Brain structure changes in a 16p11.2 deletion mouse model

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T1. Clinical Staging with the Global Deterioration Scale (GDS) Shows a Remarkably Uniform Pattern of Temporal Change Over 2 Years in Healthy Older Persons with Subjective Cognitive Impairment (SCI) in Accord with Prior Estimates and Observations Indicating a Stage of 15 Years Duration


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Background: There is increasing recognition that clinical symptoms of Alzheimer’s disease (AD) begin many years, even decades, prior to the onset of manifest mild dementia. The Global Deterioration Scale (GDS) (Reisberg, et al., Am J Psychiatry, 1982) identifies 3 pre-dementia stages. The first of these (GDS stage 3) is a stage of subtle, but observable, cognitive deficits. The terminology “Mild Cognitive Impairment,” (MCI) was coined for this GDS 3 stage in 1988 (Reisberg, et al., Drug Dev Res, 1988), and this entity has subsequently been widely studied. We initially estimated that the MCI stage lasts a mean of ~7 years prior to the advent of the mild dementia of AD (Reisberg, Geriatrics, 1986). Subsequent studies in memory clinic populations have supported this temporal estimate (see Bruscoli and Lovestone, Int Psychogeriatr, 2004, for a review). The GDS also identifies a second pre-dementia stage, the pre-MCI stage in which older persons have subjective symptoms of cognitive deficit only and perform normally on psychometric and other measures. This GDS 2 stage is defined succinctly with the scale as: “Subjective complaints of memory deficit, most frequently in the following areas: (a) forgetting where one has placed familiar objects; (b) forgetting names one formerly knew well.” The scale also notes that in this GDS 2 stage there is, “no objective evidence of memory deficit on clinical interview,” and “no objective deficit in employment or social situations.” It is also noted that there is “appropriate concern with respect to symptomology.” We suggested the terminology “Subjective Cognitive Impairment,” (SCI) for this GDS 2 stage and estimated in 1986 that it lasts a mean of ~15 years prior to the onset of MCI (Reisberg, Geriatrics, 1986). An 8.9 year longitudinal study in healthy subjects at baseline strongly supported this temporal estimate (Prichep, et al., Neurobiol Aging, 2006; Reisberg and Gauthier, Int Psychogeriatr, 2008). Assuming a uniformly distributed baseline subject population, and a stage lasting precisely 15 years, with uniform progression rates within the stage, then 6.667% of subjects should progress annually to MCI or dementia. On an annual basis, the difference between the 1986 estimated rate of progression and the observed percentage of subjects who advanced was only 0.23% per annum. In another investigation, a 7 year longitudinal study in healthy older persons at baseline, it was found that the hazard ratio for decline was 4.5 for SCI persons in comparison with matched GDS stage 1 persons with No Cognitive Impairment (NCI), the third pre-dementia stage (Reisberg, et al., Alzheimers Dement, 2010). This 4.5 hazard ratio was obtained after controlling for baseline demographic variables and follow-up time. Herein, we report changes in SCI (GDS stage 2) persons over a 2 year interval, a time interval of relevance for future pharmacologic trials endeavoring to slow cognitive deterioration in SCI persons.

Methods: Healthy subjects with SCI (GDS stage 2) from our published 7 year longitudinal study, with follow-ups between 1.5 and 3.0 years were selected. This resulted in a 98 subject cohort followed over 2.13 ± 0.30 years (63 women, 35 men). Baseline subject characteristics included a mean age of 67.12 ± 8.75 years (range 40 to 87 years) and a mean of 15.55 ± 2.60 years of formal education (range 8 to 21 years). The mean baseline Mini Mental Status Examination (MMSE, Folstein, et al., J Psychiatr Res, 1975) score was 28.92 ± 1.23 (range 25 to 30). To calculate the change in GDS stage, the following a priori assumptions were posited: (1) a reversion to a prior stage (i.e., improvement to NCI) = -1; (2) no stage change = 0; and (3) progression to MCI or dementia = +1.

Results: The Wilcoxon test was used for all analyses. The GDS stage changed from 2.00 at baseline to a mean of 2.16 ± 0.59 at follow-up (P < 0.01). More specifically, at follow-up, 8 subjects (8.16%) remitted to a GDS stage of 1 (NCI), 68 subjects (69.39%) remained at GDS stage 2 (SCI), 20 subjects (20.41%) declined to GDS stage 3 (MCI), and 2 subjects (2.04%) had mild dementia at follow-up. Using the methodology described above, for a uniformly distributed baseline population, the estimated annual progression rate for a stage lasting precisely 15 years (i.e., 6.667% of subjects progressing to MCI or dementia per annum), and the observed annual progression rate of GDS stage 2 subjects of 6.707%, differed by only 0.04%. Subject age was positively associated with GDS change (P = 0.002). There was no association between subject gender or years of education and the GDS stage at follow-up in this predominantly well-educated cohort. After adjusting for baseline subject age, GDS progression at follow-up remained significant (P < 0.01).

Conclusions: In this healthy subject population with SCI, the GDS scale identifies a subject population which progresses at a remarkably consistent rate of ~6.67% per annum towards MCI or, in a few cases, dementia, over a 2 year period. These results are consistent with our prior 8.9 year published longitudinal study findings. They are also consistent with a recent meta-analysis of 11 studies (including 2 of ours), of “older people with subjective memory complaints” which found an annual conversion rate to MCI of 6.67% (95% CI = 4.70 – 8.95%) (Mitchell, et al., Acta Psychiatr Scand, 2014). These results are of
immediate relevance for the selection of both subject populations and outcome measures for pharmacologic and other prevention trials which are being undertaken in efforts to prevent decline in the pre-MCI, SCI stage of eventual AD.

Keywords: Subjective Cognitive Impairment, Prevention of Alzheimer’s disease, Global Deterioration Scale, Treatment Outcome Prediction, Preclinical Trial Methodology

Disclosures: I am the author and copyright holder of the assessment measure which is described in this research study. This measure is made freely available for all educational and governmental purposes. Private entities may be charged for usage of this measure.

T2. Cognitive Aging and the Anterior Cingulate: Amyloid and Vascular Risk Factors

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Background: The anterior cingulate cortex (ACC) plays important roles in attention and memory. It is the principal locus of brain activation during the conflict condition of the Stroop color/word test and is the principal locus of declining metabolism during normal aging. ACC hypometabolism, detectable by fluorodeoxyglucose positron emission tomography (FDG PET), correlates with age-associated decline in executive function, e.g., verbal fluency (Pardo et al. 2007. Neuroimage 35:1231). ACC hypometabolism cannot be completely accounted for by loss of grey matter (Vaidya et al. 2007. Neuroimage 37:1346); in fact, some studies suggest the ACC remains unchanged or even hypertrophies with age. ACC hypometabolism occurs at least by age 40 years. In contrast, hypometabolism is first seen in FDG PET scans of patients with early Alzheimer’s disease (AD) in the posterior cingulate cortex (PCC; Minoshima et al. 1994. Lancet 344:895) and begins typically after 65 years of age. There is evidence that declining executive function (e.g., Stroop errors) predicts those healthy elders that will convert to MCI/AD 12 years later (Balota et al. 2010). Stroop errors are also correlated with declining functional connectivity in the ACC within the salience resting state network (Duchek 2013. Neuropsychology 27:516). This relationship is not modified by the level of CSF abeta42, suggesting amyloid deposition is not driving these correlations. This study revisits ACC function in normal aging and explores potential mechanisms of its dysfunction.

Methods: Data were downloaded from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) at the LONI archive. Protocols and procedures are detailed on the ADNI website. Older healthy adults (> 55 years) were of principal interest. Data included demographics, rating scales, neuropsychological test results, MRI, and FDG and amyloid PET scans. Because data were examined semiquantitatively (relative to a constant factor), the effects of various normalization parameters were analyzed. Subsets of subjects were sorted based on years of follow-up, amyloid positivity, and vascular risk factors (Hachinski score = 0; H0). Voxel-wise correlations between a parameter and age were calculated. Thresholds were adjusted based on the number of resels. Neurostat (courtesy Satoshi Minoshima, U. Washington) was used on the ADNI data to replicate previous work (Pardo et al 2007). New analyses on the ADNI data used FSL (Oxford) and Freesurfer (MGH).

