social stressors, with the goal of identifying novel targets for pharmacotherapeutic and/or behavioral interventions aimed at preventing relapse.
Keywords: Psychosocial Stress, Reinstatement, Cocaine Seeking, Animal Model, Self-Administration.
Disclosure: Nothing to disclose.

M228. Performance of Adolescent Cannabis Users on Dimensions of Decision-Making

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Background: Numerous studies suggest that individuals with substance use disorders exhibit impairments in decision-making (DM). DM, the ability to make optimal choices among competing options with uncertain outcomes (reviewed in Bechara, 2005), has been linked to dysfunction in several cortico-limbic structures including the OF, PFC, ACC, and IC (structures implicated in addiction neuropathophysiology). However, the evidence for DM impairments among cannabis users is mixed (reviewed in Crean, Crane, & Mason, 2011). Many of the seminal studies in this area have relied on small, adult-only samples using only a single measure to assess DM. Here, we address these issues by examining performance in a large cohort of adolescents across three measures that assess multiple facets of DM.
Methods: Participants were 384 adolescents at risk for cannabis dependence, based on their self-reported drug use history, who completed a baseline assessment as part of an ongoing prospective longitudinal study examining the influence of DM on cannabis use trajectories (R01DA031176; PI:RG). The sample was recruited from middle- and high-schools in the Miami-Dade area. Exclusion criteria comprised history of neurological, learning, or severe psychiatric disorders, head injury with loss of consciousness greater than 30 minutes, and history of significant drug and alcohol use. The 4-hour assessment spanned demographic, mental health, patterns and prevalence of substance use and substance use disorders, as well as neurocognitive functioning. DM was assessed with the Iowa Gambling Task (IGT), the Cups Task (CT), and the Game of Dice Task (GDT), which assess DM under conditions of ambiguity or specified risk and under conditions of possible loss or gain.
Participants were classified into four groups based on their cannabis use history: (1) regular recent users (RREC, $n = 125$) who reported using cannabis at least once a week for at least 3 months and reported use in the last 30 days; (2) non-regular recent users (NRREC, $n = 71$) who reported use in the last 30 days but have not used cannabis as much as once a week for 3 months; (3) past users who reported no use in the last 30 days (PAST, $n = 79$); (4) minimal or non-users (MIN, $n = 109$) who reported having used cannabis less than 3 times in their lifetime or not at all. Outcome measures scale

and subscale scores from the selected DM Tasks. One-way ANOVAs were used to compare group performance on indices of DM, with those yielding p-values $< .01$ being deemed significant. Post-hoc comparisons were conducted using Tukey's HSD. In order to determine if initial results were influenced by potential confounds, groups were also compared on factors that might influence DM performance, including, age, sex, race/ethnicity, depression symptoms, estimated IQ, lifetime frequency of use of other drugs, alcohol, and nicotine, and lifetime or current (last 30 day) diagnosis of an alcohol or substance use disorder (other than cannabis). Analyses comparing groups on DM performance were also examined after controlling for all study confound variables that differed significantly between the groups.

Results: The sample consisted predominantly of Latino (85%) participants (54% male) with a mean age of 15.4 (SD = .72) and of average estimated IQ of 108.4(SD = 14.9). Lifetime cumulative joints (.5 grams each) smoked was greatest in the RREC group (Md = 246.4), followed by the NRREC (Md = 18), PAST (Md = 14), and MIN (Md = 0) groups. Similarly, history of cannabis use disorder was 44%, 16.9%, 16.5%, and 0 for the RREC, NRREC, PAST, and MIN groups, respectively. Between-groups comparisons revealed statistically significant differences only on total risky choices on the GDT ($p = .0002$), but no other index of DM. Post-hoc comparisons indicated that the MIN group made fewer risky choices on the GDT compared to all the other groups ($p < .05$), but the cannabis using groups did not differ from each other. Results remained unchanged when including study covariates.

Conclusions: Arguably, adolescents are most at risk for neurocognitive deficits from cannabis use. However, our findings do not invariably show DM deficits among teen cannabis users, regardless of whether their use was more regular, recent, or remote. Indeed, individuals who experimented with cannabis and those with more regular patterns of use both made more risky choices on a single DM task where risk was specified, but not on several other measures of DM, when compared to minimally and non-using controls. It may be that use of cannabis must persist throughout adolescence to impact neurodevelopment and manifest with more apparent DM deficits, which will need to be determined in future longitudinal analyses.

Keywords: Cannabis use, Adolescents, Decision Making.

Disclosure: Nothing to disclose.

M229. A Comparison of the Relative Reinforcing Effect of Nicotine, Heroin, Remifentanil and Cocaine Determined by Progressive Ratio Intravenous Self-Administration Testing in Rats

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Background: The technique of intravenous self-administration in animals using fixed ratio (FR) schedules of drug reinforcement will answer the question of whether or not a drug serves as a positive reinforcer. When developing novel CNS-active drugs for use in humans, it is important not only to know whether it is a positive reinforcer, but also how rewarding it is relative to other known substances of abuse (see Committee for Medicinal Products for Human Use (CHMP)/European Medicines Agency [EMA], 2006). To achieve this objective, it requires an additional evaluation using a progressive ratio (PR) to determine the break-point for drug reinforcment. This result is only helpful if highly abused drugs with very different pharmacological mechanisms support broadly similar break-points. In this study, we have established self administration in rats with the non-scheduled drug, nicotine, or the Schedule 2 (C-II) stimulant, cocaine, or the C-II opiates, heroin or remifentanil, on a FR schedule. We have then performed a PR/break point determination for various doses of each of these abused drugs to compare their relative efficacy as positive reinforcers.

Methods: Male Sprague Dawley rats (175-225g on arrival; Charles River, UK) were mildly food restricted and trained to press levers for food rewards. Once responding was stable, animals were implanted with a jugular vein catheter.

After nicotine acquisition (0.03 mg/kg/injection, iv) and saline extinction, a dose response test was performed for nicotine (0.0075, 0.015, 0.03, 0.06 mg/kg/injection) on FR-5 schedule in 2 hr sessions. When stable (injections [inj]/session did not vary by > 20% over 3 consecutive sessions) and where each drug dose was positively reinforcing (mean > 12 inj/session/3 sessions), the break point of operant responding was determined in a 4 hr PR session. Responding for heroin (0.025 mg/kg/inj), remifentanil (0.015 mg/kg/inj) and cocaine (0.29 mg/kg/inj) was also assessed using the PR schedule in separate cohorts of rats. The doses used in the break-point determinations were the most reinforcing on PR schedule in previously performed dose-response tests. Results are reported as mean ± SEM for ≥ 5 rats/group.

Results: On the FR5 schedule, nicotine maintained self-administration (inj/session) at levels significantly ($p < 0.001$) greater than saline (3.8 ± 0.4) at all doses (0.0075 mg/kg/inj = 16.7 ± 2.6; 0.015 mg/kg/inj = 19.1 ± 0.6; 0.03 mg/kg/inj = 18.2 ± 0.9; 0.06 mg/kg/inj = 16.8 ± 1.6). On the PR schedule, the break-points of responding for nicotine were 42.3 ± 10.7 for 0.0075 mg/kg/inj ($n = 6$), 73.4 ± 14.8 for 0.015 mg/kg/inj ($n = 8$), 48.8 ± 10.0 for 0.03 mg/kg/inj ($n = 8$) and 67.2 ± 10.1 for 0.06 mg/kg/inj ($n = 7$). The break points for highly reinforcing doses of heroin (61.8 ± 17.7 for 0.025 mg/kg/inj; $n = 8$) and remifentanil (48.1 ± 18.2 for 0.015 mg/kg/inj; $n = 5$) were not different from the most reinforcing dose of nicotine (0.015 mg/kg/inj). The break-point for nicotine was significantly higher than for cocaine (46.6 ± 4.2 for 0.29 mg/kg/inj; $n = 9$; $P < 0.05$).

Conclusions: Using an ascending PR schedule, heroin, remifentanil and cocaine were robust positive reinforcers in the test, reflecting their known profiles as highly abused drugs in humans. The relative reinforcing efficacy of heroin, remifentanil and cocaine did not differ substantially from one another. The relative reinforcing effect of nicotine did not differ from heroin and remifentanil, but was significantly greater than cocaine. In summary, this investigation revealed similar break-points for three C-II Controlled Drugs with very different pharmacological mechanisms. Nicotine had the same or greater reinforcing properties in this test underpinning why quitting smoking is said to be more difficult than kicking heroin.

Committee for Medicinal Products for Human Use (CHMP) / European Medicines Agency (EMA) (2006). Guideline on

the Non-Clinical Investigation of the Dependence Potential of Medicinal Products.
Keywords: Nicotine, Heroin, Cocaine, Remifentanil, Self-Administration.
Disclosure: RenaSci, Ltd: Shareholder and Employee, Self.

M230. Trajectories of Impulsivity in Opioid Use Disorder Treatment: A Longitudinal Study of Temporal Discounting and its Dynamic Relation to Drug Use and Treatment Efficacy

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Background: Impulsivity is a core feature of substance use disorders. Temporal discounting (TD) paradigms provide a model-based approach to studying the dynamics of impulsive decision-making as individuals with substance use disorder undergo treatment. Here, we examine how TD changes as opioid use disorder (OUD) subjects stabilize on maintenance therapy and we assess how TD is predicted by (or is predictive of) relevant clinical outcomes such as illicit drug use, treatment adherence and clinical states like craving.
Methods: Individuals initiating medication-assisted treatment for OUD were assessed weekly then bi-weekly (for up to seven months) on a simple TD task. For each session, (1) we derived a computational subject-specific parameter for the TD rate as well as a model-free measure: the proportion of immediate rewards chosen; (2) we monitored illicit drug use through randomly administered weekly urine toxicology and direct self-report; (3) we established their level of adherence to their individual treatment plan as well as their current medication dose; and (4) we scored their current levels of craving, withdrawal symptoms and state anxiety.
A group of demographically matched drug-free community controls (CC) were similarly assessed repeatedly in order to establish the test-retest reliability of our measurement and discard effects of practice and repetition.
In addition, eligible subjects from both groups completed the tasks while we acquired functional magnetic resonance imaging (MRI) data in two sessions: one at the beginning of the study and the other 8-12 weeks later. During this interval, subjects continued their regular assessments outside of the scanner.
Results: As previously reported, OUD patients have significantly higher discount rates compared to controls but in our demographically matched groups the difference appears to be smaller than previously reported. Our results indicate that TD measurements have high test-retest reliability. While stable in our control group, in OUD patients the TD rates are a dynamic function of time in treatment. Interestingly, TD rates also correlate with illicit drug use events, peaking around the time that these occur. Moreover, the individual trajectory of TD leading up to these lapse events correlates with the degree of overall use during our follow up, suggesting that the course of a patient's impulsivity might be predictive of their relative success at maintaining abstinence during treatment.
Conclusions: We conclude that TD, when assessed repeatedly over the course of treatment, could be used as a behavioral signature of a patient's clinical evolution and potentially serve as a useful predictor of prognosis and treatment adherence for OUD. Our TD task is easy to automate and administer and therefore lends itself to use in larger clinical studies and might be useful to incorporate into the monitoring of these patients' progression. Our ongoing efforts focus on the investigation of the neural substrate(s) of the observed change in TD with treatment for OUD. We are exploring how the activity of regions involved in the computations necessary for impulsive decision-making (i.e. the ventromedial prefrontal cortex, ventral striatum and posterior cingulate cortex) contributes to treatment efficacy.
Keywords: Opioid Dependence, Impulsivity, Delay Discounting, Computational Psychiatry, Trajectories.
Disclosure: Nothing to disclose.

M231. Optimal Prevention of Relapse Among Opioid Users: A 12-Week Randomized Controlled Trial of Extended-Release Naltrexone Injections Versus Daily Buprenorphine-Naloxone

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Background: The abuse of opioid drugs has a higher risk of death than any other drug use disorder; a main mechanism is the development of addiction to illicit opioids like heroin or illicit diversion of prescribed opioid medications. In recent years, the preferred OMT has been a buprenorphine formulation combined with a naloxone component designed to be inert when ingested orally but cause withdrawal when injected into the bloodstream; its effectiveness in preventing abuse has been subject of debate. A different approach is medically maintained abstinence by use of the long-acting opioid antagonist naltrexone, but this have not been tried out in Western Europe and never been compared with buprenorphin.
We performed the first head-to-head comparison study to investigate whether long acting naltrexone (XR-NTX) injections are more effective than – or equally effective to – opioid maintenance treatment with daily oral buprenorphine-naloxone (BP-NLX) in reducing the use of opioids and other illicit drugs in opioid dependent patients.
Methods: 164 opioid dependent adults with no other serious mental or somatic disease were randomly assigned to receive 12 weeks of treatment with either XR-NTX every four weeks (380 mg) or daily BP-NLX flexible dosed 4-24 mg. The study was not blinded due to safety reasons.
Results: Three fourths of the patients completed the 12-week study, and there was no significant difference in time to drop-out between the groups. Among the completers, we found more frequent self-reported use of illicit opioids in the BP-NLX group compared to the XR- NTX group ($p = .03$). There were no significant differences between the groups on self- reported use of other illicit drugs, self-reported quality of life, drug craving and mental health problems. Satisfaction with treatment was significantly higher among XR-NTX patients ($p = .001$). XR-NTX was generally well tolerated,

with adverse effects most commonly being prolonged symptoms of opioid withdrawal. Four cases of opioid overdose were reported in the BP-NLX Group, but none in the XR-NTX group. Non-inferiority analyses showed XR-NTX treatment was not inferior to the current standard OMT treatment in reducing the use of any illicit drugs.
Conclusions: XR-NTX was equally effective to BP-NLX in in reducing the use of opioids and other illicit drugs in opioid dependent patients. Patients receiving XR-NTX reported a high satisfaction with their treatment and XR-NTX may represent a new treatment alternative in Europe for patients wanting stop with heroin or opioid maintenance treatment (OMT).
Keywords: Naltrexone, Buprenorphine-Naloxone, Opioid Dependence, Head-to-Head Clinical Trial.
Disclosure: Nothing to disclose.

M232. Dopaminergic and Serotonergic Interactions Underlying Stress-Induced Potentiation of Cocaine Reward

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Background: Current treatments for drug addiction are hampered by the high rate of relapse in substance-dependent individuals during abstinent periods. Risk for relapse of drug taking is particularly elevated following stressful experiences, as stress-induced disruptions in motivation and cognition lead to increased reward-seeking behaviors. Numerous studies have demonstrated that dynorphin, the endogenous kappa opioid receptor (KOR) ligand, mediates dysphoria associated with stressful states and shown that dynorphin tone is increased following repeated exposure to abused substances. Dynorphin/KOR actions on dopamine neurons have been shown to underlie aversion learning and aversive aspects of drug taking, and we hypothesized that potentiation of cocaine reward is likely to occur through similar neural mechanisms. The studies presented here investigate how dopamine neurons promote reward seeking following stress and the possible sources of these dynorphin-mediated changes in dopaminergic neurons of the ventral tegmental area.
Methods: Our behavioral experiments used a conditioned place preference (CPP) procedure. CPP is an associative learning procedure where a discriminable set of cues is paired with either drug or vehicle, and animals are tested for preference or aversion for contextual cues in a drug-free state. Male and female C57BL/6 mice were exposed to repeated forced swim stress, KOR agonist, optogenetic inhibition, or were gently handled prior to the first cocaine/optical conditioning session. Optical stimulation occurred with Channelrhodopsin-2, and optical inhibition occurred with Step-waveform inhibitory Channelrhodopsin (SwiChR). Retrograde tracing studies utilized CAV2 vectors with Cre-dependent expression of a green fluorophore (Zs-Green).
Results: Male and female C57BL/6 mice both demonstrated potentiation of cocaine reward following stress. These effects were also observed when mice were pretreated with a KOR agonist, U50,488 (5 mg/kg), 1 hour prior to a cocaine conditioning session. KOR agonism may also potentiate reward mediated by optical stimulation of dopamine neurons in DAT-Cre (dopamine transporter) mice. Transient inhibition of dopamine neurons was sufficient to potentiate reward, as DAT-Cre mice that received a 30-min session of optical inhibition prior to cocaine conditioning showed potentiation of cocaine CPP. These results suggested that KOR activation on dopamine neurons could potentiate reward, but the sources of dynorphin into the ventral tegmental area that are responsible for these effects were unknown. To this end, we injected a retrograde virus into the VTA of preprodynorphin-Cre (pdyn-Cre; a precursor for dynorphin) and observed cell body labeling in the prefrontal cortex, striatum and dorsal raphe nucleus, matching some patterns of cell body expression observed in pdyn-Cre mice with a tdTomato reporter. Immunohistochemistry suggested that dynorphin containing neurons in the dorsal raphe nucleus projecting to the ventral tegmental area were primarily serotonergic. Current experiments aim to characterize the contribution of this serotonin/dynorphin-containing neuronal population to stress-mediated changes in reward-seeking behaviors.
Conclusions: Our studies show that transient inhibition of dopamine neurons by KOR activation or optical techniques prior to cocaine conditioning can enhance the rewarding value of cocaine. We identified a population of serotonergic neurons that contain dynorphin and project to the ventral tegmental area, suggesting there may be an interaction between serotonergic and dopaminergic neurons that is mediated by dynorphin during stressful experiences. Our current studies aim to understand the role of these dynorphin containing serotonin neurons in signaling aversion and the specific role of these neurons in stress-mediated changes to reward value.
Keywords: Dynorphin, Dopamine, Cocaine Seeking.
Disclosure: Nothing to disclose.