Results: The previously reported results (Pardo et al 2007) were replicated with the normative ADNI dataset. In particular, the correlation of glucose uptake (whole brain normalization) and age was significantly negative in the ACC [Talairach (3,17,36)]; all normals, r = -0.50, r2 = 0.255, t2(209) = -8.43, p = 0.0; for normals @ 2 year follow-up with H0: r = -0.66, r2 = 0.44, t2(29) = -4.79, p < 4E-5. For all patient subgroups, the correlation of amyloid deposition (cerebellar normalization) with age in the ACC was not significant: all normals, r = 0.048, r2 = 0.002, t2(209) = 0.69, p < 0.48. The large region of age-associated ACC hypometabolism persists even after selecting for only those without vascular risk factors or amyloid deposition.

Conclusions: The principal locus of hypometabolism with normal aging localizes to the ACC confirming with the large ADNI dataset previous reports. These analyses provide evidence against two prominent hypotheses regarding age-associated ACC dysfunction: amyloid deposition and vascular disease. Further research is underway examining FLAIR data to probe vascular disease as a possible etiology. At this time, the pathophysiology of age-associated ACC dysfunction and its relation to AD remain to be clarified.

Keywords: anterior cingulate cortex, Amyloid, executive function, cognitive aging, Alzheimer’s disease

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T3. Sex-dependent Modulation of Age-related Cognitive Decline by the L-type Calcium Channel Gene Cavna1c (Cav1.2)

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Background: Evidence correlates increased calcium influx through L-type voltage-gated calcium channels (L-VGCC) to underlie age-related memory declines. The L-VGCC family consists of four distinct channels referred to as Cav1.1-Cav1.4. While Cav1.2 and Cav1.3 are the most prevalent L-VGCCs in the rodent brain, Cav1.2 accounts for about 80% of these channels. In the present study we sought to assess the specific role of Cavna1c (which encodes Cav1.2) in the regulation of age-related memory dysfunction.

Methods: Short-term, spatial, and contextual/emotional memory was evaluated in young (4-5 months) and aged (17-18 months), wild-type as well as mice with one functional copy of Cavna1c (haploinsufficient), using the novel object recognition, Y-maze, and passive avoidance tasks respectively. Hippocampal expression of Cavna1c mRNA was measured by quantitative polymerase chain reaction (qPCR).
Results: Aging was associated with object recognition and contextual/emotional memory deficits and a significant increase in hippocampal Cacna1c mRNA expression. Cacna1c haploinsufficient young and aged mice had decreased Cacna1c mRNA expression, as well as an absence of age-related increases in expression of this gene. Behaviorally, Cacna1c haploinsufficiency prevented object recognition deficits during aging in both male and female mice. A significant correlation between higher Cacna1c levels and decreased object recognition performance was observed in both sexes and within groups. A sex-dependent protective role of decreased Cacna1c levels in contextual/emotional memory loss, specifically in male mice, was observed.

Conclusions: These data provide further evidence for an association between increased hippocampal Cacna1c expression and age-related cognitive decline. Additionally, they indicate an interaction between the Cacna1c gene and sex in the modulation of age-related contextual memory declines.

Keywords: Aging, Cognition, Memory, Sex differences, CACNA1C

Disclosures: Nothing to disclose.

T4. Tryptophan 2,3-Dioxygenase Gene Prolongs Preimaginal Development in Vermilion Drosophila Melanogaster Mutants: Implications for Aging-associated Neurodegenerative Disorders and Aging

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Background: Up-regulation of kynurenine (KYN) pathway of tryptophan (TRP) metabolism was suggested as one of the mechanisms of aging and aging-associated neurodegenerative disorders (AAND)[1]. The rate-limiting enzyme of TRP conversion into KYN, TRP 2,3-dioxygenase (TDO), is an evolutionarily conserved ortholog of human TDO. In insects TDO is encoded by a Vermilion gene. TDO activity is impaired in Vermilion mutants of Drosophila melanogaster. We observed prolongation of life span of TDO deficient vermilion mutants [2]. There are four distinct stages in the life of Drosophila melanogaster: egg, larva, pupa, and imago (adult). Considering that TDO becomes active during larval stage and that longevity genes might impact the speed of preimaginal development in Drosophila, we aimed to evaluate preimaginal development of vermilion mutants.

Methods: We compared the length of larval stage (time of emergency of pupae from larvae) and body weight of vermilion mutants and wild type (Oregon) flies.

Results: Time of emergency of pupae from larvae was longer (176.5 ± 27.42 hrs) and body weight of imago was higher (10.7 ± 0.58 mg) in vermilion mutants in comparison with wild-type Oregon flies (151.2 ± 27.41 hrs and 9.9 ± 0.58 mg, p < 0.002).

Conclusions: Present finding of prolongation of larval stage and our previous reports of prolonged life span of vermilion mutants suggest that the impact of TDO gene on adult life span begins during preimaginal period. TDO-deficiency-induced increased levels of upstream KYN metabolite, TRP, and decreased levels of downstream KYN metabolites might contribute to antiaging and neuroprotective effects of down-regulation of TRP – KYN metabolism reported in drosophila, yeasts, worms and mammals. Modulation of TDO activity might be a new target of prevention and treatment of AAND

Keywords: kynurenine, aging, development

Disclosures: Nothing to disclose. (Supported by NIMH 104810).

References:

T5. Anxiety Disorders Underlie the Familial Transmission of Suicide Attempts

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Background: Although there is abundant evidence that suicidal behavior is familial, the specific mechanisms of transmission are complex. Investigation of familial patterns of suicidal behavior may highlight specific social and biologic correlates of suicide risk as well as inform prevention approaches for individuals at high familial risk for suicide. The objective of this analysis was to investigate patterns of familial transmission of suicide attempts and psychopathological correlates in a clinically enriched community sample of probands and their first-degree relatives.

Methods: The sample included a total of directly interviewed 474 probands and 565 first-degree relatives who were characterized for the full range of mental disorders based on semi-structured diagnostic interviews and family history information using best estimate diagnostic procedures. The primary outcome was a lifetime suicide attempt either self-reported by the proband, or reported by a first degree relative of the index participant. Temperament and personality characteristics, including positive and negative affect, impulsivity and aggression were also evaluated by standard self-reported assessments in probands and their relatives.

Results: Of the 474 probands, 64 (14%) had made a lifetime suicide attempt. Though not significant, unadjusted analyses indicated that there was a three-fold greater rate of suicide attempts in the adult first-degree relatives of probands with a lifetime history of suicide attempt compared to those without such a history. Mixed effects logistic regression model adjusted for age, sex and comorbid mood and anxiety yielded significant individual association between suicide attempts and Bipolar I disorder. Familial transmission of lifetime suicide attempts was primarily attributable to proband anxiety disorder disorders, specifically Panic Disorder, GAD and Social Phobia, rather than mood disorders. A significant association was found between negative affectivity and suicide attempts in relatives when controlling for the proband negative affect and relative age and sex.
Conclusions: Findings confirm the association between anxiety and suicide attempts from previous community and family studies, and suggest that anxiety disorders may be important components of the familial diathesis underlying suicidal behavior. Results underscore the importance of future research on the neurobiology of anxiety in the development and maintenance of suicide risk. Familial anxiety disorders, along with an individual history of Bipolar I disorder, may serve as important targets for suicide prevention.

Keywords: suicide, epidemiology, familial risk

Disclosures: Nothing to disclose.

T6. Sexually Divergent Expression of Active and Passive Conditioned Fear Responses

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Background: In Pavlovian fear conditioning, successful associative learning is traditionally measured by assessing time spent freezing to the conditioned stimulus, but the possibility that animals may engage active fear behaviors as well is rarely considered. In particular, it is not known whether males and females employ identical response strategies as learning occurs, an omission that could lead to misinterpretation of sex-dependent outcomes.