M233. Rate of Consumption as a Marker for Alcohol Use Disorder Risk: Evidence From a Human Laboratory Model

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Background: Problematic alcohol use accounts for a leading source of morbidity and mortality worldwide, but determining risk for problematic drinking remains difficult. Although consuming large quantities is a well-established risk factor, rate of consumption has received less attention, despite the fact that binge drinking has been associated with greater risk of health and cognitive problems. This is partly due to the complication of studying rate, since a number of factors affect the concentration of alcohol in the blood following the same dose of alcohol, including age, sex, weight, height, and recent diet. Here we tested the hypothesis that a history of problem drinking would predict rate of alcohol consumption in a carefully controlled experimental paradigm, and that

rate of alcohol consumption may therefore be a marker of risk for alcohol use disorders.
Methods: The study included 208 healthy male and female volunteers whose consumption patterns ranged from social to heavy drinking. Participants completed the Alcohol Use Disorders Identification Test (AUDIT) to assess risk for alcohol problems and the Timeline Follow-Back (TLFB) interview to quantify number of drinks over the previous 90 days. Participants then completed a laboratory session where they received a priming dose of intravenous alcohol followed by 125 minutes of ad libitum intravenous alcohol self-administration. The primary outcome measure was whether participants achieved binge-level exposure, defined by the NIAAA guideline of a Breath Alcohol Concentration (BrAC) above 0.08 g%.
Results: 88 individuals (42.3% of the sample) achieved binge-level exposure during the ad libitum phase. Cox proportional hazard models revealed that AUDIT scores and total drinks were both significant predictors of binging during the session; individuals with higher scores and more drinks achieved 0.08 g% BrAC more frequently and faster (AUDIT model: Wald = 13.4, TLFB model: Wald = 13.3, both ps < 0.001). Age and sex were significant variables in the model, where younger participants and males were more likely to binge. For both models, these effects were stronger during the first hour than the second hour of the experiment, suggesting that the first hour of a drinking session may be the most indicative of risk.
Conclusions: These outcomes suggest that the intravenous alcohol self-administration paradigm has strong external validity and that rate of consumption is a marker of risk for alcohol use disorders. These findings suggest that interventions that train individuals to slow rate of consumption may improve alcohol-related health outcomes.
Keywords: Alcohol Consumption, IV Alcohol, Alcohol Self-Administration, Alcohol use Disorder.
Disclosure: Nothing to disclose.

M234. Frontostriatal Connectivity During Response Inhibition Predicts Sensitivity to Amphetamine Reward

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Background: Individuals with poor inhibitory control are at increased risk for developing drug abuse problems, perhaps due in part to heightened drug reward sensitivity. Indeed, we have previously shown that a behavioral measure of response inhibition predicts greater subjective response (euphoria and arousal) to amphetamine. Further, preclinical and clinical studies show that both drug reward and response inhibition are linked to D2-receptor availability in overlapping fronto-striatal pathways, suggesting a biological link between the two. Here, we examined the degree to which frontostriatal connectivity during response inhibition predicted the rewarding effects of a single dose of amphetamine in healthy adults. We hypothesized that reduced frontostriatal connectivity would be related to greater sensitivity to amphetamine reward.
Methods: Participants ($n = 39$) performed the stop signal task to assess response inhibition while undergoing fMRI. Frontostriatal connectivity was assessed using psychophysiological interaction (PPI) analyses. Separately, participants attended four drug sampling sessions in which they received color-coded capsules containing either d-amphetamine (20mg) or placebo, in alternating order, and completed subjective self-report measures of drug response. Participants then attended a choice session in which they were allowed to choose which color capsule they preferred to take.
Results: Functional connectivity analyses revealed significant connectivity between the right accumbens and anterior cingulate during response inhibition. Moreover, correlational analyses revealed that the strength of this connectivity was negatively associated with amphetamine reward, such that weaker frontostriatal connectivity was associated with greater euphoria ($r = -0.382$, $p = 0.026$) and stimulation ($r = -0.502$, $p = 0.003$), as well as greater choice for amphetamine over placebo ($t = 2.0$; $p = 0.05$).
Conclusions: These findings provide further evidence for the association between poor inhibitory control and sensitivity to amphetamine reward. Importantly, they support the hypothesis that reduced top-down prefrontal control of striatal regions during response inhibition is related to greater amphetamine reward. Ultimately, understanding the behavioral and neurobiological mechanisms linking poor inhibitory control with drug reward may lead to behavioral and pharmacological interventions that simultaneously improve control and dampen euphorigenic responses to drugs, thus reducing likelihood of relapse in individuals with poor inhibition.
Keywords: Amphetamine, Inhibitory Control, Functional Connectivity.
Disclosure: Nothing to disclose.

M235. Use of Marijuana for Medical Purposes Among Adults in the United States

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Background: Legalization of marijuana for medical purposes has been adopted by 25 states and the District of Columbia. However, little is known about characteristics of marijuana use for medical purposes among adults and how they may differ from characteristics of adults reporting nonmedical marijuana use. The purpose of this presentation is to estimate the 12-month prevalence of medical marijuana use only, nonmedical marijuana use only, and combined medical and nonmedical marijuana use among adults and adult marijuana users in the U.S. and to examine whether and how adults reporting medical marijuana use only differ from adults reporting combined medical and nonmedical marijuana use and those reporting nonmedical marijuana use only.
Methods: Data are from 96,100 adults aged 18 or older who participated in the 2013-2014 National Surveys on Drug Use and Health. The main exposure variable is marijuana use, and the for the main outcome measures, medical marijuana use only was defined as no marijuana use reported in the

past year except for that "recommended by a doctor or other health care professional".

Results: Among 31 million past-year adult marijuana users in the U.S. (12.9% of the adult population), approximately 27.6 million (90.1%) used marijuana only nonmedically only, 1.9 million (6.3%) reported medical marijuana use only, and 1.1 million (3.6%) reported combined medical and nonmedical marijuana use. Compared to nonmedical marijuana use only, medical marijuana use only was positively associated with older age, older initiation age of marijuana use, disability, Medicaid beneficiary status, stroke diagnosis, poor self-rated health, anxiety disorders, daily or near daily marijuana use, residing in a state with legalization of medical marijuana use, and perceived state legalization of medical marijuana use and was negatively associated with heavy alcohol use and nonmedical use of prescription stimulants and pain relievers. Other substance use patterns were similar between medical marijuana only users and nonmedical marijuana users.

Conclusions: In the U.S., the majority of past-year adult marijuana users (90.1%) did not reported using marijuana only nonmedically for medical reasons, and approximately 6% of past-year adult marijuana users reported usinged marijuana solely for medical purposes. Medical marijuana users differed from nonmedi.cal marijuana users in ways that were consistent with endorsement of marijuana use for medical problems.

Keywords: Medicinal Marijuana, Epidemiology, Marijuana.

Disclosure: Nothing to disclose.

M236. Event-Related Theta Oscillations During Reward and Punishment Processing as Markers of Cannabis and Tobacco Use in Adolescent Daily Cigarette Smokers

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Background: Despite significant public health efforts, cannabis and tobacco remain two of the most commonly used drugs by adolescents. Cannabis and tobacco use, co-use, and related disorders are common, and associated with poor treatment outcomes. How the combined use of cannabis and tobacco impacts developing brain function, emotional states, and behaviors is poorly understood. Cannabis and tobacco use disorders have been separately linked to dysfunction in the neural processing of rewards, but few studies have examined co-use or comorbid disorders. The present study examined event-related oscillations in the theta (4-8 Hz) frequency during reward and punishment processing in non-deprived adolescent daily cigarette smokers stratified by daily cannabis use, and age, gender, and grade-level matched typically developing adolescents.

Methods: Adolescent daily cigarette smokers and matched controls were recruited from public schools and via local community advertising in the greater New Haven area. Groups were stratified by daily cannabis use into daily tobacco and cannabis users, daily tobacco non-daily/intermittent cannabis users, and healthy controls. Dense-array electroencephalography (EEG) was collected from a 128 Ag/AgCl electrode net using 100 Hz low pass filtering and 1000 Hz sampling rate. EEG was recorded in participants as they performed a non-learning four choice guessing task with monetary reward, neutral ('breaking even'), and loss conditions. Event-related theta (4-8 Hz) oscillations in the time window of 200-400 msecs obtained from the medio-frontal electrodes (11 (Fz), 12, 5, 6) was computed following each outcome stimulus using event-related spectral perturbations (ERSP) and intertrial phase coherence (ITC) approaches. Additionally, self-report data on substance use, impulsivity, and negative affect (depression and anxiety) and biochemical data on cannabis and cotinine levels were analyzed.

Results: Sixty-five participants (75% male; Mean Age = 17.7, SD = 1.40) including 36 smokers and 29 non-smoking typically developing controls met inclusionary criteria for the study and completed the experimental paradigm. Adolescent smokers had higher impulsivity but did not differ from controls in depression or anxiety. Half of the adolescent smokers were daily tobacco and daily cannabis users ($n = 18$). Adolescent daily tobacco and cannabis users had higher rates of cannabis use and increased addiction severity scores for cannabis and tobacco compared to adolescent daily tobacco non-daily/intermittent cannabis users. Complex wavelet frequency decomposition revealed that theta response to feedback followed a step-wise pattern with enhanced theta power and phase coherence during loss condition compared to neutral and gain, and in neutral compared to gain. While neither of the smoker groups differed from controls, adolescent daily tobacco and cannabis users had significantly reduced theta power ($t(31) = -2.13$, $p = 0.03$) and phase coherence ($t(31) = -2.06$, $p = 0.04$) during monetary gain receipt compared to adolescent daily tobacco non-daily cannabis users. In exploratory post-hoc analyses, theta oscillations were associated with cannabis use, cotinine levels, nicotine dependence, nicotine withdrawal symptoms, and impulsivity in adolescent daily cigarette smokers, and impulsivity and depression in typically developing adolescents.

Conclusions: This study indicates that adolescent non-deprived daily cigarette smokers who use cannabis and tobacco do not differ from typically developing adolescents in event-related theta oscillations during reward and punishment processing. Based upon our significant substance use associations with theta oscillations, it is likely that the combined effects of cannabis and tobacco or acute nicotine exposure may have 'normalized' smoker's EEG signals during the non-deprived state. Reward-related theta oscillations were moderated by cannabis use, cotinine levels, nicotine dependence, nicotine withdrawal, and impulsivity in smokers and impulsivity and depression in controls. These variables may interact in complex ways contributing to the summative theta EEG signal during reward-related feedback. Daily tobacco and cannabis users had reduced theta oscillations during reward receipt compared to daily tobacco non-daily cannabis users indicating possible cannabis-related impairment in cognitive control during reward processing. As such, additional work is needed to examine how levels of cannabis and tobacco use and co-use impact brain function, and to what extent intermittent/non-daily cannabis and tobacco use patterns are or are not related with impairments in brain function and behavior. Adolescent daily cigarette smokers did not demonstrate the same theta oscillation and depression relationships that non-depressed healthy controls

did, suggesting that smoking may disrupt neural processes involved in affect regulation. Future studies should seek to clarify the complex interrelationships between cannabis and tobacco use, impulsivity, negative affect, and reward and punishment processing as these may help guide the development of novel treatment approaches for individuals that co-use cannabis and tobacco.
Keywords: Cannabis use, Adolescents, EEG biomarkers, Tobacco Smoking, Human Neuroimaging.
Disclosure: Nothing to disclose.

M237. Shared Vulnerability: Brain Structures Associated With Youth Trauma and Adult Addictions

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Background: Youth with traumatic experiences are at higher risk for posttraumatic stress disorder (PTSD) and latter adolescent and adult addictions. PTSD and alcohol and substance use disorders (SUD) shared neurobiological deficits in orbital frontal and posterior gray matter structure and function. Translational research suggest that these areas are involved in NIMH Research Domain Criteria (RDoC) constructs involved in learning, cognitive control, reward response, and declarative memory, as dysfunction in these brain circuits impair new learning. We studied the anatomical brain regions involved in this constructs. These deficits in these constructs can be considered failures to develop new learning, a neurobiological process that is called extinction in the trauma field. During extinction, the absence of trauma in the face of traumatic reminders causes new pathways to develop that inhibit the initial fear response to traumatic reminders in those with PTSD symptoms, and thus extinction processes lead to PTSD symptom recovery. Likewise, in SUD, there are failures to develop new learning, i.e., learning to inhibit cravings (called reversal learning in animals and extinction therapy in humans). This new learning reduces conditioned responses to substances and leads to dissociating the drug with positive reinforcement (getting high) leading to sobriety. However, in addictions, this learning does not take place, causing high rates of relapse. In both PTSD and SUD, we do not know if deficits in the ability to learn and adapt to different circumstances are present prior to PTSD and SUD. We also do not know if these deficits are associated with characteristics of early trauma or familial history of SUD. In adults who experienced trauma but do not develop chronic PTSD, the dorsal and medial networks that are involved in cognitive control strongly regulate behavioral inhibition and emotional regulation so that, when confronted with traumatic cues, these individuals do not experience PTSD symptoms. The same is true of adults who try substances but do not develop addictions; when confronted with substance use stimuli, these same neural-resources are available to control impulsive use. As a first step in investigating the brain mechanisms associated with trauma and its association with latter SUD, we examined brain structures associated with cognitive control and extinction in the National Consortium on Alcohol and NeuroDevelopment in Adolescence study (NCANDA) sample, an epidemiological sample, to examine the relationship between brain structures and traumatic experiences, PTSD symptoms, and familial density of alcohol and substance use disorder.
Methods: Preliminary cross-sectional anatomical total gray and white matter cortical volumes data from the NCANDA examined 617 (340 females) low- to no-drinking adolescents in relationship to lifetime traumatic experiences, PTSD symptoms, and familial density of alcohol and substance use disorder. NCANDA is a multisite accelerated longitudinal, cross-sequential study that uses multimodal brain imaging (anatomical, diffusion tensor, fMRI of resting state), cognitive testing, and psychological measures at 4 yearly time points to determine both the neuro-markers of SUD and the neuro-effects of alcohol and other substances on adolescent brain maturation in a large sample of community youth (Brown et al, 2015; Pfefferbaum et al, 2015). Psychiatric diagnoses and trauma history were assessed with the Computerized Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) in the NCANDA study. NCANDA subjects were recruited at baseline without SUD or binge history.
Results: In the NCANDA sample, 61.4% experienced some form of trauma while 38.6% did not. The most common trauma was hearing about the traumatic death of a family or friends (32.2%). Number of lifetime traumatic experiences predicted smaller frontal gray matter ($p = .004$), insula cortical thickness ($p = .02$), and posterior corpus callosum ($p = .05$). PTSD symptoms predicted smaller parietal surface area ($p = .03$) and posterior corpus callosum ($p = .006$), and larger lateral ventricles ($p = .02$). Family density of alcohol and substance use disorders predicted smaller insular gray matter volume ($p < .03$) and cingulate surface area ($p = .04$).
Conclusions: Given these findings from NCANDA, where subjects with early trauma and PTSD symptoms showed similar findings to adults with alcohol and substance use disorders, it is possible that anatomical differences in key structures associated with cognitive and emotional control and extinction learning may represent a shared risk mechanism for PTSD and later addictions (De Bellis et al, 2015; Morey, Haswell, Hooper, & De Bellis, 2016). These findings are complex and suggest that there may be a shared risk mechanism for both PTSD and addictions and this may be related to trauma or familial factors that predate early trauma.
Keywords: Human Neuroimaging, Addiction, Epidemiology.
Disclosure: Nothing to disclose.