Methods: We evaluated locomotor activity in videos of gonadally intact adult male (n = 56) and female (n = 58) Sprague Dawley rats as they were trained and tested in auditory fear conditioning (5 habituation CS followed by 7 CS-US pairs), extinction (20 CS), and extinction retention (3 CS) across 3 days. In some animals, we observed a rapid “darting” behavior during fear conditioning that suggested an escape-like response. To quantify these responses, we used Ethovision software to record each animal’s velocity. Darting during fear conditioning corresponded to reduced freezing across fear conditioning, extinction, and extinction retrieval tests.

Results: Females were 4 times as likely as males to exhibit darting behavior in response to the conditioned stimulus. In females only, darting frequency increased with CS-US pair presentations and was correlated with shock response velocity. Darting during fear conditioning corresponded to reduced freezing across fear conditioning, extinction, and extinction retrieval tests.

Conclusions: Our data suggest that females employ multiple response strategies in the expression of learned fear. Importantly, the expression of active darting responses does not simply compete with freezing, but appears to induce lasting changes in freezing behavior, even in the absence of darting itself. Overall, our findings have major implications for the interpretation of fear conditioning and extinction studies, suggesting that freezing alone may not be a complete measure of learned fear in female subjects.

Keywords: Sex differences, Fear conditioning, Fear extinction, individual differences

Disclosures: Nothing to disclose.

T7. Genetic Predictors of Dysmaturation of the Brain’s Intrinsic Network Architecture: Relation to ADHD and Attention Dysfunction

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Background: The human brain is organized into several large-scale intrinsic connectivity networks (ICNs), which exhibit massive maturation from childhood to young adulthood. There is mounting evidence that the maturational trajectories of ICNs are perturbed in attention-deficit/ hyperactivity disorder (ADHD), with alterations in these ICNs playing a central pathophysiological role in the attention deficits seen in the disorder. ADHD is highly heritable (roughly 76% heritability), and this raises an intriguing hypothesis that specific genes are predictive of the disrupted patterns of ICN maturation reliably found in ADHD.

Methods: Participants (n = 519, ages 8.6-22.6) from the Philadelphia Neurodevelopmental Cohort (PNC) underwent 5.2-minute resting state scans, and cross-correlation matrices (“connectomes”) were produced from 1068 ROIs, with motion scrubbing and second-level regression used to minimize motion artifact (verified by inspection of QC-RSFC plots). In previous studies, we linked dysmaturation of the connectome to attention dysfunction using a novel “network growth charting” methodology. In brief, we first produced maturational growth charts for six major components of the connectome (which we labeled “A” through “F”) using joint independent component analysis, an unsupervised machine learning method. For each subject, we calculated “maturational deviation scores” that reflect differences in expression of the six connectomic components relative to the neurotypical profile. We showed that downward scores—reflecting underexpression of components relative to age—are robust predictors of worse attention functioning [F(6, 512) = 26.89, p < 2.2 * 10e-16; R2 = 0.2396]. In the present study, we used genome-wide association data for these same participants from the PNC dataset. Analysis was restricted to three of the six platforms (Human610_Quadv1_B, HumanHap550v3.0, and Huma-nOmniExpress; all from Illumina) representing 92% (n = 481) of the participants. The genomic data was recoded, quality checked, and filtered (variant missing call rate < 0.05, sample missing call rate < 0.1, minor allele frequency > 0.005, and Hardy-Weinberg equilibrium exact test p-value < 10e-6), yielding 476 subjects included in the analysis set. The data was then phased by SHAPEIT and imputed by minimac3 through the Michigan Imputation Server with the reference panel 1000G Phase3 v5. A genome-wide association analysis was next conducted with EPACTS on the imputed dosage data. Quantitative score tests were used to identify genes that predict relative underexpression of connectomic components relative to age (i.e., the component-specific maturational deviation scores calculated in the previous paragraph). Age and sex were added as covariates, as well as the top 10 principle components to account for population stratification.
**Results:** In preliminary analysis, 45 loci reached the genome-wide significance threshold ($p < 5 \times 10^{-8}$). Loci that predicted underexpression of components B, E, and F were particularly common. Component B involves interconnections between default mode network (involved in internally-directed mentation and mind wandering) and frontoparietal network (involved in cognitive control and executive functions). Component E involves connections between visual network and major regulatory networks, in particular frontoparietal network and ventral attention network. Component F involves connections linking “ventral stream” language regions in superior and middle temporal gyrus. Gene network analyses are currently being undertaken to identify families of genes overrepresented among the significant loci.

**Conclusions:** In our previous studies, we used “network growth charting” methodology to show that: (1) ADHD involves disrupted patterns of maturation of major components of the functional connectome; and (2) Underexpression of these components relative to age robustly predicts worse attention performance. In this study, we link dysmaturation of the brain’s network architecture to genes. In particular, we provide first time evidence of numerous genetic loci that directly predict underexpression relative to age of major components of the functional connectome. It is notable that despite the high heritability of ADHD, previous studies failed to find loci predictive of ADHD at genome-wide significance thresholds. Dysmaturation of specific components of the functional connectome might thus constitute a more homogenous, and thus more readily detectible, intermediate phenotype for the purposes of genetic investigations. More broadly, the novel “network growth charting” methodology used in this study could potentially be fruitfully applied to identify candidate genes for other psychiatric disorders, such as schizophrenia and autism, that are known to involve neurodevelopmental network disturbances.

**Keywords:** Large Scale Networks, ADHD, neurodevelopment, fMRI/imaging genetics

**Disclosures:** Nothing to disclose.

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**T8. Integrative Systems Analysis Associates Blood Glucocorticoid Receptor Dependent Immune Response with PTSD Diagnosis and Treatment Response**

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**Background:** Delineating the molecular basis of individual differences in the stress response is critical to understand the pathophysiology of post-traumatic-stress-disorder (PTSD), with the ultimate goal of identifying biomarkers that predict recovery from PTSD and associate with treatment response to psychotherapy.

**Methods:** Data: Blood molecular data (expression arrays and RNA-seq) and functional endocrine data will be presented from a cross-sectional biomarker study ($n = 68$ with PTSD, $n = 68$ without PTSD) and a longitudinal psychotherapy trial ($n = 50$) in combat veterans with PTSD. In addition, gene expression data in blood and brain (amygdala and hippocampus) of a PTSD rat model will be presented, in which resilient and vulnerable phenotypes are identified according to the long-term behavioral response to predator scent stress (PSS).

**Analyses:** Analyses of the human and animal data were performed separately, and then compared and integrated with the use of gene network analyses: (a) differential gene expression; (b) weighted gene co-expression network analysis (WGCNA); (c) comparative network analysis; (d) module-trait correlations; and (e) key driver analysis.

**Results:** Differentially expressed (DE) genes were identified in blood of veterans with PTSD in comparison with veterans without PTSD. DE genes associated with PTSD were consistent with downregulation of glucocorticoid receptor (GR)-signaling and upregulation of proinflammatory signaling. These findings were replicated by the DE-signatures identified in rat blood and brain, in association with exposure-related individual differences. Interestingly, even though the across-tissue overlap in rat DE-signatures was small at the individual gene level (6%), there was a high conservation at the upstream transcription factor (TF) or cytokine level (36% and 63%, respectively). Gene co-expression networks were also identified. Among the most promising networks, there was a large (>100 genes) PTSD co-expression module showing a high level of dysregulation (compared to controls modular differential connectivity >50) and conservation in the blood and brain of PTSD-like rats. Functionally, this module is associated with innate immune response ($p < 0.001$), and correlated with plasma cortisol decline in dexamethasone suppression test ($p < 0.001$) and the lymphocyte lysozyme inhibition ($p < 0.05$) by dexamethasone. Identified DE genes, pathways and networks predicted treatment response. Several key network drivers changed over time in association with treatment response in both combat veterans with PTSD. Finally, GR agonist administration in rats shortly after PSS prevented PTSD-like phenotypes by reversing the vulnerability-associated transcription patterns.

**Conclusions:** We identified genes, pathways and gene co-expression networks in the blood of combat veterans with PTSD and key network drivers’ activity changes over time in association with treatment response. Blood-based biomarkers can predict aspects of brain signaling. GR-dependent immune pathways are associated with trauma-related individual differences in blood and brain, and can be the basis of treatment strategies for PTSD.

**Keywords:** PTSD, biomarkers, gene expression, animal model, humans

**Disclosures:** Nothing to disclose.

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**T9. Methylphenidate Decreases Anxiety-Induced Impairment of Working Memory Performance in Healthy Subjects**

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**Background:** A large literature documents the influence of anxiety on cognition, and reciprocally the influence of
cognition on anxiety- the latter being exploited for the treatment of anxiety disorders. It is well documented that anxiety can impair cognition, and that cognitive strategies can be used to decrease anxiety. For instance, one effective way to reduce anxiety is to immerse oneself in a cognitive task, specifically a difficult or effortful task. Tasks that involve working memory (WM) appear to be especially efficient at down-regulating anxiety, probably because of competition for attentional resources. However, the underlying mechanisms that mediate the interaction between anxiety and cognition remain unclear. This study is a first step towards clarifying this question using a pharmacological approach, through the distinct pharmacological manipulation of either cognitive function via Methylenephene-date (MPH), or state anxiety via Propanolol (PRO).

Hypotheses are that, compared to placebo (PLA), (1) MPH, a dopamine agonist that improves executive function, will improve memory performance and potentially decrease anxiety, and (2) PRO, a beta-adrenergic receptor-blocking agent used to reduce physiological arousal, will reduce anxiety and potentially improve performance.

**Methods:** The basic design of the study involves (1) the within-subjects induction of anxiety through the application of unpredictable electrical shocks, (2) the quantification of anxiety level by using a well-established translational method that provides a physiological measure of anxiety via EMG measures of anxiety-potentiated startle (APS) (Davis et al., 2010; Grillon, 2008), (3) the use of a working memory task (WM: n-back task, 1-, 2-, and 3-back), which we have shown to be reliably impaired by induced-anxiety (Vytal et al., 2012; Vytal et al., 2013), and (4) the double-blind administration of either PLA, PRO (40 g) or MPH (20 mg) in a parallel group design. Three groups of 20 healthy participants (N = 60 total) completed one experimental session, during which they performed the WM task under the threat of shock (anticipating unpleasant electric shocks) or safety (no shock). Each group received one drug challenge, either PLA, PRO (40 g) or MPH (20 mg). Outcome measures (performance and startle variables) were analyzed using 3-way ANOVAs with Condition (threat, safety) and Load (1-back, 2-back, 3-back) as within-subject factors, and treatment (PLA, MPH, PRO) as a between-subject factor.

**Results:** Results are presented for WM accuracy and then for APS. Regarding accuracy, overall, the experimental paradigm showed the expected effects of load and condition on performance, i.e., decreased accuracy with higher load and with threat vs. safe. Most important, the 3-way interaction of Condition by Load by Treatment was significant. Two follow-up analyses examined PRO vs. PLA and MPH vs. PLA on accuracy. PRO did not differ from PLA, i.e., PRO did not influence performance differently than PLA under threat (Condition by Load by Treatment: NS). However, MPH influenced performance differently from PLA under threat (Condition by Load by Treatment F(2,76) = 5.2, p = .008, GG-ε = .90). Specifically, MPH compared to PLA revealed better performance on the 3-back load during threat vs. safe, exhibiting a unique interaction of load by condition. Regarding startle, the analysis of baseline startle (without WM) showed no effects of treatment (Condition by Treatment: NS). Similarly, the analysis of startle during WM showed no effect of treatment (Condition by Load by Treatment: NS). Taken together, these results indicate a unique beneficial effect of MPH on WM accuracy during threat and the most difficult task (3-back), but no effects on anxiety. However, PRO did not influence either the anxiety startle measure or WM performance compared to PLA. Additional analyses on the effects of Treatment on other anxiety measures will also be presented.

**Conclusions:** Findings provide three take-home messages. First, performance under threat was uniquely modulated by MPH, while physiological anxiety was not. This suggests a dissociation of the effects of MPH on the modulation of cognition by threat and on anxiety per se. This needs to be further examined at the neural level to identify potential mechanisms underlying MPH action on cognition and anxiety. Second, in contrast to expectation, PRO did not differ from PLA. Conceivably, a higher dose, like 60 mg, could have been more powerful to detect an effect. Third, this approach to manipulate the interactions between anxiety and cognitive processes is promising and needs to be followed up in 2 ways: one that extends the present findings into neural mechanisms, and the other that tests potentially more effective doses, drug regimens or different drugs.

**Keywords:** Threat of Shock, Working Memory Capacity, stimulant, anxiety state, propanolol

**Disclosures:** Nothing to disclose.

**T10. DICER1 and MicroRNA Regulation in Post-Traumatic Stress Disorder**

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**Background:** Our understanding of biological mechanisms underlying PTSD and depression is still incomplete. PTSD with comorbid depression (referred to as PTSD&Dep from here on) is highly prevalent among susceptible individuals following traumatic life experiences and likely represents an extreme phenotype for PTSD. In this study, we aimed to increase our understanding of molecular mechanisms underlying PTSD using an extreme phenotype design, since an enrichment of extreme cases and controls in such design can improve our statistical power toward uncovering biological mechanisms.

**Methods:** Using PTSD&Dep following trauma exposure as an extreme phenotype for PTSD, we surveyed genome-wide differential gene expression profiles in blood in 184 cases of PTSD&Dep and controls with no PTSD and no depression. Our hypothesis was that genes differentially expressed in PTSD&Dep inform biological pathways underlying this psychiatric phenotype. We followed up on our genome-wide gene expression finding of DICER1 with expression quantitative trait locus (eQTL) analysis and functional imaging study. Moreover, since DICER1 cleaves precursor
microRNAs (miRNAs) into mature microRNAs, which regulate expression of thousands of genes post-transcriptionally, we surveyed genome-wide differential expression profiles of miRNAs in blood in a subset of PTSD&Dep cases and controls.

**Results:** Expression of DICER1 was significantly reduced in cases of PTSD&Dep relative to controls at genome-wide FDR < 0.05 (p = 7.93E-06, adjusted p = 0.040) after gender, age, population substructure were adjusted for, and this finding was replicated in two independent cohorts. Our follow-up studies found a DICER1 eQTL, rs10144436, located in its 3'UTR, significantly associated with PTSD&Dep (OR = 1.32, p = 0.012, Bonferroni-adjusted p = 0.024) and replicated this finding in another cohort. Additionally, we found that lower blood DICER1 expression was significantly associated with increased activation of the amygdala to threat stimuli, a neural correlate of PTSD, in a functional MRI study. Lastly, at genome-wide FDR <0.05, two miRNAs were significantly down regulated in the PTSD&Dep cases compared to controls after sex, age, and population substructure were controlled for: miR-212-3p (p = 4.48E-05, adjusted p = 0.048) and miR-3130-5p (p = 4.97E-05, adjusted p = 0.048). Each of these two miRNAs had a target mRNA that was significantly upregulated in PTSD&Dep cases at FDR < 0.1 in our original genome-wide differential gene expression analysis.

**Conclusions:** Taken together, our novel data suggest that DICER1 and miRNAs are involved in molecular mechanisms of PTSD&Dep via the DICER1/miRNA regulation pathway. This stress-related DICER1/miRNA regulation in blood is paralleled by published findings of DICER1/miRNA regulation in brain of stressed mice, as mediated by β-catenin. Studies are needed to elucidate the relationship between blood and brain DICER1/miRNA regulation, as well as mechanisms underlying the connection between blood DICER1/miRNA regulation and stress-related psychiatric disorders to contribute to their prevention and treatment efforts.

**Keywords:** Gene expression, molecular mechanisms, PTSD, MicroRNA, Depression

**Disclosures:** Nothing to disclose.
Trauma-focused exposure therapies such as prolonged exposure (PE) are the most efficacious interventions for the disorder, but our understanding of the neural mechanisms underlying their efficacy is nearly nonexistent. This knowledge gap is due partly to a lack of well-controlled imaging studies that facilitate isolation of neural effects related to the therapy itself from those arising from repeated assessments and non-specific changes over time. As trauma-focused psychotherapy is the first-line intervention for PTSD and only a minority of individuals undergoing this treatment will demonstrate clinical remission, it is imperative to characterize the effects of trauma-focused treatments on brain function and how such effects relate to changes in symptomatology. Such studies are ultimately needed to: a) identify neural changes that serve as mechanisms for symptom improvement following psychotherapy; b) parse neural abnormalities that are sensitive to interventions vs. those that are enduring and may reflect risk factors and/or confer treatment resistance; and c) identify relevant brain mechanisms that can be augmented to promote greater efficacy of future novel and/or modified interventions. Here, we report initial results from a two-arm, randomized imaging intervention study in PTSD investigating the mechanisms underlying efficacy of PE therapy. We focused specifically on tasks that capture constructs theoretically relevant to the neuropathophysiology of PTSD as well as exposure-based psychotherapy—emotional reactivity and regulation. As such, we investigated therapeutic effects of PE on prefrontal and limbic brain function using a waitlist (WL) control condition in patients to control for changes in brain function non-specific to treatment.