M238. A Phase 1 Single- And Multiple-Rising Dose Study of the Safety & Pk of EMB-001, a Potential Treatment for Substance Use Disorders, With Exploratory Efficacy Measures in Tobacco Use Disorder

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Background: EMB-001 is a combination of two FDA-approved drugs: metyrapone (MET), a cortisol synthesis

inhibitor, and oxazepam (OX), a benzodiazepine. MET is approved for only one day of use as a test of pituitary function; OX is approved for acute and chronic treatment of various anxiety disorders and alcohol withdrawal. EMB-001 reduced cocaine and nicotine self-administration and attenuated cocaine and methamphetamine cue reactivity in rats. In an earlier human study in cocaine-dependent subjects, EMB-001 significantly reduced cocaine use.

Methods: This was a single- and multiple-rising dose study. Healthy volunteers who were daily cigarette smokers aged 18-65 were recruited; this population is relevant for studying both tobacco use disorder and cocaine use disorder, as 75-80% of the latter also smoke cigarettes. Subjects received a single am dose on Day 1, followed by a 48-hr pause to assess safety & PK. Then they received BID dosing on Days 3-9 and a single am dose on Day 10, again with safety & PK assessed. Three sequential dose cohorts of 8 subjects (6 drug, 2 placebo) received the following doses of MET and OX, respectively: 270 and 12 mg; 540 and 24 mg; and 720 and 24 mg. Total daily doses were double these amounts on BID dosing days, which were still low relative to FDA-approved maximum daily doses of both drugs (4500 mg in one day for MET; 120 mg per day for OX). Primary outcomes were safety and the pharmacokinetics of MET, its active metabolite metyrapol, and OX. Safety measures included vital signs, ECGs and standard safety labs. Cortisol and other HPA axis parameters were monitored closely throughout the study. In addition, exploratory measures of efficacy in smoking cessation were assessed. Cigarettes smoked, breath CO and urine cotinine were assessed. The Smoking Urges Questionnaire and the Minnesota Nicotine Withdrawal Symptoms scales were administered prior to the start of BID dosing on day 3, and on the last day of BID dosing after a 12-hr enforced abstinence from smoking. The study was not powered for these efficacy assessments.

Results: The most frequent adverse event was somnolence. Most AEs were mild; all were mild or moderate. There were no SAEs and no discontinuations due to AEs. Serum cortisol was reduced 2-4 hours after the first dose, consistent with the known pharmacology of MET, but returned to baseline on subsequent mornings and at follow-up; there were minimal to no symptoms suggesting adrenal insufficiency, and ACTH stimulation tests were normal. There were no clinically significant changes in vital signs, ECGs or other safety labs. The half-lives of MET, OX and metyrapol were approximately and respectively 2, 7.5 and 8 hr. Exposure increased with increasing dose and there was modest accumulation with repeated dosing. There were reductions in cigarettes smoked, smoking urges and withdrawal symptoms, and although there was large variability, few systematic dose-related effects and most findings did not reach statistical significance in this small study, the Cohen's d effect sizes were moderate, ranging from .31 - .47.

Conclusions: EMB-001 was well-tolerated in this study and no new safety signals were identified. These findings are generally consistent with MET and OX approved labeling and with safety data in 6 published studies in which MET doses of 500-4000 mg/day were given for 2-8 weeks. PK results suggest that twice-daily dosing may provide appropriate duration of exposure for efficacy. Effects on smoking were encouraging for a small study that was not powered for efficacy. Future plans include Phase 1b and 2 studies in cocaine use disorder (which has been supported by the recently-awarded grant U01DA038879 from NIDA) and a Phase 2a study in tobacco use disorder.

Keywords: Cocaine, Tobacco, Cortisol.

Disclosure: Embera NeuroTherapeutics: Consulting and Equity, Self.

M239. A Longitudinal fMRI Study of Initiation of Heavy Drinking and Neural Response to Alcohol Cues in College Students

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Background: Recent functional magnetic resonance imaging (fMRI) work describes increased brain response to alcohol cues associated with heavy drinking. However, it is unclear how neural response to alcohol cues changes as heavy drinking emerges. In this 2-year longitudinal study, we explored whether initiating heavy drinking was related to fMRI response to alcohol images among college students.

Methods: Participants were 60 individuals who underwent fMRI scans during freshman year of college (average age 18.4 years), and again approximately two years later. Based on our previous work (Dager et al, 2014) heavy drinking was defined as averaging >30 drinks per month in the past 6 months. Unlike our previous work that only followed drinking patterns for 12 months following scanning, here we ascertained both drinking and fMRI response at baseline and two-year follow-up. All participants were moderate drinkers (no more than 30 drinks per month) at baseline; 49 remained moderate drinkers at follow-up, and 11 increased to heavy drinking by follow-up. During fMRI scanning at baseline and follow-up, participants viewed alcohol beverage images and matched non-alcohol beverage images as described previously. Independent samples t-tests determined group differences between individuals who were consistently moderate drinkers compared to those who increased to heavy drinking on fMRI response to alcohol vs. non-alcohol beverage images at baseline and follow-up (clusters >518 voxels, voxel-wise $p<.05$, whole-brain alpha $<.025$ at each time-point).

Results: Although groups did not differ on fMRI response at baseline, several widespread regions showed group differences at follow-up, including right parahippocampal and middle temporal gyri, bilateral fusiform, cerebellum, lentiform, insula, inferior parietal lobule, and occipital cortices, and left cingulate. In each region, those who transitioned to heavy drinking showed more fMRI response at follow-up to alcohol images than those who remained moderate drinkers. Stepwise regressions in SPSS indicated that change in drinks per month between baseline and follow-up was positively associated with follow-up fMRI response, above and beyond baseline drinks per month.

Conclusions: Our results show that fMRI response to alcohol cues at 2-year follow-up was higher among those who increased from moderate to heavy drinking compared to those who remained moderate drinkers. This heightened activation was found in brain areas subserving craving,

attention, and memory, overlapping with regions we have previously associated with heavy drinking in college students (Dager et al, 2013). These regions did not show group differences at baseline, suggesting that this pattern of hyperactivation emerges as drinking escalates. Our results support models proposing changes in neural systems underlying alcohol-related reward as drinking progresses.
Keywords: Alcohol, Young Adults, fMRI, Cue Reactivity.
Disclosure: Supported by NIAAA (AA016599 & AA19036; Pearlson) and the Alcohol Beverage Medical Research Foundation (Anderson).

M240. Relating Anticipatory Processing to Risky Decision-Making: Differences in Gambling Disorder and Healthy Controls

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Background: Decision-making varies both among healthy individuals and those with gambling disorder (GD), and this variability is reflected in performance on emotion-related learning tasks such as the Iowa Gambling Task (IGT). Neuroimaging studies demonstrate alterations in fronto-striatal neurocircuitry in GD, including the ventral striatum and ventromedial prefrontal cortex (vmPFC) during anticipatory processing, which may influence decision-making impairments. However, to date little is known about fronto-striatal anticipatory processing and emotion-based decision-making.
Methods: While undergoing functional magnetic resonance imaging (fMRI), 56 participants performed the Monetary Incentive Delay Task (MIDT). Twenty-eight participants met criteria for GD and 28 were healthy control (HC) subjects matched on age, gender, race, ethnicity and education. The MIDT effectively separates the prospect of reward/loss (A1) and anticipation of reward/loss (A2). Pearson correlation coefficients assessed the association of out-of-scanner IGT performance with the neural activity during prospect (A1) and anticipatory (A2) processing on the MIDT across combined GD and HC groups. Correlation coefficients also assessed the association with IGT performance separately in the GD and HC groups.
Results: The HC and GD groups showed no significant difference in out-of-scanner IGT performance across the five blocks of trials, although there was a trend for higher IGT scores in the HC group on the last two blocks of IGT trials. Whole-brain correlations across combined HC and GD groups showed that MIDT BOLD signal in the ventral striatum/caudate/vmPFC and anterior cingulate regions during the prospect of winning (A1Win phase) was associated with higher total IGT scores. These correlations appeared to be mostly driven by activity in the GD group. Ventral striatal/orbitofrontal cortex/vmPFC activity during the prospect of losing (A1Loss phase) was also correlated with higher IGT scores across both groups. There were no significant correlations between A2 phase activity across combined GD-HC groups with total IGT scores.
Conclusions: In the current study, fronto-striatal activity during the prospect of reward and loss on the MIDT was related to decision-making on the IGT. Reduced activity in these areas was related to poorer IGT performance, particularly in individuals with GD. These findings highlight how reduced reward prospect signaling may relate to impaired decision-making (i.e. lower IGT scores) and possibly the progression of GD. The findings from this work are novel in linking brain activity during a prospect-of-reward phase with performance on a well-validated emotion-based-learning decision-making task.
Keywords: Gambling Disorder, Decision Making, Ventral Striatum, Reward Prospect, Human Neuroimaging.
Disclosure: Boehringer Ingelheim, Ironwood, Lundbeck, INSYS, Shire, RiverMend Health, Opiant/Lakelight Therapeutics and Jazz Pharmaceuticals: Consulting, Self; Mohegan Sun Casino, the National Center for Responsible Gaming, and Pfizer, Forest Laboratories, Ortho-McNeil, Psyadon, Oy-Control/Biotie and Glaxo-SmithKline pharmaceuticals: Research Support, Self.

M241. Forward Genetic Approaches to Reward Behaviors

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Background: Misregulation of neuronal plasticity in the mesolimbic system is thought to play a key role in transition to addiction. Neuronal plasticity falls into two broad categories; functional plasticity in synaptic function and physiology (LTP/LTD) and structural plasticity of cellular architecture (dendritic spines, cell size). Although evidence for drug induced functional plasticity in regulating addiction is quite strong, structural plasticity remains controversial. There are conflicting reports in rodents and nonhuman primates regarding changes in dendritic spine density in the NAcc in response to psychostimulants and opioids. However, these studies are correlative, and lack of consensus has driven some to refer to drug induced spine density changes as an "epiphenomenon". As such, there is a great need for identification of genetic factors that influence the architecture of dendritic spines, in order to study the causal relationship between cell structure and addiction.
Methods: We use quantitative genetics, next generation sequencing, biochemistry, imaging, and electrophysiology.
Results: Using unbiased forward genetic approach, we recently identified Cyfip2 as a key regulator of acute and sensitized cocaine responses in an unbiased forward genetic approach utilizing closely related mouse substrains. This study had two major implications for the work proposed here: We identified a novel variant that regulates cocaine responses at the nucleotide level, and we demonstrated that CYFIP2 functions in an actin-remodeling complex and influences cocaine responses through regulation of neuronal structure in the NAcc.
Cyfip2 and its paralog Cyfip1 control neuronal structure through at least two pathways, FMRP and the WAVE complex, both previously shown to regulate neuronal connectivity and behavior. We identified a mutation in Cyfip2 (S968F) that exists in all C57BL/6N strain of mice that is being used for the Knockout Mouse Project. By

constructing an allelic series, we show that the S968F variant behaves like a hypermorh, a dominant gain of function mutation. We present data that this mutation leads to hyperactivated Rac signaling.
Conclusions: Using unbiased forward genetic analysis we link a novel actin remodeling factor to addiction relevant phenotypes. This approach is highly flexible and powerful for gene discovery for addiction.
Keywords: Genetic Mouse Model, Cocaine Addiction, Actin Remodeling.
Disclosure: Nothing to disclose.

M242. The Neural Correlates of Aversion Resistant Alcohol Seeking

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Background: A hallmark of alcohol addiction is continued alcohol seeking despite adverse consequences. Animal models have been used to investigate compulsive alcohol seeking and taking. These models have identified the medial prefrontal cortex, insula, and nucleus accumbens as key regions for aversion resistant alcohol seeking (Seif et al, 2013). Despite the importance of compulsivity in addiction, there have been very few studies of aversion resistance in alcohol addiction in humans. Here, we translate a preclinical paradigm into an fMRI task to investigate the neural substrates of aversion resistant alcohol seeking.
Methods: Light drinkers (LDs; $n = 13$) and non-treatment seeking heavy drinkers (HDs; $n = 7$) participated in the study. Imaging was performed on a 3T Siemens scanner. Participants completed a paradigm that measured willingness to obtain alcohol and food points at different threat levels. Participants were able to work for alcohol, food, or neutral points by pressing a button when a target box appeared. During each trial a colored border was present that indicated the potential threat of receiving an electric shock. There were three threat levels: safe, indicated by a green border, where participants were at no risk of receiving a shock, low threat, indicated by a yellow border, where participants were at a small chance of receiving an electric shock, and high threat, indicated by a red border, where participants had a higher chance of receiving an electric shock. Participants were only at risk of receiving a shock if they attempted to earn the reward. Imaging results were analyzed using AFNI software (Cox, 1996). Both cue (alcohol, food, and neutral) and feedback (hit or miss) periods were analyzed at each threat level (safe, low threat, and high threat) in a GLM.
Results: Behaviorally, the HDs attempted to earn more alcohol points under the high threat condition than LDs. There were no group differences in low threat or safe alcohol conditions. During high threat alcohol cue presentation, HDs showed increased BOLD activation of the right amygdala and decreased BOLD activation in the superior, middle, and medial prefrontal cortices compared to LDs. During alcohol miss feedback presentation, HDs showed increased BOLD activation in the bilateral insula, dorsal anterior cingulate, and the dorsal and ventral striatum, compared to LDs.
Conclusions: We have developed a translational paradigm to investigate aversion resistant alcohol seeking in a clinically relevant population. We found that HDs were willing to risk an adverse consequence to earn alcohol points. During high threat alcohol cue presentation, HDs displayed increased amygdala activation and decreased mPFC activation. This pattern may indicate that HDs are still able to detect the potential adverse consequence, but they are unable to activate frontal control mechanisms to prevent the risky, but rewarding action. Further, HDs displayed increased activation in regions associated with aversion resistant alcohol seeking preclinically, the insula, mPFC, and nucleus accumbens, when they were informed that they were not successful in earning an alcohol reward point. Together these findings suggest a potential neural circuitry for aversion resistant alcohol seeking in heavy drinking individuals.
Keywords: Alcohol-Seeking Behavior, Compulsivity, Functional MRI (fMRI), Addiction.
Disclosure: Nothing to disclose.

M243. Cognitive Reappraisal Impacts Both Volitional and Spontaneous Trial-By-Trial Attention Bias to Drug Cues: An EEG and Eye-Tracking Study

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Background: Drug addiction is a chronically relapsing disorder. Relapse can be precipitated by re-exposure to cues previously associated with drug use that evoke craving. An underlying mechanism of such cue-induced craving encompasses enhanced attention to drug-related cues that occurs at the expense of attention afforded to other salient non-drug-related reinforcers (e.g., generally pleasant stimuli and money). Such a shift in attention (or attention bias) is associated with lack of control over craving and drug-seeking in addicted individuals. Cognitive reappraisal (i.e., reinterpretation of the meaning and/or personal relevance of the situation) has previously been used to modulate attention bias. However, its impact on subsequent spontaneous attention allocation, especially to drug-related cues in addicted individuals, is not known. To fill this knowledge gap, we used a novel attention regulation task during which electroencephalogram (EEG) and eye-tracking data were acquired to assess both instructed and spontaneous attention modulation in individuals with cocaine use disorder (iCUD). Our dependent variables were the amplitude of the late positive potential (LPP), an event-related component of the EEG, and gaze duration, both well established psychophysiological measures of attention bias.
Methods: EEG data was acquired from 37 iCUD and 23 healthy controls (HC) while subjects completed a cognitive reappraisal task, in which they viewed drug- (e.g., pipe) and threat-related (e.g., weapon) pictures either normally or when down-regulating their reactivity using cognitive reappraisal (30 trials each for each picture category).