**Methods:** Fifty individuals meeting criteria for PTSD were randomized to 9-12 sessions of PE treatment (N = 24) or WL (N = 26) for a comparable time period. Individuals completed a clinical assessment and battery of functional imaging tasks before and after PE or WL. Here, we focus on an emotional reactivity paradigm (processing of fearful and neutral faces) and an emotion regulation paradigm (cognitive reappraisal and passive processing of negative and neutral pictures from the International Affective Picture System) to assess therapeutic effects on limbic and prefrontal brain responses. We utilized a repeated-measures framework to isolate group (PE vs. WL) x time interaction effects, i.e. changes across time that were significantly different for PE vs. WL. We also assessed the relationship of putative therapeutic neural changes isolated as above to changes in PTSD symptom dimensions.

**Results:** Clinically, individuals receiving PE demonstrated significantly better improvement in PTSD symptoms relative to those on WL from pre-to-post time points. On imaging measures, we observed prominent prefrontal changes from pre-to-post treatment that were greater in individuals undergoing PE vs. WL, with no significant treatment-related changes observed in limbic regions implicated in PTSD neurocircuitry models, i.e. amygdala and insula. Prefrontal PE-related changes were characterized by two dissociable processes: 1) decreased recruitment of the ventromedial prefrontal cortex during emotional reactivity across two tasks (fear vs. neutral face processing, negative vs. neutral passive IAPS picture viewing); and 2) increased recruitment of the rostral/dorsal anterior cingulate and dorsolateral prefrontal cortex during emotion regulation (reappraisal vs. passive viewing of negative IAPS pictures). Furthermore, within the PE group greater reductions in vmPFC activation to negative vs. neutral passive picture viewing were associated with greater reductions in PTSD re-experiencing symptoms, while greater increases in dorsal/rostral anterior cingulate and dorsolateral prefrontal activation to reappraisal vs. passive viewing of negative pictures were associated with greater improvement in avoidance and hyperarousal symptoms.

**Conclusions:** Using a well-controlled study design, these findings provide initial evidence that therapeutic mechanisms underlying efficacy of trauma-focused exposure therapies such as PE involve prominent changes in prefrontal circuitry with little-to-no effect on limbic circuitry during emotional reactivity and regulation. Moreover, trauma-focused exposure treatment in PTSD appears to promote greater engagement of prefrontal structures involved in salience signaling and top-down engagement during explicit attempts at regulating emotions while also decreasing ostensibly implicit regulatory processes in ventromedial prefrontal regions during emotional reactivity. Taken together, the current findings provide timely new information regarding mechanisms underlying trauma-focused exposure therapy in PTSD and suggest that the efficacy of such treatments is conveyed mechanistically primarily via strengthening compensatory prefrontal emotional regulatory processes, rather than attenuating exaggerated limbic reactivity.

**Keywords:** Posttraumatic stress disorder, BOLD imaging, Psychotherapy, emotion processing, emotion regulation

**Disclosures:** Nothing to disclose.

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**T13. Acute Methylphenidate Improves Performance on a Change Detection Task: A Double-Blind Randomized Controlled Trial in Healthy Males**

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**Background:** Change detection has been identified as a key cognitive process underlying effective decision making in noisy, volatile environments. Converging evidence indicates that noradrenergic neurons in the locus coeruleus (LC) signal unexpected environmental change, prompting both allocation of attention to unexpected stimuli and new learning. Recent evidence indicates that individuals with high trait anxiety exhibit impaired change detection and abnormal pupillary responses to unexpected events. Dysfunctional change detection may represent a target for treatments that modulate noradrenergic neurons in the LC. To the best of our knowledge, the effect of pharmacologic manipulation on change detection has not previously been studied. The purpose of this study was to examine the effect of methylphenidate (MPH), which inhibits norepinephrine reuptake and modulates LC activity, on change detection in healthy adult males. We hypothesized that MPH would improve both overall
performance and learning (improvement in performance across the first block) on a change detection task.

**Methods:** 19 healthy males were randomized to receive under double-blind conditions either an acute dose of MPH 40 mg (10 subjects) or placebo (9 subjects). Subjects completed a change detection decision making task with 180 total trials across 3 blocks. On each trial, subjects attempted to predict the location of a target stimulus out of 3 possible locations. Stimulus location was determined by a probability distribution in which one location was most likely to contain the target. At random intervals, the most likely target location changed. Performance was calculated based on how often a subject chose the most likely target location. Performance was compared between subjects receiving MPH and placebo using both a t-test and a mixed effects logistic regression model. Learning was also assessed by examining improvement in performance across the first block. Improvement in performance was compared between groups by testing a group-by-time interaction in a mixed effects logistic regression model across the first block.

**Results:** Subjects receiving MPH chose the most likely stimulus location significantly more often than subjects receiving placebo (p < 0.05). Subjects receiving methylphenidate also improved in performance more quickly across the first block (p < 0.05).

**Conclusions:** The results suggest that subjects receiving MPH more quickly adjusted to the environmental statistics (volatility) in the task, resulting in improved overall decision making performance. Medications such as MPH which improve change detection performance should be investigated as potential treatments targeting impaired change detection in psychiatric disorders.

**Keywords:** Decision Making, Methylphenidate, Anxiety

**Disclosures:** Nothing to disclose.

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**T14. A Cardiorespiratory Non-Chemosensory Interoceptive Pathway to Panic Anxiety Independent of the Amygdala**

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**Background:** The role of the amygdala in the acquisition, maintenance and extinction of fear learning in animal models is well defined. However, two reports of fear and panic in humans with bilateral amygdala damage have suggested that amygdala dysfunction alone may be insufficient to prevent these aversive emotional experiences in humans. One of these studies demonstrated that inhalation of 35% carbon dioxide (CO2) produced clinically significant panic attacks that included palpitations, dyspnea, dizziness, trembling, derealization, and fear of dying. We hypothesized that induction of a subset of these symptoms (principally palpitations and dyspnea) using isoproterenol, a rapidly acting beta adrenergic agonist without the pH altering properties of CO2, would be sufficient to elicit anxiety and panic in a pair of monozygotic twins with bilateral amygdala damage due to Urbach-Wiethe Disease who had previously undergone the CO2 inhalation challenge. We also hypothesized that these patients would demonstrate impaired cardiorespiratory interoceptive awareness of the sensations induced by isoproterenol.

**Methods:** 2 monozygotic twin sisters with bilateral amygdala damage were compared to two separate groups of healthy comparisons (n = 16 and n = 15). Participants rated their affective and sensory experiences following bolus intravenous infusions of isoproterenol and saline during two conditions: panic provocation, and assessment of cardiorespiratory interoceptive awareness (IA). During the panic provocation condition participants completed a panic symptom rating scale following each of 7 bolus intravenous infusions, administered in a single blinded, fixed order (0.1 mcg, saline, 4 mcg, saline, 1 mcg, 2 mcg, saline). During IA assessment randomized and double blinded infusions of isoproterenol (0.1, 0.25, 0.5, 0.75, 1.0, 2.0 and 4.0 mcg) and saline were administered while participants rotated a dial to track their momentary experience of heartbeat and respiratory sensation intensity.