Participants also normally viewed 60 neutral pictures (e.g., pen). Each trial started with a fixation cross (1000ms), followed by a picture (7500ms). Shortly after picture onset (1500ms), an auditory instruction was presented instructing participants to either view the picture normally ('Look') or to reduce their reactivity using cognitive reappraisal ('Decrease'). The 7500ms picture-viewing was followed by either an 'attention screen' (1500ms) or a rating screen (self-paced). On the 'attention screen' participants were shown a random set of a drug, threat and neutral picture on each quadrant (one quadrant was left blank) without any explicit instruction and their gaze duration on each picture was measured. On the rating screen, participants were asked to rate their 'Liking' and 'Wanting' of the content of the picture they saw in that trial [on a scale from 1 (don't like/want it at all) to 5 (like/want it a lot)]. The LPP amplitude was extracted separately for the pre- and post-instruction periods from central electrodes. For this study, only the data in response to drug-related and neutral pictures are reported.

Results: The results for pre-instruction LPPs validated previous findings by showing a Picture (drug, neutral) by Group (iCUD, HC) interaction ($p = 0.028$), driven by significantly increased LPP amplitude to drug vs. neutral pictures only in iCUD ($p = .003$). The Instruction ('Look' vs. 'Decrease') by Group (iCUD, HC) ANOVA on post-instruction LPPs, elicited by the drug-related pictures (after directly subtracting the baseline neutral 'Look' condition), revealed an Instruction main effect whereby both groups showed reduced LPPs in the 'Decrease' compared to the 'Look' condition ($p = 0.031$). Although the Instruction by Group interaction was not significant ($p = 0.46$), the Instruction main effect was driven by significant reduction ('Look' > 'Decrease') in iCUD ($p = .035$), unlike HC ($p = 0.27$). Interestingly, a similar analysis using gaze duration as the dependent measure showed an Instruction by Group interaction ($p = 0.041$), driven by significantly reduced gaze duration on drug-related pictures post-'Decrease' compared to post-'Look', only in iCUD ($p = .006$). As expected, iCUD showed increased 'Liking' and 'Wanting' for drug-related cues compared to HC ($p < 0.01$), but there was no modulation by instruction.

Conclusions: This study used an objective measure of motivated attention, the LPP, to first validate drug-related attention bias in iCUD compared to HC. The study further demonstrated that, compared to normal viewing, iCUD were able to use cognitive reappraisal techniques to down-regulate their reactivity to drug-cues as evident from reduced LPP amplitude. Importantly, the study used eye-tracking to show the impact of such cognitive reappraisal on attention bias, suggesting that volitional down-regulation of reactivity to drug-related cues may reduce subsequent spontaneous attention allocation these cues. As attention bias to drug-cues has been implicated as a precursor of cue-induced craving and relapse, reducing such bias could have clinical relevance. Specifically, this study calls for the design of a training trial aimed at optimizing the observed reductions in the select psychophysiological measures for the ultimate reduction in craving and drug-seeking in drug addicted individuals. The current results also highlight the utility of objective markers of attention, such as the LPP and gaze duration, in understanding the psychophysiology of attention and its regulation.

Keywords: Attentional Bias, Drug Cues, EEG Biomarkers, Eye-Tracking, Cocaine Addiction.

Disclosure: Nothing to disclose.

M244. Interpeduncular Alpha5 Nicotinic Receptor Neurons Mediate Nicotine Reinforcement and Aversion

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Background: Tobacco/nicotine dependence exerts a profound negative impact on millions of individuals' lives worldwide. An increased risk of nicotine dependence has been repeatedly demonstrated with allelic variation in the CHRNA5/CHRNA3/CHRNB4 gene cluster. Given this association, investigations with animal models into the neurobiological mechanisms mediating nicotine dependence are essential to more fully understand the disease state. The current studies expand on our prior findings and further delineate the role of the habenulo-interpeduncular pathway and its downstream targets in nicotine reinforcement and aversion.

Methods: Adult mice expressing cre under the Chrna5 promotor were injected with an inhibitory hM4Gi DREADD or control virus into the interpeduncular nucleus. After an incubation period, mice were trained in the intravenous nicotine self-administration protocol and subsequently provided access to saline or nicotine during 1hr daily sessions. To examine the effects of DREADD-mediated neuronal inhibition, clozapine-N-oxide (CNO) was administered in a crossover design prior to the self-administration session. A second cohort of mice was tested in the conditioned place aversion procedure to assess whether DREADD-mediated neuronal inhibition would prevent the formation of a nicotine associated conditioned aversion.

Results: We previously found that alpha5-containing nicotinic acetylcholine receptors in the habenulo-interpeduncular pathway mediate the aversive effects of nicotine, particularly at higher doses of the drug. Here, we extend these findings into the alpha5-containing neurons in the IPN and demonstrate differential modulation of nicotine reinforcement at a moderate and high dose. These effects appear to be specific to nicotine, as differences were not found with saline or food self-administration. Moreover, inhibition of these neurons prevented the formation of a conditioned place aversion for a high dose of nicotine.

Conclusions: Taken together, our data provide evidence that alpha5-containing nicotinic acetylcholine receptors and circuits downstream from the IPN modulate nicotine reinforcement and aversion. By identifying the characteristics of these neurons, our findings may not only serve to elucidate the mechanistic function of the signaling pathways but may also provide insight into the human condition, as well as potential targets for therapeutic development.

Supported by the National Institute on Drug Abuse (DA032543 and DA039658 to CDF).

Keywords: Nicotine Dependence, Nicotinic Acetylcholine Receptors, Self-Administration, DREADD, Interpeduncular.

Disclosure: Nothing to disclose.

M245. Dramatic Increase of Dopamine D1-D2 Receptor Heteromers by Tetrahydrocannabinol (THC) in Primate Caudate Nucleus is Attenuated by Cannabidiol (CBD)

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Background: Heavy marijuana users self-report blunted "high", heightened anxiety, along with evidence of attenuated dopamine signaling following a methylphenidate challenge (Volkow et al, 2014), thereby linking cannabinoid activity to adaptive responses in dopamine signaling. Previous studies in rodents have uncovered a novel mechanism in brain, a dopamine D1-D2 receptor heteromer complex, for regulating such rewarding or aversive effects. A discrete subpopulation of medium spiny neurons (MSNs) in caudate nucleus coexpress both D1 and D2 receptors, as well as CB1 cannabinoid receptors. Molecular interactions lead to formation of a functional D1-D2 heteromer. D1-D2 heteromer exerts tonic inhibitory control over brain reward processes, functioning as a constraint on expression of drug-induced sensitization and addiction-like behaviors: (1) Activation of the D1-D2 complex produces aversive behavior and a reduction in the hedonic value of psychostimulant drugs and natural rewards; (2) Disruption of the D1-D2 complex is rewarding; (3) D1-D2 heteromer is elevated in the reward system of female rats, which may account for a higher incidence of anxiety and depression in female marijuana users. Based on altered dopamine signaling, self-reports of blunted dopamine reward and increased anxiety in heavy marijuana users, we investigated whether repeated administration of THC, the main psychoactive and addictive cannabinoid in marijuana affected D1-D2 heteromer density in caudate nucleus of nonhuman primates. We compared THC effects alone or if co-administered with the non-psychoactive, non-addictive phytocannabinoid cannabidiol (CBD) also found in the marijuana plant.
Methods: Three groups of male adult monkeys were treated with THC (0.32-1.0 mg/kg over 24 days), or THC (0.32-1.0 mg/kg over 24 days) combined with CBD (1-3 mg/kg over 16 days) or vehicle (daily for 24 days). In situ proximity ligation assay (PLA) was performed in 30 μm sections from dissected frozen adult monkey caudate nucleus to assess occurrence of D1 and D2 receptors in close proximity ($<$40nm) forming complexes. Following validation of the specificity of the primary rat anti-D1 and rabbit anti-D2 receptor antibodies, PLA was performed with the plus and minus probes consisting of the antibodies covalently attached directly to oligonucleotides. The distinct fluorescent PLA signals were detected by confocal microscopy and analyzed (Duolink). Using digital quantification, the nuclei labeled by DAPI were detected, the size of surrounding cytoplasm estimated for cell counting and analysis of single cell expression levels. Appropriate negative controls were tested. From each animal caudate ($n=2$ per treatment group), Z stacks were acquired at 63x for 1000-1500 neurons.
Results: In control animals, PLA signal was detected in 7.9 + 0.1% of MSNs ($n=2200$) in caudate nucleus. Following THC administration, PLA signal was detected in 81.2 +/- 0.2% of MSNs ($n=3090$). With concomitant THC and CBD administration, PLA signal was detected in 24.0+/- + 0.2% ($n=2700$). Analysis of PLA signal density in the caudate neurons revealed an average of 2.1 +/- 0.02 fluorescent spots per MSN in control monkeys, 6.7 +/- 0.03 per MSN after THC and 3.0 +/- 0.01 per MSN after THC and CBD.
Conclusions: We provide abundant morphological evidence that D1 and D2 dopamine receptors form complexes in primate caudate nucleus, as visualized and quantified by in situ PLA and with exceptional specificity. The number of neurons expressing D1-D2 heteromers in caudate increased dramatically after chronic THC administration, together with increased levels of heteromer expression in single neurons. With CBD co-treatment, the increase in D1-D2 heteromers and the enhanced density of heteromer expressing neurons was markedly attenuated, and restored to levels similar to control animals. Whether the altered phenotype of MSN with upregulated D1-D2 heteromer is mediated by CB1 cannabinoid receptor activity and linked to marijuana-induced changes in dopamine signaling, blunted reward and increased anxiety remains to be shown. Our findings illustrate that the phenotype of MSNs in adult primate caudate nucleus is not static but can change dynamically with altered cannabinoid signaling. Our preliminary data also show that CBD conceivably modulates the molecular actions of THC in striatum. In humans, THC, but not CBD engenders psychoactive effects, memory impairment, anxiety, and addiction. The relevance of our molecular findings to the divergent actions of THC and CBD reported for humans warrants investigation.
Keywords: Marijuana, Dopamine Eeceptors, THC, Cannabidiol, D1-D2 Dopamine Receptor Heteromer.
Disclosure: Nothing to disclose.

M246. Synthetic Cannabinoids (SC) Association With Psychosis and Agitation

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Background: Synthetic Cannabinoids (SC) are cannabinoid receptor agonists, usually with high potency and efficiency. Their recreational use dramatically increased since 2010, and severe adverse events have raised serious public health concerns. There is accumulating evidence of the association between SC use and psychosis in case reports and preliminary studies. We evaluated this relationship in large groups of Emergency Department (ED) patients and psychiatric inpatient setting.
Methods: We compared the clinical presentation and medical management of patients with substance use in two different settings: ED (acute) and inpatient psychiatry unit (subacute). To evaluate the acute presentation, we analyzed data from the Toxicology Investigators' Consortium (ToxIC), a nationwide registry of patients from 50 U.S. medical centers who were evaluated by medical toxicologists for acute drug poisoning between 2010-2015. From the ToxIC registry we gathered sociodemographic factors (age,

gender), and adverse neuropsychiatric effects (psychosis, agitation, coma, CNS depression).
To assess SC subacute presentations, we reviewed digital medical records of all patients who were admitted to a dual diagnosis psychiatric inpatient unit at Mount Sinai Beth Israel (MSBI) Hospital, New York over 12 months (2014-15). Data extracted for demographic variables, clinical presentations, diagnoses, medication administration, length of stay, as well as current and life-time substance use. Patients who reported recent use of any drug or had positive urine toxicology were included.
Results: Our ED sample included 650 individuals, with mean age of 33.33 (SD: 11.94), among them 257 (39.5%) patients reported recent use of SC. Other main reported drugs were cannabis (61.5%), alcohol (19.7%), cocaine (17.5%), opioids (12.0%), PCP (11.2%), benzodiazepines (7.4%), and amphetamine (4.9%). Of these, SC use was the only substance with significant positive association with psychosis (adjusted odds ratio (AOR) 4.69, CI: 1.11-19.86). SC use also had a positive trend for an association with agitation in ED patients (SC AOR 2.807, CI 0.80-14.31), while amphetamine had a significant association with agitation in these patients (AOR 2.81, CI 1.30-6.07).
The sample from our inpatient unit included 595 patients, with a mean age of 40.57 (SD: 12.89); of these, 83 (14%) patients had SC use. Other used substances in this setting were alcohol (56.6%), cannabis (43.3%), cocaine (39.2%), benzodiazepines (21.0%), opioids (20.2%), amphetamine (7.6%), and PCP (3.7%). Similar to acute ED setting, SC use had a significant positive association with psychosis in this sample (AOR 3.04, CI 1.73-5.33). The only other drug with significant positive association with psychosis was natural cannabis with AOR of 2.19 (CI: 1.53-3.14). In our subacute inpatient sample, SC was the only drug with significant positive association with agitation (AOR 2.93, CI 1.76-4.86).
Post-hoc analysis was conducted to assess the contribution of gender differences. Only in the subacute sample was a gender difference evident with SC use: women had a stronger association with psychosis (AOR: 4.56, CI: 1.13-18.41) and agitation (AOR: 40.39, CI: 7.78-209.73) compared to men (AOR 2.97, CI 1.59-5.57 for psychosis; AOR 1.78, CI, 1.00-3.18 for agitation).
Conclusions: Our results suggest that the period closely following acute use of SC or amphetamine is associated with psychosis and agitation, and that these symptoms persist during inpatient hospitalization with SC use. These findings support different lines of evidence supporting perturbation of cannabinoid receptors in psychosis, which seems to have a robust interaction with gender. The presence of severe agitation with SC compounds, specifically in women is an intriguing feature that needs further exploration.
Keywords: Cannabis, Synthetic Cannabinoids, Psychosis, Agitation.
Disclosure: Nothing to disclose.

M247. 5-HT6 Receptors: Multiple Signaling Pathways Regulate Neuronal Morphology

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Background: 5-HT6 serotonin receptors regulate a variety of cognitive processes, including memory, mood, reward seeking, and habitual behaviors. We and others have found that 5-HT6 receptors are trafficked to, and reside exclusively in, primary cilia of neurons. The primary cilia is a small sensory organelle stemming from the cell body, is packed with a discrete subset of GPCRs and signaling enzymes, and is thought to act like an antenna to receive extrasynaptic signals from other cells and the surrounding environment. Primary cilia may be interesting therapeutic targets, as they play a critical role in many disorders, including Huntington's and Alzheimer's disease, making 5-HT6 signaling an intriguing means of modulating neuronal function. However, since they are not localized to the synapse, they don't interfere with moment-to-moment signal integration. In addition, 5-HT6 receptors stimulate multiple distinct signaling pathways including the canonical Gs-coupled adenylyl cyclase and several others including the fyn kinase/Cdk5 signaling pathway. Previously our lab found that endogenous 5-HT6 receptors modulate the length of the primary cilia, with antagonists causing reduced cilia length; this is thought to diminish the impact of cilia-based signaling in general and suggests that 5-HT6 receptor activation lengthens the cilia.
Methods: In this study we focused on the impact of differing levels of exogenous 5-HT6 receptor expression in striatal primary neurons cultured from wild-type or 5-HT6 KO mice, and on the effects of mutations that selectively inhibit either Gs (cAMP) or fyn kinase signaling. We used primary neurons transfected with wild-type and mutant receptors and investigated the changes in morphology of the transfected neurons with immunohistochemistry and quantitative morphemetry.
Results: While endogenous 5-HT6 receptors are almost exclusively localized to primary cilia, increasing levels of exogenous overexpression led to "leakage" of 5-HT6 receptors so that the were detected on the entire cell surface. This was observed both in wild-type and 5-HT6 KO neurons. Wild-type and 5-HT6 KO neurons had similar morphology, but reintroduction of 5-HT6 receptors into KO neurons produced an increase in cilia length but the dendritic morphology was not significantly changed. Interestingly, expression of 5-HT6 receptor mutants with disrupted fyn kinase signaling induced cilia lengthening whereas 5-HT6 mutant receptors lacking canonical Gs-coupled signaling stimulateD dendritic elongation. These effects were partly dependent on the maturity of the cultured neurons.
Conclusions: Together, these results suggest that 5-HT6 receptors, and perhaps other GPCRs in cilia, modulate primary cilia and dendritic morphology via distinct signaling pathways, but the developmental effects are complex since the timing of 5-HT6 receptor expression had a marked effect on whether these receptors modulated cellular morphology. Further, our results indicate the importance of studying these receptors at modest expression levels in order to preserve

normal intracellular trafficking and distribution of 5-HT6 receptors.
Keywords: Serotonin, Cilia, Morphology, Primary Neuron, Dendrite.
Disclosure: Nothing to disclose.