**Results:** Isoproterenol infusions were sufficient to induce anxiety in both twins with amygdala damage, and panic in one twin. The twin who did not panic displayed impaired IA as evidenced by significantly poorer interoceptive accuracy with the dial (t(5) = -3.9, p = .006, attenuated palpitation t(8) = -1.90, p = .047, and attenuated dyspnea ratings t(8) = -1.85, p = .051 at all the highest dose. Furthermore, this twin demonstrated a complete lack of dyspnea sensation across both conditions, suggestive of impaired respiratory interoception. Mean heart rate responses to isoproterenol did not statistically differ between samples at the 2 mcg dose (BG t(16) = .55, p = .59, AM t(16) = .79, p = .44) or at the 4 mcg dose (BG t(16) = 1.61, p = .13, AM t(16) = -.83, p = .42).

**Conclusions:** These findings provide further evidence that the amygdala is not required for experience of anxiety and panic in humans, suggesting that additional neural circuits beyond the amygdala must be involved in the observed fear and anxiety responses. They also suggest that damage to the amygdala can impair cardiorespiratory interoceptive awareness. Characterizing the respiratory pathways involved in interoceptive processing and identification of the neural circuits associated with compensatory processing of interoceptive sensations in patients with amygdala damage is warranted.

**Keywords:** Amygdala, Anxiety, panic, fear, interoception

**Disclosures:** Nothing to disclose.

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**T15. Heart Rate Variability Predicts Vulnerability for Posttraumatic Stress Disorder in Active-Duty Marines**

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**Background:** Disrupted autonomic nervous system (ANS) functioning as measured by heart rate variability (HRV) has been associated with posttraumatic stress disorder (PTSD).
It is not clear, however, whether reduced HRV before trauma exposure plays a role in risk for development of PTSD. Here we tested the hypothesis that reduced HRV before trauma exposure may be a risk factor for development of PTSD.

**Methods**: To test our hypothesis we analyzed HRV data from the Marine Resiliency Study, a prospective, longitudinal study of behavioral and biological risk factors for development of PTSD in Active Duty Marines. After consent, participants were assessed using psychiatric, psychosocial, physical and laboratory measures in a 4 hr assessment period 1-2 months prior to a combat deployment and again three to six months after their return. The first phase of the Marine Resiliency Study (MRS-I) included 1415 male Marines, 59 of whom developed PTSD post-deployment. The second phase of the Marine Resiliency Study (MRS-II) included 745 male Marines, 25 of whom developed PTSD post-deployment. Heart rate was measured via finger plethysmography (1000 Hz) during a five-minute period of rest, and frequency-domain measures of HRV were generated. PTSD diagnosis was determined using the Clinician-Administered PTSD Scale (CAPS). Marines were categorized as either not meeting or meeting criteria for PTSD for each of the two time points using CAPS responses (Criteria were derived from the DSM-IV: at least 1 “B” symptom, 3 “C” symptoms, and 2 “D” symptoms. Marines that met PTSD diagnosis at the pre-deployment assessment were not included in the analysis. Combat trauma during deployment was measured via the Deployment Risk and Resilience Inventory and used as an additional predictor of post-deployment PTSD in the model.

**Results**: After accounting for deployment-related trauma exposure, lower HRV at pre-deployment as measured by increased low-frequency/high-frequency (LF/HF) ratio predicted the risk of PTSD diagnosis post-deployment (combined MRS/I/II cohort meta-analysis odds ratio = 1.48, p = 0.01). The pattern of results did not change when controlling for additional factors of deployment history or pre-deployment PTSD symptoms.

**Conclusions**: These findings support the hypothesis that an altered “state” of ANS functioning before trauma may contribute to PTSD vulnerability. If these findings are replicated, interventions that change ANS function in at risk populations such as rescue and military personnel may open new opportunities for treatment and prevention.

**Keywords**: PTSD, heart rate variability, at-risk, combat, psychophysiology

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impacts contextual fear memory but not innate anxiety behavior. Ongoing studies are aimed at understanding the interplay between behavioral state and activity within vHPC, utilizing pharmacological and behavioral manipulations to modulate anxiety levels of mice during exploration of the EZM while recording local population dynamics.

Conclusions: Our findings demonstrate a unique population-level activity signature for anxiogenic environments within vHPC. Further, the varying activity changes between innate and learned fear behaviors suggest diverse circuit mechanisms for processing exploration of an innately anxiogenic environment and previously conditioned fearful environments. Our results also reveal a potential circuit mechanism for increased population activity during innate anxiety behaviors, possibly through disinhibition of the local vCA1 circuit. The specificity of vHPC-LHA and vHPC-BMA terminal modulation effects on innate and learned fear behavior suggests a projection specific segregation in vHPC function, possibly mediated through projection-specific cell populations within the vHPC. This study provides a functional map of the cell-types and long-range circuits that underlie the vHPC contribution to innate and learned anxiety-related behavior.

Keywords: ventral hippocampus, calcium imaging, anxiety disorders, amygdala, hypothalamus

Disclosures: Nothing to disclose.

**T17. Attention Bias Modification Alters Amygdala-Cortical Functional Connectivity**

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Background: Novel cognitive treatments have been emerging. For example, attention bias modification (ABM) aims to reduce attentional threat bias and reduce anxiety symptoms (Hakamata et al., 2010). Understanding the neural correlates associated these changes in symptoms may help refine therapeutic strategies. Previous reports have indicated changes in amygdala activation with acute and extended ABM training, and that baseline amygdala activation and active ABM relative to placebo yield greater symptom reductions (Britton et al., 2015); however, changes in functional connectivity have yet to be examined. This study characterizes the functional connectivity between the amygdala and the prefrontal cortex (PFC) following ABM training relative to placebo training.

Methods: Socially anxious adults (18-30 years old, Liebowitz social anxiety scale ≥ 50) were randomized to receive either active ABM training or placebo training. Fifteen individuals in each group completed both acute (i.e., single session training) and extended training (i.e., twice weekly over a 4-week period). During all sessions, individuals indicated the probe (E or F) that appeared in one of two locations following threat-neutral or neutral-neutral face pairs. The placebo training involved the standard dot-probe task, where there was an equal likelihood of probes occurring in the location previously occupied by the threat face (i.e., congruent) or the neutral face (i.e., incongruent) across the task. The active ABM training task was designed to train participants to attend away from threat; therefore, all threat-neutral trials were incongruent. Using different face stimuli, attention biases were measured in the scanner before and after acute training and after extended training. Previously, Britton et al. (2015) reported results from analyses regarding changes in task-related activation. The current analyses of data from this prior report use an anatomical seed region to implement generalized psychophysiological interaction analysis. This analysis tested whole-brain Group (active, placebo) × Time(pre-training, following acute training, following extended training) × Condition(congruent, incongruent, neutral) interactions. The search territory was restricted to the prefrontal cortex using p < 0.005 and cluster size of 27 voxels, which corrects for multiple comparisons within the PFC.

Results: Group differences in functional connectivity between the amygdala and ventromedial prefrontal cortex were detected across time and condition [(-1, 36, -6), 29 voxels, F(4,112) = 3.99, p < .05 corrected]. To understand these differences, conditions used to calculate threat bias (i.e., incongruent vs. congruent) were compared. Before training, no group differences in connectivity were noted [both ps > 0.8]. After acute training, a group difference in amygdala-PFC connectivity to the incongruent relative to the congruent condition was noted [p < 0.04], with the active and placebo groups showing opposite patterns of connectivity. Within the attention bias contrast, the active group exhibited stronger amygdala-PFC connectivity to the incongruent condition [p < 0.05] and the placebo group exhibited a trend toward a stronger connectivity to the congruent condition [p < 0.07]. Following extended training, this pattern was reversed [p < 0.008]. Within the bias contrast, the active group exhibited stronger connectivity to the congruent condition [p < 0.2], while the placebo group exhibited a trend toward a stronger connectivity to the incongruent condition [p < 0.06].

Conclusions: In these preliminary analyses, acute and extended training alter the amygdala-PFC connectivity in different ways. Following acute training, the amygdala and PFC are more positively correlated in the incongruent relative to the congruent condition in the active group, while following extended training they are more negatively correlated. These results provide insight into mechanisms of change associated with ABM.

Keywords: fMRI, threat, anxiety

Disclosures: Nothing to disclose.

**T18. Increased Within-Network and Cross-Network Functional Connectivity in Returning Veterans with Posttraumatic Stress Disorder**

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Background: Posttraumatic stress disorder (PTSD) is characterized by disruptions in arousal/interoception, executive function, and sense of self. These functions are subserved by intrinsic brain connectivity networks, which are distributed, functionally coherent regions that interact...
to coordinate complex behavior and cognitive functions. The three key networks that coordinate the functions disrupted by PTSD are the salience network, the central executive network, and the default mode network. The salience network (anchored in dorsal anterior cingulate cortex, amygdala, and anterior insula) is responsible for detecting and orienting to salient stimuli. The central executive network (anchored in dorsolateral prefrontal cortex and lateral parietal cortex) is associated with goal-directed behavior and high level cognitive function, including planning, decision-making, and working memory. The default mode network (anchored in medial prefrontal cortex, posterior cingulate cortex and hippocampus) is associated with stimulus-independent, internally-focused thought and autobiographical memory. The salience network mediates between the central executive network and default mode network to maintain an adaptive balance between internal mentation and externally-oriented focus and task execution. PTSD symptoms have been linked to alterations in each of these networks. However, the interplay between these three critical networks has not yet been examined in patients with PTSD. Thus, the current study was designed to investigate patterns of connectivity within and between these three central intrinsic connectivity networks.

Methods: 19 OEF/OIF/OND combat veterans with chronic PTSD (Clinician-Administered PTSD Scale Score ≥ 50) and 14 combat-exposed healthy control veterans underwent 3T fMRI prior to initiating PTSD treatment as part of a comparative outcomes study for PTSD. A standard series of processing steps was performed using statistical parametric mapping. Based on previous research with the triple-network model, seed regions for salience network were anatomical anterior insula and amygdala. The seed region for the central executive network was a 10-mm-radius sphere placed in dorsolateral prefrontal cortex (MNI: 46, 6, 34). The seed regions for the default mode network were 10-mm-radius spheres placed in medial prefrontal cortex (-2, 48, -4) and posterior cingulate cortex (0, -56, 20). Functional connectivity analysis was performed using the ConndTool package. We extracted the spatially averaged time series from seed regions for each participant. Next, linear detrending was performed, followed by nuisance regression with motion regressors and five principal components of the BOLD time series extracted from white matter and cerebrospinal fluid masks. The residuals from this regression were then retained for further analysis. Since resting-state functional connectivity measures low-frequency spontaneous BOLD oscillations (0.01–0.10 Hz band), the time-course for each voxel was band-pass filtered in this range. Next, motion scrubbing was performed. Participants with more than 60% of their frames removed by scrubbing were excluded from further analysis. Pearson product-moment correlation coefficients were calculated between average time courses in the seed regions of interest (ROIs) and all other voxels of the brain, resulting in a 3-dimensional correlation coefficient image (r-image). These r-images were then transformed to z-scores using the Fisher r-to-z transformation. Z-score images from the individual activation maps were entered into second-level random-effects analyses implemented in SPM8.

Results: Veterans with PTSD demonstrated greater within-network salience network connectivity, as well as greater cross-network connectivity between central executive network seeds and salience network regions and between default mode network seeds and salience network regions. Specifically, the PTSD group demonstrated stronger connectivity than the control group between anterior insula (salience network) and anterior cingulate cortex (-3, 15, 28; Z = 3.69)(salience network), between dorsolateral prefrontal cortex (central executive network) and right amygdala (30, 5, -20; Z = 4.44)(salience network), between medial prefrontal cortex (default mode network) and anterior cingulate cortex (-9, 26, 25; Z = 3.55)(salience network), and between posterior cingulate cortex (default mode network) and anterior cingulate cortex (-15, 23, 25; Z = 4.51)(salience network).

Conclusions: Here we replicate previous findings in PTSD of increased within-network salience network connectivity and increased cross-network connectivity between salience network and default mode network. We extend these findings by demonstrating additional cross-network connectivity or desegregation between salience network and central executive network. Desegregation between these intrinsic connectivity networks may reflect sustained and likely inappropriate activation of salience network, which may negatively impact the adaptive balance between networks that is needed for appropriate cognitive resource allocation. This finding may reflect or help to explain sustained hypervigilance and hyperarousal in PTSD patients. These aberrant neural circuits may serve as targets for examination of change with treatment and future development of therapeutic interventions for PTSD.

Keywords: Posttraumatic stress disorder, Human Neuroimaging, Connectivity, Resting state, salience network

Disclosures: Nothing to disclose.

T19. Dentate Gyrus Controls Extinction of Contextual Fear Memory

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Background: Traumatic experiences produce long-lasting fear memories that can form the basis of anxiety disorders. The primary method for attenuating learned fear is extinction, a procedure in which the subject is re-exposed to conditioned stimuli in a safe environment. Much of the previous research about extinction learning has focused on simple fear conditioning paradigms involving tone-shock pairings, which mainly recruit subcortical learning mechanisms. Humans and other mammals, however, can acquire more complex fear memories using cortical mechanisms, but very little is known about how these more complex fear memories are extinguished. To study extinction of cortical fear memories, we used contextual fear conditioning, a hippocampus-dependent form of conditioning that occurs when an animal is placed into a distinctive environment (or context) and
given a footshock. Here we use optogenetic and pharmacogenetic methods to assess the neuroanatomical substrates of context fear acquisition, expression and extinction. Our studies focus on dentate gyrus (DG) because of previous work implicating DG in acquisition of context fear memory.

**Methods:** Fear conditioning was produced by placing mice into a conditioning chamber and administering a single footshock. Context fear memory was assessed by quantifying freezing behavior when mice were re-exposed to the chamber. To rapidly and reversibly manipulate neural activity during distinct phases of training, adeno-associated virus (AAV) was used to express optogenetic or pharmacogenetic neural actuators in the DG cell populations. The light-activated chloride pump halorhodopsin (eNpHR3.0) was used for optogenetic inhibition, and the GPCR Gs DREADD (rM3Ds) was used for pharmacogenetic excitation. Activation of rM3Ds via systemic injection of its ligand clozapine-N-oxide (CNO) stimulates cAMP production, leading to increased activation of dentate granule cells (DGCs). Neural activity in DG was silenced or enhanced (with eNpHR or rM3Ds, respectively) during acquisition, expression or extinction of context fear.

**Results:** Optogenetic inhibition of the dorsal DG during the context-shock pairing impaired context fear acquisition. Silencing the DG during repeated re-exposures to the context in the absence of shock did not alter fear expression but attenuated fear extinction, suggesting that neural activity in DG is required for acquisition and extinction of context fear but not for its expression. Increasing activity of DGCs during context-shock pairing impaired acquisition of context fear, similarly to DG inhibition. Increasing excitability of DGCs during context re-exposure after acquisition had no effect on expression of the fear memory but led to a significant reduction in freezing during a subsequent drug-free context test, suggesting enhanced extinction or impaired consolidation.

**Conclusions:** Our data suggest that extinction of context fear recruits neural circuits that are distinct from those controlling extinction of simple tone-shock associations. Whereas extinction of tone-shock pairings is mediated by plasticity in amygdala and prefrontal cortex, our studies identify DG as a critical locus for context fear extinction. Our data indicate that DG controls acquisition and extinction of context fear memory, but is not required for expression of learned fear or fear extinction. DG may provide a teaching signal that enables fear memory coding in a downstream structure such as CA3. In addition, our data suggest that enhancing activation of DG granule cells can facilitate the attenuation of learned context fear, either by enhancing extinction or preventing fear memory consolidation. In a clinical context, modulation of neural activity in DG may provide a strategy for facilitating extinction or preventing the consolidation of traumatic memories. In summary, our data identify the dorsal DG as a critical component of the neural circuitry mediating context fear extinction and as a potential therapeutic target for the treatment of emotional disorders related to aberrant fear learning.