M248. The Medial Prefrontal Cortex is Critical for the Flexibility Necessary for Adaptive Risky Decision-Making

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Background: Previous research shows that prefrontal cortex (PFC) damage mimics decision-making deficits observed in individuals suffering from substance use disorder (SUD). Thus, it is conceivable that impaired decision making in SUD is due to alterations in PFC integrity. While the role of the rodent medial PFC (mPFC; homologous to the primate dorsolateral PFC) has been elucidated in some forms of decision making, its contributions to decision making under risk of explicit punishment are unknown. To this end, we investigated the role of the mPFC in this form of decision making.
Methods: We assessed the effects of mPFC manipulations in a rat model of risky decision making [the "Risky Decision-Making Task" (RDT)], in which rats choose between a small, "safe" food reward and a large, "risky" food reward accompanied by varying probabilities of mild footshock punishment. In Experiment 1, well-trained Long-Evans rats ($n = 13$) were tested in an "ascending" version of the RDT in which the probabilities of punishment increased across the session, following mPFC inactivation with muscimol/baclofen (M/B) or vehicle. In Experiment 2, effects of intra-mPFC M/B or vehicle were assessed on a control version of the task in which reward magnitudes associated with each lever were equated (such that rats chose between a small, safe reward and a small, risky reward). To determine whether effects of mPFC inactivation in Experiment 1 were due to behavioral inflexibility, a new cohort of Long-Evans rats ($n = 15$) was trained in a "descending" version of the RDT in which the probabilities of punishment decreased across the session (Experiments 3-5). In Experiment 3, rats received intra-mPFC M/B or vehicle prior to testing in the descending RDT. In Experiment 4, rats received intra-mPFC amphetamine (AMPH; 0, 2, 10, 20 μg) prior to testing. Finally, in Experiment 5, rats received systemic injections of AMPH (0.0, 0.3, 1.0, 1.5 mg/kg) before being tested.
Results: In the RDT with ascending probabilities of punishment, mPFC inactivation increased choice of the large, risky reward. When reward sizes were equated, choice behavior was not affected by mPFC inactivation, indicating that this manipulation did not reduce sensitivity to punishment. In the RDT with descending probabilities of punishment, mPFC inactivation decreased choice of the large, risky reward. Together, Experiments 1-3 suggest that the mPFC is necessary for modifying choice behavior in response to changing punishment probabilities. In Experiment 4, similar to mPFC inactivation, intra-mPFC AMPH decreased choice of the large, risky reward in the descending RDT, suggesting that mPFC monoamine neurotransmission is critical for flexible decision-making. Finally, and consistent with prior work in the ascending RDT, systemic AMPH decreased choice of the large, risky reward in the descending RDT.
Conclusions: Together, these data demonstrate that the mPFC is necessary for adapting choice behavior in response to changing punishment contingencies. Based on these experiments, it appears that monoamine neurotransmission within the mPFC mediates this flexibility associated with decision making. Previous work, however, has shown that systemic AMPH can enhance punishment salience. Thus, an alternative interpretation of the current data is that while mPFC inactivation impairs behavioral flexibility, enhancing mPFC monoamine neurotransmission potentiates the salience of punishment, causing a decrease in risk-taking. In support of this latter interpretation, systemic amphetamine decreases choice of the large, risky reward irrespective of the direction of change in punishment probability (Experiment 5 and Orsini et al. 2016), whereas the effects of mPFC inactivation differ depending on whether punishment probabilities are descending or ascending (Experiments 1 and 3). Future studies will dissociate these two interpretations and determine the roles of specific monoamine systems (dopamine vs. norepinephrine) in risky decision making.
Keywords: Decision Making, Medial Prefrontal Cortex, Punishment.
Disclosure: Nothing to disclose.

M249. Impairment of Neuroplasticity in the Dorsolateral Prefrontal Cortex by Binge Drinking

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Background: Acute alcohol consumption and binge drinking has been demonstrated to impair neuroplasticity in the motor cortex. It is unknown, however, whether a similar impairment of neuroplasticity by binge drinking is produced in the dorsolateral prefrontal cortex, a brain region that plays an important role in cognitive functioning and has been implicated in the pathophysiology of alcohol use disorders (AUDs). Paired associative stimulation (PAS), a TMS protocol, with electroencephalography (EEG) allows for the induction and measurement of LTP-like neuroplasticity in the DLPFC. The primary aim of the current study was to examine the effect of binge drinking on neuroplasticity in the DLPFC.
Methods: Fifteen healthy binge drinkers were administered PAS to the DLPFC following consumption of an alcohol or placebo beverage in a within-subject cross-over design study. Alcohol was administered at a dose of 1.5g/l of body water. Neuroplasticity induced by PAS was indexed at Post0, Post15, Post30 and Post60 minutes following PAS. The effect of alcohol on PAS-induced potentiation of theta (4-7 Hz)-gamma (30-50 Hz) coupling was also examined prior to and following PAS.
Results: A significant impairment of mean ($t = 2.456$, $df = 13$, $p = 0.029$) and maximum potentiation ($t = -2.945$, $df = 13$, $p = 0.011$) was produced by binge drinking alcohol compared to the placebo beverage in the DLPFC and

globally. PAS with placebo beverage produced an increase in theta-gamma coupling ($t = 2.954$, $df = 14$, $p = 0.010$). This increase in theta-gamma coupling was not observed with PAS following binge drinking ($t = 1.486$, $df = 14$, $p = 0.159$).
Conclusions: The present findings provide a potential neurophysiological mechanism for impairment of cognitive functioning by binge drinking. Additionally, impairment of neuroplasticity in the DLPFC produced by repetitive binge drinking may play a role in the transition to AUDs. Findings from the current study suggest that targeting neuroplasticity in the DLPFC with neuromodulatory brain stimulation may be valuable as a potential treatment for AUDs.
Keywords: Alcohol, Neuroplasticity, Binge Drinking, Dorsolateral Prefrontal Cortex, Alcohol use Disorders.
Disclosure: Nothing to disclose.

M250. Blocking D1 Vs. D2 Receptors in the Agranular Insular Cortex Differentially Affects Cocaine Seeking During Cued and Cocaine-Prime Reinstatement in Rats

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Background: The insular cortex, a lateral region of the prefrontal cortex, mediates nicotine craving in humans and appears to be involved in drug craving in general as well as drug-related interoceptive information. Following up on the AId's role in nicotine addiction, recent work from our laboratory demonstrated that inactivating the dorsal agranular insular cortex (AId) attenuates cued reinstatement but has no effect on cocaine-prime reinstatement in a cocaine self-administration paradigm. Moreover, we found that AId inactivation has no effect on food seeking, indicating a specific role for the AId in cocaine-seeking behavior, as opposed to general reward seeking. Considering that the AId receives a significant dopaminergic innervation from the ventral tegmental area, it is likely that dopaminergic signaling within the AId is involved in the reinstatement of cocaine seeking. Additionally, it has been shown that blocking D1 receptors in the AId reduces the motivating properties of cocaine during self-administration. However, D1 receptor blockade within the AId has not been examined during reinstatement to cocaine seeking. To address this question, we investigated how dopamine within the AId influences cocaine seeking by blocking D1 and D2 receptors during two distinct reinstatement tests: cued and cocaine-prime.
Methods: Male Sprague-Dawley rats underwent surgery for implantation of bilateral guide cannulae aimed at the AId and implantation of an intravenous jugular catheter. After undergoing cocaine self-administration for at least 12 days (2 h daily), rats underwent extinction training prior to reinstatement testing. Reinstatement tests consisted of cue-induced and cocaine-prime reinstatement tests. Rats received either the D1 receptor antagonist SCH 23390, the D2 receptor antagonist sulpiride, or a vehicle control immediately prior to reinstatement testing.
Results: Blocking D1 receptors within the AId reduced cued and cocaine-prime reinstatement. Additionally, in line with previous studies which showed that D2 receptor blockade within the prefrontal cortex had little effect on drug-seeking, our preliminary data suggest that intra-prefrontal cortex D2 receptor blockade has no effect on cocaine seeking during cued and cocaine-prime reinstatement, however studies are still ongoing to verify a lack of a role for intra-AId D2 receptors in the reinstatement of cocaine seeking.
Conclusions: The current findings suggest that dopamine within the AId is driving the previous effects observed with general AId inactivation. Surprisingly, although inactivating the AId does not alter cocaine-prime reinstatement, blocking activity at D1 receptors reduces this type of reinstatement. These findings are particularly interesting given that D1 receptor blockade within other regions of the prefrontal cortex has produced similar effects, in which dopamine manipulation alters cocaine seeking despite findings showing general inactivation of the same structure has no effect.
Keywords: Prefrontal Cortex, Cocaine Seeking, D1 Dopamine Receptors, Cue Reinstatement.
Disclosure: Nothing to disclose.

M251. Oxytocin Decreases Drug Seeking via mGlu2/3 Receptor Antagonism in the Nucleus Accumbens Core

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Background: Methamphetamine (meth) and cocaine addiction are prevalent health concerns for men and women worldwide, yet remain without approved pharmacological treatments. My lab has shown that oxytocin decreased cued meth and cocaine seeking in male and female rats, but the neuronal underpinnings remain unknown. However, it is known that glutamatergic synaptic function in the nucleus accumbens core (NAc) is deregulated following cocaine and meth. Here we suggest that oxytocin interacts with presynaptic glutamatergic regulation of the NAc.
Methods: We blocked mGlu2/3 receptors with LY-341495 (antagonist of mGlu2/3) before oxytocin using systemic and site-specific application of the drugs before cued reinstatement testing. In separate experiments, male and female (Sprague-Dawley) rats self-administered meth or cocaine on an escalating FR ratio (1,3, or 5) for 13 days, and then underwent at least 8 days of extinction to a criteria of less than 25 lever presses on two consecutive sessions. In the systemic experiments, rats received LY-341495 (1mg/kg, ip) or vehicle 5 min before oxytocin (1mg/kg, ip) or saline before the cued reinstatement sessions. During this session a response on the drug-associated lever resulted in a presentation of the light +tone stimulus complex originally associated with drug. In the cite specific experiments, rats received LY-341495 (1.3nmol/0.25µl/side) or saline (0.25µl/side) followed by oxytocin (0.6nmol/0.25µl/side) or saline (0.25µl/side) directly into the NAc (+1.2 posterior, ± 2.4 mediolateral, -7.2 ventral from bregma, with a 6° angle) before the cue test.
Results: All rats readily acquired drug self-administration, and the majority met extinction criteria during the 8 extinction sessions. Typically, males and females did not differ in lever pressing during acquisition or extinction, but meth intake was higher in females. As expected, both males and females reinstated to meth associated cues, and LY-341495 alone did not impact reinstatement in either study. Oxytocin injected systemically or infused into the NAc decreased cued reinstatement. LY-341495 injected

systemically before oxytocin prevented oxytocin from reducing cued reinstatement. Moreover, this finding involves the NAc, because site-specific application of LY-341495 before oxytocin also prevented a reduction in cued reinstatement.

Conclusions: Taken together, peripheral (ip injection) and site-specific application of oxytocin into the NAc decreased meth and cocaine cued reinstatement in males and females. Oxytocin may be a common therapeutic for stimulant abuse in males and females. These results demonstrate an interaction between oxytocin and mGlu2/3 receptors, and indicate that oxytocin may work by restoring tone on presynaptic regulation of glutamate in the NAc. Future studies identifying cell-type specificity of oxytocin receptor localization will shed light on the mechanisms by which oxytocin is functioning in the nucleus accumbens.

Keywords: mGlu2/3 Antagonist, Oxytocin, G protein, Addiction.

Disclosure: Nothing to disclose.

M252. ADHD Symptoms Moderate the Efficacy of Verenacline in a Randomized Controlled Trial

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Background: The co-occurrence of Attention-Deficit/Hyperactivity Disorder (ADHD) and Nicotine Dependence is common. Both an ADHD diagnosis and higher, but subclinical, levels of ADHD symptoms predict smoking initiation, nicotine dependence, and difficulty quitting smoking. Little is known about how to best approach treating these co-morbidities to optimize clinical outcome. Few published cessation trials specifically consider the impact of ADHD symptoms on treatment outcomes, including those testing established, first line, pharmacological therapies, such as varenicline.

Varenicline is thought to improve cessation by reducing craving for smoking and attenuate withdrawal, restlessness, and negative affect during abstinence, potentially via the antagonist action of varenicline at α4β2 nAChRs which play a role in mediating the reinforcing effects of nicotine. Thus, varencline may be a uniquely efficacious pharmacotherapy for particular smokers, such as those with high levels of ADHD symptoms, who may have preexisting nicotinic-acetylcholinergic aberrations and, further, find it hard to cope with symptoms of withdrawal during quit attempts. Thus, although data suggest pharmacological overlap among varenicline and ADHD as well as greater withdrawal severity and difficulty quitting in individuals with ADHD, there is a dearth of cessation trials that specifically consider the impact of ADHD symptoms on treatment outcome.

Methods: The current study focused on the impact of pretreatment ADHD symptoms on smoking cessation outcome in 12 weeks a randomized controlled trial of varenicline ($n = 205$), a front line treatment thought to improve cessation by attenuating the severity of craving and withdrawal. Given established data on differential roles for the two ADHD symptom dimensions, inattention (IN) and hyperactivity-impulsivity (HI), in relation to smoking outcomes, we examined the extent to which gradations in IN and HI symptom dimensions of ADHD impacted treatment outcomes across medication and placebo conditions. Further, in light of data suggesting that 1) the putative mechanism for the efficacy of varencline in smoking cessation is related to its effect on attenuation of withdrawal symptoms during quit attempts and 2) individuals with higher levels of ADHD symptoms experience greater severity of withdrawal during abstinence from smoking, we tested withdrawal as a mediator of varenicline treatment effects in individuals with high and low ADHD symptoms.

Results: There were no main effects of IN or HI on treatment outcome and there was no suggestion that IN symptoms moderated the effect of treatment (Varenicline vs placebo) over time. However, there was a significant time X treatment x Hyperactivity-impulsivity (HI) symptoms interaction, suggesting that baseline HI symptoms did moderate the effect of treatment condition over time ($\beta = -0.04$, S.E. = 0.02, $p = 0.02$). Specifically, although ADHD symptoms did not associate with baseline differences across treatment groups in terms of cigarettes/day, in the varenicline condition higher levels of HI symptoms ($\geq$ 6) were associated with smoking the fewest cigarettes at the end of the treatment phase (3.68 cig/day at week 12). In contrast, individuals with lower scores of $<$ 6 on the HI scale reported smoking 3.77 cig/day at week 12. At follow up, individuals with high levels of HI symptoms randomly assigned to varenicline smoked 5.68 cigs/day at Week 36 follow up whereas those with lower levels of HI smoked 4.75 cigs/day. Thus, despite similar reductions in smoking during the course of varenicline treatment, those with higher HI symptoms were less successful at reducing smoking once off the medication. In the placebo condition, individuals with higher levels of ADHD symptoms had the worst smoking outcomes, and smoked 7.56 cigs/day at the end of treatment, whereas individuals with a score of less than 6 on the HI scale smoke 5.98 cigs/day. At follow up, those with high levels of HI ($> = 6$) who received placebo continued to have the worse outcomes, smoking the most cigarettes (10.2 cigs/day) across any of the groups, time points, and treatment conditions. Further, attenuation of withdrawal partially mediated the effect of medication on cessation outcome, but path models suggested differing mechanisms for individuals with high and low ADHD symptoms.

Conclusions: Thus, results suggested that treatment condition interacted with ADHD hyperactivity-impulsivity symptoms, such that individuals with high levels of hyperactivity-impulsivity did well on varenicline but much worse on placebo. Further, attenuation of withdrawal significantly mediated the effect of varenicline treatment, but this effect differed in high and low HI individuals. Given that the pharamacological targets of varenicline and the neurobiology of ADHD overlap, it is possible that varenicline is uniquely efficacious in individuals with high levels of ADHD. These data add to a gap in the smoking cessation literature regarding the impact of ADHD symptoms on the efficacy and mechanism of frontline pharmacological treatments.

Keywords: ADHD, Smoking Cessation, Withdrawal, Impulsivity.

Disclosure: Nothing to disclose.

M253. Dopamine Transporter Methylation Predicts Increased Striatal Activation During Reward Anticipation in Individuals With Alcohol Dependence

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Background: Alcohol Dependence (AD) is a highly prevalent disorder characterized by a chronic course and high comorbidity rates. Dopamine signaling in the ventral striatum has traditionally been associated with the reinforcing effects of alcohol. The SLC6A3 gene codes for the dopamine transporter (DAT) which regulates the duration and intensity of dopamine signaling by mediating its reuptake into the presynaptic terminal. While some data indicate that chronic alcohol use may cause a reduction of striatal DAT expression, the underlying mechanisms remain unclear. Preclinical evidence indicates that increased DAT methylation is associated with reduced DAT expression in the rodent striatum, and DAT methylation in peripheral tissue has been associated with alcohol craving in AD patients. Here we investigated how epigenetic variation in DAT may contribute to individual differences in brain-reward circuitry in individuals with AD.
Methods: For the present study, 25 recently detoxified individuals with AD (mean age = 41.04, SD = 11.43) and 16 healthy controls (HC; mean age = 31.31, SD = 7.16) provided plasma samples and performed the Monetary Incentive Delay (MID) task in a 3-Tesla functional magnetic resonance imaging (fMRI) scanner. Bisulfite sequencing was used to assess the percentage of methylation across 48 CpG sites of the DAT gene (SLC6A3). We examined the relationship between SLC6A3 CpG site methylation and reward anticipation-related striatal activity using blood oxygen level dependent (BOLD) fMRI. Linear regression models were used to examine effects of CpG site percent methylation on striatal BOLD responses during reward anticipation in the MID task.
Results: Analyses revealed that percent methylation of SLC6A3 CpG sites -338 from the transcription start site (TSS; $\beta = .47$, $p = .017$), -342 TSS ($\beta = .58$, $p = .002$), -886 TSS ($\beta = .58$, $p = .002$), and 29050 TSS ($\beta = .74$, $p < .001$) significantly predicted reward anticipation-related activity in the Caudate in the AD but not the HC group (all ps > .278). Similarly, percent methylation of CpG sites -338 TSS ($\beta = .46$, $p = .021$), -418 TSS ($\beta = .47$, $p = .017$), and 29271 TSS ($\beta = -.51$, $p = .009$) significantly predicted reward anticipation-related activity in the Putamen in the AD but not the HC group (all ps > .196). Finally, percent methylation of CpG site 29271 TSS ($\beta = -.49$, $p = .012$) significantly predicted reward anticipation-related activity in the Nucleus Accumbens for the AD but not the HC group ($p = .219$). The differences in regression slopes for the AD and HC groups were tested by adding an interaction term (group x SLC6A3 CPG site methylation) to each regression model. Between-group analyses showed that these differences were significant for the Caudate (all ps < .023), the Putamen (all ps < .043), and the Nucleus Accumbens ($p = .047$).
Conclusions: These results suggest that increased DAT methylation predicts differences in striatal activation during reward anticipation in individuals with AD but not in healthy controls. Differences in methylation might result in lower DAT transcription and expression in the striatum, which may in turn protract dopamine reuptake. Altered neural activity during reward anticipation is thought to impair advantageous decision-making. Improving our understanding of the underlying neurocircuitry is clinically relevant as drug-seeking is often ascribed to differences in reward sensitivity. Therefore, peripheral SLC6A3 methylation may be a biomarker with clinical relevance for neuronal phenotypes in AD populations. Future studies should aim to replicate these results, and compare blood methylation with striatal DAT mRNA in AD patients to clarify whether peripheral methylation mimics methylation in the brain.
Keywords: DAT1 Gene, Methylation, Functional MRI (fMRI), Striatum, Reward Neural Circuitry.
Disclosure: Nothing to disclose.

M254. Alcohol Seeking Behavior is Attenuated by Inhibiting μ-OPIOID Receptors

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Background: While alcohol drinking to intoxication is the major behavioral characteristic of those addicted to alcohol, such individuals also crave alcoholic beverages and spend a great amount of time actively seeking alcohol, ultimately in the face of adverse consequences, i.e. compulsively. In addition to reducing compulsive alcohol drinking, effective treatments for alcoholism should not only reduce volumes drunk, but also reduce the craving and alcohol seeking that leads to drinking.
Drug seeking behavior is triggered and maintained by environmental drug-associated stimuli acting as conditioned reinforcers that influence both appetitive and consummatory behaviors. Additionally, when control over drug seeking is lost such that it persists in the face of negative physical and social consequences, this drug seeking becomes compulsive, a hallmark feature of addiction.
In order to understand the psychological and neural mechanisms underlying these aspects of alcohol addiction and thereby provide a means of developing novel treatments, we developed two novel animal experimental models separately that capture these challenging features of alcohol seeking: (i) alcohol seeking maintained by alcohol-associated conditioned stimuli (CSs), followed by a period of alcohol intake; (ii) compulsive alcohol seeking operationalized as the persistence of seeking behavior despite the occurrence of unpredictable punishment. We then investigated the effects of a novel, putative treatment for alcohol addiction, GSK1521498, which selectively inhibits brain μ-opioid receptors.
Methods: Since alcohol is a relatively weak reinforcer in rats, we maximised the possibility of studying alcohol seeking by using alcohol-preferring P rats. After establishing their alcohol preferring phenotype in an intermittent 2-bottle (alcohol/water) choice procedure, P rats were trained to seek alcohol under two different schedules of reinforcement in

order to assess the psychological and neurchemical mechanisms of cue-controlled and compulsive alcohol seeking behavior: (i) Alcohol seeking maintained by alcohol-associated CSs was assessed under second-order schedule of 15% ethanol (EtOH) reinforcement. Rats were trained instrumentally to seek alcohol for a 15-min interval maintained and invigorated by alcohol-associated cues contingently upon every tenth lever press [FI15(FR10:S)], after that rats were given a 20-min period freely to consume alcohol. (ii) Compulsive alcohol seeking was assessed under a probabilistically punished seeking-taking chained schedule of reinforcement. Responding on one lever (the seeking lever) under a random interval (RI) 60 sec schedule gave access to the opportunity to press a second lever (the taking lever), responding on which under a fixed ratio (FR) 1 schedule resulted in delivery of 0.1 mL 15% EtOH. Once seeking behavior was established and stable, unpredictable intermittent delivery of foot-shock punishment was introduced so that out of the 25 cycles, 17 were now reinforced by delivery of 0.1 mL of 15% EtOH following a taking lever response, whereas 8 were randomly punished by a 0.25-0.45 mA/0.5 sec foot shock. The completion of the 25 cycles/session was an index of animals' persistent alcohol seeking in the face of negative consequences. Seeking behavior under both tasks was then challenged by treatment with the novel selective μ-opioid receptor antagonist GSK1521498.

Results: (i) P rats acquired high levels of CS-controlled alcohol-seeking behavior for EtOH and achieved blood alcohol concentrations up to 80 mg% in the immediately following 20 min earned drinking periods. Selective antagonism at the μ-opioid receptors by the novel compound GSK1521498 markedly reduced both cue-controlled alcohol seeking and alcohol drinking. (ii) Upon introduction of unpredictable punishment of seeking responses, approximately one-third of rats progressively decreased their alcohol seeking. By contrast, a subpopulation of rats maintained their seeking response despite punishment, thereby displaying compulsive alcohol seeking behavior. This compulsive subgroup of animals, as compared to rats in low and intermediate subgroups, designated according to the persistence of responding during the punishment sessions, also showed persistence of alcohol seeking behavior as measured under extinction-conditions, and an increased motivation for alcohol measured under an exponential progressive ratio schedule of alcohol reinforcement. Systemic treatment with GSK1521498 significantly reduced alcohol seeking behavior specifically in high-compulsive rats, without affecting the seeking behavior of low-compulsive rats.

Conclusions: The data reported here demonstrate that vulnerable individuals developed CS-controlled alcohol-seeking behavior and that only a subset of them developed compulsive alcohol seeking and displayed an associated increased vulnerability to relapse. The data further show that a propensity to drink and spontaneously prefer alcohol is dissociable from the propensity compulsively to seek it. Finally, the novel selective μ-opioid receptor antagonist GSK1521498 has significant translational potential as a highly effective treatment of alcoholism by preventing compulsive, as well as non-compulsive, alcohol seeking, suggesting that it may be a more generally effective treatment across a broad spectrum of individuals addicted to alcohol, but especially in compulsive individuals.

Keywords: Alcohol-Seeking Behavior, Compulsivity, Mu-Opioid Receptors.

Disclosure: Nothing to disclose.

M255. Correlates of Cognitive Function Among Tobacco Smokers With HIV and Comparison to Tobacco Smokers Without HIV

Joseph Harrison, Jessica Dochney, Sonja Blazekovic, Frank Leone, David Metzger, Ian Frank, Robert Gross, Anita Hole, Karam Mounzer, Robert Schnoll, Rebecca Ashare*

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Background: Medical advances in the treatment of HIV/AIDS have improved the life expectancy of HIV-infected individuals. Unfortunately, HIV-infected individuals are three times more likely to use tobacco than those in the general population, but little is known about the mechanisms that underlie these high smoking rates. Indeed, HIV-infected smokers lose more years of life to tobacco-related disease than HIV. Since neurocognitive deficits are common among those with HIV and are associated with smoking persistence, these deficits may be a unique barrier to smoking cessation among HIV-infected smokers. To this end, we compared neurocognitive function between HIV-infected and HIV-uninfected smokers and evaluated correlates of neurocognitive function among HIV-infected smokers to help guide the development of population-specific smoking cessation interventions.

Methods: Participants were HIV-infected ($n = 103$) and HIV-uninfected smokers ($n = 73$) enrolled in separate smoking cessation trials. Prior to receiving any treatment (baseline assessment), participants completed neurocognitive tasks measuring working memory, attention, and processing speed (N-back and Continuous Performance Task [CPT]). For the HIV-infected smokers, we assessed the association among cognitive function, demographics (e.g., age, gender, race), smoking history (e.g., CO, FTND, years of smoking), and disease specific information (e.g., viral load, CD4 count).

Results: For the N-back task, HIV-infected smokers (vs. HIV-uninfected smokers) were less accurate ($p = 0.008$), particularly as task difficulty increased (group by n-back level interaction, $p = 0.01$). HIV-infected smokers also exhibited slower responses time on the N-back ($p = 0.001$) and the CPT ($p = 0.002$). Among all smokers, higher education and younger age were associated with better neurocognitive function. Among HIV-infected smokers, male gender, higher income, and higher CO were associated with better neurocognitive function, whereas among HIV-uninfected smokers, lower levels of nicotine dependence and fewer cigarettes smoked per day were associated with better cognitive function.

Conclusions: These data suggest that, at baseline, HIV-infected smokers may have lower levels of working memory and processing speed compared to HIV-uninfected smokers. Smoking cessation interventions that consider cognitive neurorehabilitation for HIV-infected smokers may reduce the likelihood of nicotine relapse and decrease tobacco-related morbidity in this population.

Keywords: HIV, Cognition, Tobacco, Smoking Cessation.
Disclosure: Nothing to disclose.

M256. Evidence for Dopamine D1-D2 Receptor Heteromers in Striatum of Multiple Species With Functional Relevance for Cocaine Addiction

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Background: In the basal ganglia dopamine D1 and D2 receptors are predominantly segregated to distinct neuronal pathways, the direct striatonigral and indirect striatopallidal pathways respectively, and are thought to be the predominant mediators of dopamine action in this region. However, there has been controversy as to whether a fraction of striatal medium spiny neurons (MSNs) express both receptors. Colocalization of D1 and D2 receptors has been shown to occur predominantly in nucleus accumbens (17-38% of D1 neurons) and within most of these D1/D2 receptor-coexpressing MSNs the receptors exist within a complex forming the D1-D2 receptor heteromer. Dopamine signaling in nucleus accumbens has been widely demonstrated to play a pivotal role in cocaine addiction, yet the contribution of the dopamine D1-D2 receptor heteromer in mediating addiction processes has not previously been evaluated. Discerning the physiological relevance of this receptor complex in cocaine addiction is of utmost importance.
Methods: In situ proximity ligation assay (PLA) and confocal fluorescence resonance energy transfer (FRET) techniques were performed in adult mouse, rat and monkey nucleus accumbens to assess D1 and D2 receptor complexes with resolution of 30-40 nm for PLA and <10 nm for FRET. The primary D1 and D2 receptor antibodies used were validated in D1 and D2 receptor gene-deleted mice and in cell lines expressing each of the five dopamine receptors individually. PLA probes consisted of the affinity-purified antibodies covalently attached directly to the PLUS and MINUS oligonucleotides. PLA experiments were replicated with a second antibody set. Confocal microscopy FRET was performed by direct coupling of Alexa Fluors to the D1 and D2 receptor antibodies. To assess a role for the D1-D2 heteromer in cocaine conditioned place preference and intravenous self-administration, and since no D1-D2 heteromer selective agonists or antagonists are available, a selective heteromer disrupting peptide (TAT-D1) which targeted the site of interaction between the D1 and D2 receptors was developed. The effect of repeated TAT-D1 administration on nucleus accumbens ΔFosB, the transcription factor that has a critical role in regulating addictive drug-induced gene expression, was also examined.
Results: PLA and FRET studies showed conclusively that native D1 and D2 receptors formed a heteromeric complex in D1/D2 receptor-coexpressing MSNs in mouse, rat and monkey nucleus accumbens. Functional disruption of the dopamine D1-D2 receptor heteromer by TAT-D1 induced conditioned place preference and increased expression of ΔFosB in nucleus accumbens. D1-D2 heteromer disruption also resulted in the promotion and exacerbation of the incentive motivational effects of cocaine in the self-administration paradigm.
Conclusions: The present findings demonstrate definitively that native D1 and D2 receptors formed D1-D2 heteromers in all species tested. Further, disruption of the D1-D2 heteromer was rewarding, potentially through the induction of ΔFosB in D1 receptor MSNs. D1-D2 heteromer disruption also potentiated the behavioral effects of cocaine. Together these findings indicate that a physiological role of the D1-D2 heteromer may be to exert tonic inhibition on brain reward mechanisms and thus function as a brake to constrain the development and expression of addiction-like behaviors. The identification of a singular molecular entity in nucleus accumbens that blocks cocaine effects suggests its potential value as a target in combatting cocaine addiction.
Keywords: Cocaine, Dopamine D1-D2 Receptor Heteromer, Proximity Ligation Assay, FRET, DeltaFosB.
Disclosure: Nothing to disclose.

M257. A Second Update on Susceptibility Genes for Nicotine Dependence Identified by Genome-Wide Linkage, Candidate Gene Association, Genome-Wide Association, and Targeted Sequencing Approaches

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Background: Tobacco smoking is a severe health hazard worldwide, as nearly one-third of the global adult population smokes tobacco products, and these have been associated with numerous serious health problems. This high prevalence of tobacco use highlights the importance of studying the genetic determinants of nicotine dependence (ND). To identify genetic factors for ND, various approaches have been used, including genome-wide linkage, candidate gene-based association, genome-wide association (GWAS), and targeted sequencing analysis.
Methods: We systematically analysed the findings from all the abovementioned approaches according to rigorous selection criteria for each included study such as sample size, statistical significance, and independent replication.
Results: Our analysis revealed 14 regions nominated by genome-wide linkage analysis and 34 significantly associated loci in 43 genes by candidate gene-based association. The GWAS and meta-GWAS revealed 11 genome-wide significant loci; however, only the loci on chromosomes 8, 15, and 19 have received independent replication. Although it is still in early stages, limited targeted sequencing studies using next-generation techniques have implicated 18 variants in the aetiology of ND.
Conclusions: Together, we identified 14 linkage regions and 47 unique loci in 60 genes involved in the development of ND, which forms our current understanding of the susceptibility map for ND. Because almost all of these loci and genes have received replication by independent approaches in different samples, they should be considered high priorities for future functional study of ND.
Keywords: Genetics, GWAS, Tobacco Smoking, Susceptibility Genes.
Disclosure: Nothing to disclose.

M258. Increased Death Receptor Signaling in Alcoholic Human and Alcohol-Treated Rat Hippocampus: Evidence for Both Neuroimmune and Caspase-Neurodegeneration Pathways

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Background: The tumor necrosis factor (TNF) and associated receptor superfamily (TNFRSF) include Death Receptors (DR) that contain a death domain (DD) protein sequence found to trigger caspase cascades that induce apoptosis. DR ligands include FasL, TL1A and others that bind to DRs and signal through specific adaptor proteins, e.g., FADD and TRADD. In immune cells, signaling through these pathways increases apoptosis and proinflammatory responses, although new roles are emerging. TNFRSF members that contain a death domain include Fas receptor, death receptor 3 (DR3) and others. Interestingly, DR3 is constitutively expressed in rodent hippocampus. Previous studies finding neuroimmune activation in alcoholism prompt further investigation into DR signaling in alcoholic brain.
Methods: Human post-mortem hippocampal paraffin sections of moderate drinking controls and alcoholics were obtained from the New South Wales Tissue Resource Center at the University of Sydney in Australia and matched for relevant medical and lifestyle information. Markers of DR signaling were assessed using immunohistochemistry (IHC), including DR3/TNFRSF25 and TL1A/TNFSF15, the DR3 ligand; Fas Receptor and FasL; TRADD, FADD and pFADD, the active phosphorylated form of FADD; and NFkB-p65. The activated forms of caspases 3, 7, 8 and 9 were also quantified. Rat studies used the adolescent intermittent ethanol (AIE) model (5 g/kg, i.g., 2 days on-2 off, PND25-55, 16 doses over 30 days) with rats sacrificed 40 days after the last ethanol dose at P95.
Results: AIE treatment of rats increased DR3/TNFRSF25+IR (204% of control, $p<0.01$), TL1A/TNFSF15+IR (184% of control, $p<0.01$) and active caspase-3+IR (138% of control, $p<0.01$) in the dentate gyrus granule cell layer (GCL) of rat hippocampus. In human hippocampus, DR3/TNFRSF25+IR and its ligand TL1A/TNFSF15+IR were significantly increased 267% ($p<0.05$) and 364% ($p<0.01$) in alcoholics compared to moderate drinking controls. Human post-mortem alcoholics also showed increased expression of DD-containing FADD (469%, $p<0.05$) and pFADD (342% of control, $p<0.01$), and TNF receptor-associated DD, i.e., TRADD (414%, $p<0.05$). FADD activation of caspase protease cascades is linked to apoptosis. Active caspase-8 +IR in alcoholic human hippocampus increased 292% ($p<0.05$) and active caspase-9+IR showed a trend to increase (150%), whereas the two executioner caspases, active caspase-3 (356% of control, $p<0.05$) and caspase-7 (385% of control, $p<0.05$) were markedly increased. In addition, NFkB-p65+IR (342% of control, $p<0.01$) was increased, consistent with previous findings of increased expression of proinflammatory innate immune genes in alcoholism. Fas ligand (FasL+IR) in human alcoholics also was markedly increased (370% of control, $p<0.01$); however, Fas receptor +IR showed no significant change. Studies in rats find AIE causes long-lasting increases in DD signaling suggesting ethanol increases DR signaling in alcoholic brain.
Conclusions: Human post-mortem alcoholic brain and AIE-treated rats show increased expression of DRs, activated caspases and DR signaling proteins in hippocampus consistent with increased DR signaling in alcoholic hippocampus through both proinflammatory and DD signaling caspase cascades.
Keywords: Apoptosis, Adolescent Alcohol, Death Receptor Expression, Caspase Cascades, Death Domain.
Disclosure: Supported by NIAAA Grant 2U01AA020023-06.

M259. A Diffusion Tensor Imaging Study of Interaction Between Genetic Variants of the Serotonin 5-HT2A Receptor (5-HT2AR) and 5-HT2CR

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Background: Previous studies using diffusion tensor imaging (DTI) have shown that cocaine use disorder (CUD) is associated with impaired white matter microstructure. Recently, we combined DTI and Bayesian statistical methods and demonstrated that the impaired white matter in CUD subjects may be associated with genetic factors (Azadeh et al, Neuroimage 125:813, 2016). Consistent with our long-term research objectives, we conducted this preliminary study to investigate the interaction effects on DTI data between variants of the HTR2A (SNP: rs6311) and HTR2C (SNP: rs6318) genes in in healthy controls and CUD subjects.
Methods: DTI data were acquired from 39 CUD subjects (40.4 ± 7.9 years old, 29 males) and 19 controls (32.7 ± 8.5 years old, 12 males) on a Philips 3.0 T scanner. The DTI images were acquired in the transverse plane using a single shot spin-echo diffusion sensitized echo-planar imaging (EPI) sequence (21 gradient directions, b-factor = 1000s/mm2, repetition time: 6100 ms, echo time: 84 ms, 44 contiguous axial slices, field-of-view: 240mm × 240mm, 112 × 112 acquisition matrix, 256 × 256 reconstructed matrix, 0.9375mm × 0.9375mm reconstructed in-plane resolution, slice thickness: 3mm, no interslice gap). The DTI data were processed using FSL software. For each subject, the diffusion-weighted images were corrected for eddy current distortions and head motion. Then, brain was extracted from the images using FSL's Brain Extraction Tool. After these preprocessing steps, FSL's Diffusion Toolbox was used to extract the DTI parameters for each voxel. Fractional anisotropy (FA), one commonly used DTI parameter, was used to measure white matter integrity. FSL's TBSS software was used to yield skeletonized FA maps. DNA was extracted from whole blood samples drawn from each subject using standard methods. Twenty-one candidate genetic variants, including HTR2A (SNP: rs6311) and HTR2C (SNP: rs6318) genetic variants, were determined as a restriction fragment length polymorphism (RFLP) using polymerase chain reaction (PCR) amplification as described in previous imaging genetic interaction studies. An integrative Bayesian analysis was implemented to study the dependence between

the voxel level measurement, FA, and various subject-level genetic and demographic characteristics. A posterior probability map was obtained for the interaction effect through voxel-based Bayesian model averaging. A post-hoc smoothing procedure was then used to account for the spatial correlation among voxels. Finally, the region showing significant interactions between the select HTR2A and HTR2C genetic variants was identified through Bayesian false discovery rate (FDR) correction.
Results: A white matter cluster (10 mm3) with a center of mass at (x = 21, y = 0, z = 52, Montreal Neurological Institute [MNI] coordinates) was identified close to the right supplemental motor area (SMA) and right premotor cortex of CUD subjects. A significant interaction between the FA of this white matter cluster and the interaction between the HTR2A and HTR2C genetic variants was observed (FDR corrected $p < 0.05$).
Conclusions: Results are consistent with an interaction between the HTR2A and HTR2C polymorphisms in brain structure as measured by FA in the right SMA and right premotor cortex. These findings are interesting in light of our published observations in preclinical models that a protein: protein interaction occurs between the 5-HT2AR and 5-HT2CR in cortical regions (Anastasio et al, ACS Chemical Neuroscience 15:1248, 2015). Thus, our observations suggest an interaction between these two regulatory systems in cortical circuits relevant for addictions and related behavioral disorders.
Keywords: Diffusion Tensor Imaging, Serotonin 5-HT2C Receptor, Cocaine Addiction.
Disclosure: Boehringer Ingelheim: Contract, Self.

M260. Distinct Roles of Opioid and Dopamine Systems in Lateral Hypothalamic Self-Stimulation

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Background: Opioid and dopamine systems play crucial roles in reward. Similarities and differences in neural mechanisms of reward mediated by these two systems have remained largely unknown.
Methods: Mice lacking mu-opioid receptors (MOP), the main target of morphine, and mice lacking dopamine transporters (DAT), a main target of cocaine, were used. Lateral hypothalamic intracranial self-stimulation (lhICSS), tail-suspension, and water-wheel tests were conducted.
Results: MOP knockout mice showed enhanced lhICSS which induces dopamine release. They also displayed increased movement in despairing conditions in both the tail-suspension and water-wheel tests. Both of these results are consistent with the involvement of MOP in drive-reducing reward functions such as satisfaction and relaxation. In contrast, DAT knockout mice maintained lhICSS responding even when more active efforts are required for the reward.
Conclusions: These results are consistent with the involvement of dopamine systems in drive-inducing reward functions such as excitation and volition. LhICSS differences in MOP and DAT knockout mice underscore the multi-dimensional nature of reward.
Keywords: Mu-Opioid Receptors, Dopamine Transporter, Intracranial Self-Stimulation, Reward System.
Disclosure: Nothing to disclose.

M261. The Effect of Cannabis Use on Cognitive Set-Shifting Functional Connectivity

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Background: Although several functional neuroimaging studies have found evidence for long-term effects of cannabis use on human brain function, no studies have examined the acute effects of marijuana use on distributed network functional connectivity. The current study used functional magnetic resonance imaging (fMRI) to determine whether functional connectivity during cognitive set shifting task performance was altered by different doses of marijuana. We hypothesized that marijuana use would impair attention-related neural network connectivity. We also were interested in whether drug effects on functional connectivity differed by delta-9-tetrahydrocannabinol (THC) dose, or between regular versus occasional users. Because there are no prior precedents upon which to base specific regional connectivity hypotheses, these latter analyses were exploratory.
Methods: Fourteen medically healthy adults who were regular ($n = 6$) or occasional ($n = 8$) users of marijuana. Regular use was 1 or more joints at least 4 times per week, while occasional use was 1 or more joints at least once a month. Structured clinical interview (SCID-IV) confirmed the absence of all DSM-IV psychiatric diagnoses. On three separate occasions, participants used a paced inhalation method with vaporizer to smoke marijuana, receiving either medium/high-dose (5.7% THC), low-dose (3.4% THC), or placebo in a randomized, double-blind order across visits. After dosing, participants underwent fMRI while performing a rapid, event-related cognitive set shifting task. In this task, participants were cued to count the number of different colors, sizes, or shapes of subsequent stimuli. Half the trials represented a shift of cognitive set from one rule to another, while the other half comprised non-shift trials where the stimulus attribute that guided response selection remained the same as the previous trial. Independent component analysis (ICA) was used to identify a component representing the dorsal attention network, which prior published studies using this task have found is strongly engaged for set switching. This ICA-identified network also included prefrontal cortex regions typically found in the frontoparietal cognitive control network. Maps representing the strength of regional connectivity for this component were examined using SPM12 flexible factorial model (Subjects × Group × Dose). Contrasts examined the main effect of Dose and interaction of Dose and the regular vs. occasional use Group factor. A whole brain clusterwise ($p < .05$ corrected) statistical inference framework was used to control for Type I error (cluster determining threshold $p < .005$ voxelwise).
Results: Consistent with published results that used conventional fMRI activation analysis methods, analysis of ICA-

derived timecourse data found the attention network component was strongly engaged during switch trials, but less so for non-switch trials. After marijuana use, network functional connectivity was weaker in a dose-dependent manner in several brain regions, including right caudate/putamen, right inferior frontal gyrus (BA 44), and left superior occipital gyrus (BA 19). Contrary to predictions, marijuana also enhanced the strength of functional connectivity in several brain regions, including anterior cingulate (BA24), right bilateral thalamus, brainstem, bilateral posterior cingulate, bilateral orbitofrontal cortex (BA 11), and superior/medial frontal gyri (BA6). In addition, the cerebellum culmen was recruited into this network after marijuana use, although there was no evidence it was normally connected during the placebo condition. Exploratory analyses at whole brain-corrected statistical thresholds found numerous brain regions showing a Dose × Group interaction for which marijuana's effects on functional connectivity differed between regular and occasional marijuana users. Effects were found in bilateral medial frontal gyrus (BA 6), bilateral medial frontal gyrus (BA 10), two separate clusters within left middle frontal gyrus (BA 9 and BA 46), right superior frontal gyrus (BA 8/9), right middle/inferior frontal gyri (BA 47), left insula (BA 13), left midcingulate gyrus, right superior temporal gyrus (BA 38), left cuneus, left putamen, right cerebellar tonsil, and left midbrain. None of these interaction effects overlapped regions showing simple placebo/dose-related differences.

Conclusions: Recent marijuana use both increases and decreases functional connectivity of different brain regions within the dorsal attention network. Effects were dose-dependent, in that greater marijuana doses were associated with greater regional connectivity changes. Moreover, functional connectivity was altered in both attention-related network regions and brain regions known for high concentration of cannabinoid receptors (e.g., cerebellum and basal ganglia). While it was not unexpected that dose effects on brain connectivity differed between regular and occasional users, there was no overlap between these findings and simple dose effects on functional connectivity. Neurofunctional differences related to long-term, chronic use have been attributed to both putative neural adaptation as well as pre-existing neurobiological risk. These results suggest that with respect to cognitive set shifting, the acute effects of marijuana use might be separate from either of these two factors.

Keywords: Marijuana, Functional MRI (fMRI), Task-Based Functional Connectivity, Mental Set Shifting.

Disclosure: Nothing to disclose.

M262. GRIK1 Snp rs2832407, Trauma Burden, and Risk for Alcohol Dependence: Results From the National Health and Resilience in Veterans Study

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Background: We sought to replicate previous findings that a single nucleotide polymorphism (SNP) in the GRIK1 gene, which encodes the GluK1 subunit of the kainite receptor, is associated with alcohol dependence (AD; Kranzler, Gelernter, Anton, et al, 2009) in a nationally representative sample of European-American (EA) U.S military veterans. SNP rs2832407 has previously been found to be associated with increased risk for AD (C-allele for risk), as well as preferential treatment response to topiramate in individuals with AD (CC genotype). Preclinical studies have implicated changes in kainate receptor expression and the involvement of glutamatergic changes in the response to stress and trauma, suggesting a potential for moderating influences of these variables on the relation between GRIK1 variants and AD risk.

Methods: Data were analyzed from a nationally representative sample of 1,666 European American (EA) U.S. military veterans. A total of 220 (13.2%) veterans were diagnosed with AD using the Mini Neuropsychiatric Interview. Veterans were genotyped using the Illumina PsychChip GWAS array, a PGC augmented tag SNP plus exome array; GRIK1 rs2832407 genotype data were imputed and genotype frequencies did not differ from Hardy-Weinberg expectations ($p = 0.55$). Multivariate binomial logistic regression was used to examine the relation between GRIK1 genotype, lifetime trauma load, and the interaction of GRIK1 genotype x trauma load on AD risk; age, sex, lifetime history of major depressive disorder (MDD) or posttraumatic stress disorder (PTSD), and ancestral proportion scores were entered as covariates.

Results: After adjustment for age, sex, lifetime history of MDD and/or PTSD, and ancestry, there was a significant additive main effect of the C allele on AD diagnosis (Wald chi-square = 4.95, $p = 0.026$; OR = 1.49, 95%CI = -1.05-2.12), as well as a significant interaction of number of GRIK1 rs2832407 C alleles with trauma load (Wald chi-square = 24.76, $p < 0.001$), with C allele carriers with higher trauma load having the highest risk of AD relative to C allele carriers with lower trauma load.

Conclusions: We replicated the association of GRIK1 rs2832407*C to AD in a nationally representative sample of predominantly male EA U.S. military veterans. We also found that the effect of this polymorphism on AD risk was moderated by trauma load, such that higher trauma load in C allele carriers was associated with highest risk of AD. These results suggest that a polymorphism in the GRIK1 rs2832407 genotype, directly and interactively with trauma load, contributes to AD risk in the general population of U.S. military veterans.

Keywords: Alcohol Dependence, Glutamatergic Transmission, Trauma Exposure.

Disclosure: Nothing to disclose.

M263. Vagus Nerve Stimulation Enhances Extinction From Cocaine-Seeking and Modulates Plasticity in the Infralimbic Prefrontal Cortex to Basolateral Amygdala Projection

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Background: Relapse to drug seeking may result from drug-induced dysergulation of brain regions that regulate impulse

control and self-regulation. These changes cause overvaluation of drug-associated cues, leading to craving and relapse. In animal models extinction training can reverse neuroadaptations caused by drug self-administration. Similarly, behavioral therapy for substance abuse often focuses on extinguishing the drug-seeking response to the associated cues; however, this type of exposure therapy for drug addiction is not consistently effective in reducing cue reactivity and preventing drug relapse. Therefore, modulating extinction processes in order to better consolidate newly-formed memories has clinical potential to reshape maladaptive behavior and prevent relapse. A major avenue of research investigates the possibility of combining pharmaceutical agents with extinction-based behavioral therapy to enhance therapeutic outcomes for both anxiety disorders and addiction-related behaviors; however, currently there is no FDA-approved medication for the treatment of cocaine addiction. We propose vagus nerve stimulation (VNS) as an alternative approach to modulate synaptic plasticity in extinction circuits to facilitate extinction training and to reduce drug-seeking. VNS is FDA-approved for the treatment of epilepsy and depression and has recently been used to induce targeted plasticity in models of stroke and tinnitus, and to enhance the extinction of conditioned fear. Here we delivered VNS during extinction from cocaine self-administration and measured relapse to drug seeking during cue-induced reinstatement. VNS facilitated extinction and reduced reinstatement. To study the mechanisms that regulate these effects we used immunohistochemistry for the phosphorylated transcription factor cAMP response-element binding protein (pCREB) in the prefrontal cortex (PFC) and the basolateral amygdala (BLA), which regulate cue learning and extinction. In addition, we used in-vivo recordings of evoked field potentials in the pathway from the PFC to the BLA to measure drug- and VNS-induced changes in metaplasticity (i.e. the ability to induce synaptic plasticity).
Methods: Rats were trained to self-administer cocaine in operant conditioning chambers in daily 2 hour sessions. During self-administration, cues (a light and tone) were presented after correct lever presses. After 10 days of drug self-administration, rats received extinction training in the same chamber for 12 days. During extinction sessions levers were available as before, but lever presses at the previously drug-paired ("active") lever no longer produced a drug reward or the conditioned stimuli. Animals received either sham-stimulation or VNS under one of two conditions. One group of animals received VNS (0.8 mA, 15 biphasic pulses, 100 μs each, at 30 Hz for 500 ms) contingent with each press on the previously active lever. Another group of animals received stimulation (0.4 mA, 15 biphasic pulses,100 μs each, at 30 Hz) for 30 seconds every 5 minutes for the duration of the session, irrespective of lever presses. A cued reinstatement session was used to assess relapse to drug-seeking behavior. After reinstatement, animals were either immediately sacrificed for immunohistochemical analysis of pCREB in the PFC and BLA, or were used 24 hours later for in-vivo recordings of evoked field potentials in the pathway between the PFC and BLA. Changes in the size of the baseline response in the BLA to PFC stimulation were measured, as well as changes in the ability to induce LTD in this pathway.
Results: Compared to sham-stimulated animals, VNS treated animals had reduced rates of responding at the previously drug-paired lever during extinction and reinstatement sessions. Similar changes were seen in a control group that extinguished from food self-administration. Following reinstatement, VNS animals showed reduced levels of pCREB in the IL and BLA, but no differences were found in the PL. During recordings of evoked field potentials VNS-treated animals had reduced baseline responses in the BLA to IL stimulation, and these responses were resistant to LTD induction compared to sham and naïve animals.
Conclusions: VNS shows potential as an adjunct treatment for extinction-based behavioral therapy, as animals treated with VNS during extinction from both food-seeking or drug-seeking showed greatly reduced rates of cue-induced reinstatement. VNS paired with extinction training caused changes in the levels of pCREB in the PFC and BLA, as well as changes in the connectivity between these two areas, suggesting that this connection may be important for the effect of VNS on the expression of drug-seeking behavior.
Keywords: Extinction Learning, Drug Abuse, Vagus Nerve Stimulation.
Disclosure: Nothing to disclose.

M264. IV Citalopram Effects on Craving, Decision Making, and Dopamine Binding in Alcoholics

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Background: Alcohol abuse and dependence represent a spectrum of maladaptive behaviors with enormous public health impact, especially for the U.S. veteran population. Depressive symptoms are frequently comorbid with alcohol use disorders, and the use of serotonin reuptake inhibitors (SSRIs) to treat these symptoms are common in clinical practice. Clinical trials with SSRIs for alcohol use disorders, however, have yielded mixed results concerning their impact on drinking behavior. As intravenous (iv) citalopram infusion (40 mg) bypasses hepatic metabolism, a single infusion is well-tolerated, and produces a clinically relevant concentration in human brains. A single infusion reduces striatal dopamine receptor availability (presumably through an action to increase intrasynaptic dopamine) by a magnitude comparable to the effect of chronic oral citalopram treatment, as measured by positron emission tomography (PET). The subjective experience of craving for alcohol in alcohol-dependent individuals has been associated with decreased dopamine receptor availability in the striatum via PET.
Methods: We have been recruiting heavy-drinking alcohol-dependent research volunteers and matched non-drinking controls for a double-blinded, placebo-controlled, within-subjects, outpatient study with iv citalopram (40 mg and saline, in counter-balanced order) and [18F]fallypride PET scanning. The goal of the project is to assess whether a single iv dose of SSRI causes either an alteration in the level of craving for alcohol and/or changes in dopaminergic neurotransmission in the striatum in alcoholics, compared to non-drinking controls. Secondarily, we have also assessed measures of risky decision-making in these subjects.

Results: We present preliminary data based upon a partial dataset of 10 completing alcoholic and 10 control subjects. Contrary to expectations, a single IV dose of citalopram produced a reduction in craving for alcohol dependent participants. Preliminary results also show a strong trend to increased striatal dopamine receptor availability with IV citalopram, an opposite result to that reported previously in the literature. In a novel finding, alcohol dependent participants show increased loss aversion in the Loss Aversion Gambling Task compared to control participants.
Conclusions: The results of this study may have clinical importance in the treatment of alcohol dependence, by providing an increased understanding of the neuropsychopharmacology of practical treatment options for clinical populations.
Keywords: SSRI, Alcoholism, Craving, Dopamine (D2, D3) receptors, Positron Emission Tomography Imaging.
Disclosure: Nothing to disclose.

M265. Histone Acetyl-Lysine Reader Proteins Mediate Neurobehavioral Adaptations to Psychostimulants

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Background: Epigenetic-based therapies are emerging as potential treatments for several psychiatric disorders, including drug addiction. In particular, the BET family of histone acetyl-lysine reader proteins (BRD2, BRD3 and BRD4) represents a novel, druggable class of epigenetic targets. BET proteins bind to acetylated histones and recruit protein complexes involved in transcriptional activation, elongation and super-enhancer activity. Because BET proteins have been implicated in the pathophysiology of several disorders, multiple companies have developed selective, small molecule BET inhibitors that are currently being tested in clinical trials. Given that histone acetylation mechanisms are importantly involved in drug-induced neuroadaptations, we hypothesized that BET proteins may also play a vital role in addiction-related phenomena.
Methods: Using the conditioned place preference (CPP) procedure, male C57BL/6 mice were injected with cocaine (15 mg/kg), amphetamine (3 mg/kg), nicotine (0.5 mg/kg) or morphine (5-10 mg/kg) and confined to one side of the chamber by a solid divider for 30 minutes, or injected with saline and restricted to the other side of the chamber for 30 minutes. During the 3 conditioning days, injections were administered in a balanced fashion in morning and afternoon sessions (at least 4 h apart). The BET bromodomain inhibitor, JQ1 (10, 25, or 50 mg/kg, i.p.), or vehicle was administered before each conditioning session. As a control, the inactive enantiomer of JQ1 (-JQ1, 50 mg/kg) or iBET-151 (a BET inhibitor that doesn't cross the blood brain barrier, 50 mg/kg) was injected prior to conditioning in a different set of mice. Other animals received intra-accumbal injections of JQ1 or AAV5-BRD4-shRNA prior to conditioning. In additional behavioral studies, JQ1 was administered during acquisition of LiCl-induced conditioned place aversion (CPA) or contextual fear conditioning to determine the effects of JQ1 on other learning and memory procedures. In molecular studies, we measured BRD2, BRD3, BRD4 and phospho-BRD4 protein levels and BRD4 binding to specific promoter regions (ChIP-qPCR) in the nucleus accumbens (NAc) following repeated exposure to drugs of abuse. In additional mice, JQ1 or vehicle was administered prior to an injection of cocaine, amphetamine, nicotine or morphine and addiction-related genes were measured in the NAc, prefrontal cortex and dorsal striatum.
Results: JQ1 significantly reduced behavioral responses to cocaine, amphetamine and nicotine but not morphine. JQ1 did not attenuate LiCl-induced conditioned place aversion or contextual fear conditioning, indicating that BET inhibition does not affect all types of contextual learning. Repeated exposure to psychostimulants increased BRD4, but not BRD2 or BRD3, proteins levels. Psychostimulants increased BRD4 binding to addiction-related genes in the NAc, and JQ1 attenuated psychostimulant-induced gene expression in multiple brain regions.
Conclusions: Together, these studies indicate that the displacement of BET proteins from chromatin may have therapeutic efficacy in addiction-related behaviors.
Keywords: Epigenetics, Cocaine, Substance use Disorder.
Disclosure: Nothing to disclose.

M266. Effects of Dietary Choline Supplementation in an Adolescent Nicotine Exposure Model- A Whole Genome Epigenetic Perspective

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Background: Chronic nicotine exposure during adolescence produced persistent changes in neuronal function leading to learning deficits in adulthood. Choline supplementation reversed these deficits, but the mechanism involved is unclear. We examined whether the action of choline as a methyl donor in DNA methylation may be involved; thus leading to changes in DNA methylation and subsequently changing gene expression.
Methods: Adolescent, C57BL/6J mice were implanted with pumps delivering saline or nicotine. Then animals received either standard or choline diet and underwent fear conditioning testing. Dorsal hippocampi were extracted and DNA/RNA isolated. Methylated DNA Immunoprecipitation Sequencing was performed using ABI SolidTM.. MEDIPS BioconductorTM package was used for identification of Differentially Methylated Regions (DMRs). Enrichment for gene pathways was performed using PANTHER. Expression changes in selected genes were assessed by RT-PCR.
Results: Whole genome comparison of dorsal hippocampi revealed DMRs that were altered in nicotine exposed animals compared to controls and were reversed by choline supplementation. 453 of these DMRs were located in proximity (5kb of TSS) to brain expressed genes. Gene enrichment analysis showed the greatest enrichment for chromatin remodeling genes. qRT-PCR analysis in a subset of the chromatin remodeling gene category revealed, significant expression changes which were opposite correlated with promoter methylation changes in a number of

chromatin remodeling genes. Of which to note is Smarca2 a member of the neuronal specific SWI/SNF ATP dependent chromatin remodeling complex - BAF, which has been shown to participate and regulate differentiation of neuronal precursor cells to mature neurons both in vitro and in vivo. Genetic polymorphisms in SMARCA2 and other SMARC family members have been shown to be associated with Schizophrenia, Autisim spectrum disorders and a possible association with alcohol use disorders.

Conclusions: To our knowledge this is the first study to examine whole genome epigenetic effects of choline supplementation after chronic nicotine exposure in adolescent in-vivo model. The findings that the main gene targets involved in choline's restorative function are chromatin remodeling genes, which by themselves serve as epigenetic regulators, points to a complex multi-layered mechanism of action. The elucidation and understanding of the interplay between these epigenetic systems and the identification of downstream targeted effectors can assist in better understanding of the mechanisms by which choline reverses deficits in adult cognition due to adolescent nicotine exposure.

Keywords: Epigenetic, Nicotine Addiction, Adolescence, Choline.

Disclosure: Nothing to disclose.

M267. GABRA2 Association With Addiction-Related Endophenotypes is Environmentally Influenced

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Background: The GABRA2 gene contains more than 250 SNPs that in Caucasians form two major common "Yin-Yang" haplotypes - having either all or none of the non-ancestral (NA) alleles. Since 2004, association of the non-ancestral, slightly less common haplotype with alcohol use disorder (AUD) and with increased beta waves in EEG has been reported and replicated many times.

Methods: In a longitudinal sample of >400 families at high risk for AUD and low socio-economic status, we investigated the influence of these GABRA2 haplotypes on addiction related endophenotypes in the offspring, and started to investigate potential functional implications.

Results: We found that the NA haplotype is associated with impulsivity and with increased activation of the insula in response to reward expectation, both of which partially mediate the association of the NA alleles with alcoholism. Impulsivity was also associated with reward expectation, independent of genetics. Moreover, the effect of parental monitoring on externalizing behavior trajectories across childhood and adolescence, i.e., consistently low, developmentally limited, and rising trajectories, was moderated by the NA alleles of GABRA2. Subjects with the NA alleles were more strongly influenced – both positively and negatively–by the extent of parental monitoring, while subjects with the ancestral alleles were not significantly influenced by the monitoring.

In addition to parents, peers are also known to influence addiction-related behaviors such as rule breaking during adolescence. Association with delinquent peers increased rule breaking, particularly in those with NA alleles of GABRA2, while association with peers who displayed positive behaviors (such as religious activity and scholastic competence) decreased externalizing behaviors. Subjects with the ancestral alleles of GABRA2 were less affected by peers.

Conclusions: Our results illustrate a more complex influence of genotypes on risk for common traits such as those associated with addiction. Our data suggest that the previously identified "risk" alleles impact the strength of both adaptive and maladaptive environmental influences on risky behaviors such as rule breaking, and hence might be thought of as plasticity factors.

None of the alleles of the GABRA2 haplotypes change an amino acid, and previous standard genotype-expression linkage studies (eQTL) found no associations in several large brain tissue collections. The Allelic Expression Imbalance (AEI) approach is more sensitive to slight expression differences than eQTL. Preliminary AEI using RNASeq from brain mRNA suggests that the NA (risk) alleles may increase expression. Our result is in contrast to a recent report of decreased expression of the risk alleles in iPSCs, suggesting differential effects across diverse tissues and highlighting the importance of studying the brain. Epigenetic modifications may be one pathway of how environmental influences on behavior are being molecularly mediated, and just as our results demonstrate plasticity at the behavioral level, plasticity may exist on the molecular level, in that expression of GABRA2 haplotypes may be influenced molecularly by different environments.

Keywords: Gene Environment Interactions, Allelic Bias Analysis in RNASeq, Haplotypes, Substance-Related Disorders, GABRA2.

Disclosure: Supported by NIAAA grants AA07065, AA012217, T32 AA07477, and NIDA grants DA027261 and DA041202.

M268. Effects of the Nicotinic Partial Agonist Varenicline on Smoking Lapse Behavior in Smokers With and Without Schizophrenia: Preliminary Studies

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Background: Varenicline, a nicotinic receptor partial agonist, is the most efficacious smoking cessation pharmacotherapy, and has been shown to reduce long-term smoking relapse rates in smokers with schizophrenia (Evins et al, 2014 JAMA). Despite the promise of varenicline and other smoking cessation interventions in these patients, high relapse rates persist in the schizophrenia population. The use of human laboratory models may allow the parsing of potential mechanism to improve treatment outcomes in these difficult to treat smokers. The first instance of smoking during a quit attempt ('smoking lapse') is one of the best

predictors of relapse. Accordingly, the goal of this study was to investigate the effects of varenicline on smoking lapse behavior in smokers with and without schizophrenia using a validated model (McKee et al, 2013. Nicotine Tobacco Res.).

Methods: A total of 33 subjects (15 controls, 18 schizophrenia patients) participated in the study. Varenicline was titrated up to 2mg/day over 4 days and continued for a total of 6 days during two separate test weeks, using a randomized, double-blind, counter-balanced, cross-over human laboratory study design. The primary outcome was the time to smoking lapse, conducted in a negative airflow indoor smoking room.

Results: In a preliminary analysis of study completers [smokers with schizophrenia ($n = 14$); control smokers ($n = 14$)], varenicline non-significantly increased time to lapse in healthy controls ($p = 0.22$) and schizophrenia patients ($p = 0.12$). These effects were more pronounced in smokers with higher levels of nicotine dependence (e.g. FTND score > 5). There was a trend towards an effect of treatment on time to lapse among the entire sample, with an increased resistance to smoke with varenicline treatment ($p = 0.08$). Interestingly, in heavily dependent smokers, the ability of varenicline to enhance resistance to smoking lapse was less robust in smokers with schizophrenia versus non-psychiatric control smokers.

Conclusions: Our preliminary findings support the conduct of larger studies to further delineate the specific mechanisms by which varenicline protects against smoking relapse in smokers with schizophrenia, possibly in combination with adjunctive treatments to boost overall outcomes in these difficult to treat tobacco smokers.

Keywords: Nicotine Dependence, Schizophrenia, Relapse, Human Laboratory Study, Varenicline.

Disclosure: Pfizer: 2012 GRAND Award, Self; CIHR: Grant Funding, Self. Supported in part by a 2012 GRAND Award (to TPG and VCW) and Canadian Institutes of Health Research (CIHR) grant MOP#115145 (to TPG).