**Keywords:** Fear extinction, dentate gyrus, Memory Consolidation and Extinction, Hippocampus, optogenetics

**Disclosures:** Nothing to disclose.

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**T20. PACAP Effect on Fertility in Female Mice is Relayed through Leptin Receptor Expressing Neurons of the Ventral Premammillary Nucleus and Central Ventromedial Nucleus of the Hypothalamus**

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**Background:** Leptin is known to play a critical role in control of metabolism and reproduction, but the mechanisms by which this occurs are not yet understood. Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuromodulator implicated in human anxiety, feeding and reproductive behavior. Though the hypothalamic-pituitary-gonadal axis is known to be important in the control of fertility and reproduction, the role of PACAP in this circuit has only been investigated through whole body manipulations, where PACAP knock out animals display decreased rates of reproduction. PACAP has been described to stimulate luteinizing hormone (LH) release directly at the level of the gonadotrophs in the pituitary and increase sensitivity to gonadotropin releasing hormone (GnRH), but the origin of the neuropeptide is not known. Centrally, high expression of PACAP is found in the ventral premammillary nucleus of the hypothalamus (PMV) and the ventromedial hypothalamus (VMH) both regions known to be involved in leptin-related control of puberty and fertility, though the role of PACAP has not been investigated there. We harness genetic tools that focus on PACAP neurocircuitry in leptin responsive neurons to investigate the role that central PACAP plays in leptin-driven metabolism and reproduction.

**Methods:** Taking advantage of cre-lox technology we created lox-PACAP mice that possess loxP sites flanking the 2nd exon of the PACAP gene, allowing for deletion of functional PACAP in the presence of cre-recombinase. The PACAP-lox mice were bred with LepRb-cre mice, which express cre-recombinase under control of the promoter for the long form of the leptin receptor (LepRb), which is restricted to the brain. We used dual-fluorescent immunohistochemistry and in situ hybridization to confirm colocalization of PACAP and LepRb. Mice were subjected to assays for energy homeostasis and fertility, and compared to littermate controls (PACAPfl/fl) using ANOVA.

**Results:** Conditional knock out females lacking PACAP in LepRb-expressing neurons show slight protection against body weight gain seen in control animals when subjected to high fat diet. They have significantly delayed puberty onset with longer alternating periods in diestrus and estrus and decreased litter size. There is no change in male body weight, onset of puberty, or fertility. Combined ISH/IHC shows LepR activity co-localizes with PACAP expression in the ventral premammillary nucleus and the central part of the ventromedial nucleus of the hypothalamus.

**Conclusions:** Deletion of PACAP from leptin receptor expressing neurons of the hypothalamus in female mice leads to similar fertility-related dysregulation seen in whole body PACAP knockouts, yet these changes are due to...
deletion of PACAP from two discrete regions of the hypothalamus known to be involved in HPG axis regulation. While previous work has shown that the effect of leptin on both metabolism and fertility is through GABAergic signaling, PACAP neurons are glutamatergic. These findings propose a new role for the PACAP-containing leptin-responsive neurons of the hypothalamus in signaling nutritional status for reproductive status, indicating that a subset of glutamatergic neurons may be involved in fine tuning leptin's action on reproductive function.

**Keywords:** metabolism, reproduction, PACAP, Leptin

**Disclosures:** Nothing to disclose.

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**T21. Adolescent Caffeine Consumption Enhances Anxiety-Related Behavior and Disrupts Neuroendocrine Signaling**

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**Background:** Caffeine is the most commonly used psychoactive substance worldwide, and consumption by children and adolescents has risen dramatically in recent years. Recent studies have found associations between energy drink use and anxiety in young adult males. Here, we examine the effects of adolescent caffeine consumption on anxiety-related behaviors and several neuroendocrine measures.

**Methods:** Beginning on postnatal day 28 (P28), Sprague-Dawley rats consumed caffeine (0.3 g/L) for 28 days (P28-55). Age-matched control rats consumed water. Following 28 days of caffeine consumption, the caffeine solution was replaced with water for the remainder of the experiment. Behavioral testing for anxiety-related behavior commenced at least 7 days after removal of caffeine (P62). Blood plasma levels of corticosterone (CORT) levels were assessed throughout the caffeine consumption procedure and at 24 hr and 7 days withdrawal from caffeine. Brain tissue was collected at 7 days withdrawal from caffeine to assess changes in basal and stress-induced c-fos and corticosterone releasing factor (CRF) mRNA expression throughout stress- and anxiety-related brain structures.

**Results:** Caffeine consumption in adolescent rats enhanced anxiety-related behavior as indicated by decreased center exploration in an open field, decreased social interaction with age-matched conspecifics, and decreased open arm exploration on an elevated plus maze. Analogous studies conducted in adults suggest that caffeine consumption in adulthood does not enhance anxiety-related behavior. Caffeine consumption increased plasma CORT after 24 hrs of caffeine consumption. Plasma CORT levels normalized after 14 and 28 days of chronic caffeine consumption suggesting the development of tolerance. Twenty-four hrs after the removal of caffeine, animals had elevated plasma CORT that remained elevated for 7 days. Exposure to an elevated pedestal (a mild stressor) elevated plasma CORT levels in control animals, but this was significantly blunted in animals that consumed caffeine during adolescence. Blunted CORT release to adrenocorticotropic hormone (ACTH) (1mg/kg, ip) was also observed in animals that consumed caffeine during adolescence. Adolescent caffeine consumption increased basal c-fos mRNA in the paraventricular nucleus of the hypothalamus. Stress increased c-fos mRNA in various stress reactive brain regions, but caffeine consumption had no effect on these stress-induced changes. Caffeine consumption during adolescence also produced a significant increase in CRF mRNA in the central nucleus of the amygdala, but no effects of stress or caffeine consumption were observed on CRF mRNA expression in other brain regions analyzed.

**Conclusions:** Together these findings suggest that adolescent caffeine consumption may increase vulnerability to psychiatric disorders including anxiety-related disorders, and this vulnerability may result from dysregulation of the neuroendocrine stress response system.

**Keywords:** Hypothalamic-Pituitary-Adrenal axis, Vulnerability, Adolescent Development, Adolescent Stress

**Disclosures:** Nothing to disclose.

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**T22. Fear Conditioning and Extinction in Pediatric Obsessive Compulsive Disorder**

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**Background:** Fear acquisition and extinction are central constructs in the cognitive-behavioral model of obsessive-compulsive disorder (OCD). Fear acquisition refers to the process by which a neutral stimulus (CS; e.g. a door handle) is associated with an aversive stimulus (US; e.g. the belief that the door handle is contaminated and will cause severe illness); this association leads to a conditioned fear response upon repeated exposure to the CS. Conversely, fear extinction is the process by which an individual decreases their emotional response to the CS by creating a new, positive association with that stimulus. Fear extinction is the foundation of first-line behavioral treatments for OCD, namely exposure with response prevention (ERP). Despite the centrality of fear learning processes in the acquisition, maintenance, and treatment of OCD, there have only been two studies of fear learning among adults with OCD, and none in youth with OCD. Studies comparing youth with anxiety disorders and healthy controls suggest that fear conditioning produces comparable fear learning in anxious and non-anxious youth during acquisition; however, results for fear extinction are less definitive. Behavioral and neurobiological research indicates that youth exhibit differential fear learning processes relative to adults. Furthermore, given that OCD typically onsets in childhood, and that a considerable number of youth with OCD exhibit inadequate or incomplete response to CBT, an examination of fear acquisition and extinction processes in youth with OCD is clinically relevant. The present study examined fear conditioning and extinction in youth with OCD and healthy controls using a novel computer-administered differential conditioning task. We hypothesized that, similar to findings from the adult OCD and child anxiety literature, youth with OCD would exhibit comparable physiological fear respon-
sivity to healthy controls during the fear acquisition phase. However, we hypothesized that youth with OCD would exhibit poorer fear extinction compared to healthy controls.

**Methods:** Eighty youth (39 OCD, 41 healthy controls) completed a battery of clinical interviews, rating scales, and a differential conditioning task. In this fear conditioning paradigm, participants learned to associate an aversive stimulus (US; a 95 decibel scream) with the paired conditioned stimulus (CS+), but not the unpaired conditioned stimulus (CS−). Two female faces served as the conditioned stimuli. The paradigm included three stages: habituation (4 presentations of each face without the US); acquisition (5 presentations of each face, with the US presented five seconds after the CS+); and extinction (8 presentations of each face without the US). Skin conductance response (SCR) to the presentation of stimuli was the primary dependent measure of fear.

**Results:** During habituation, participants with OCD produced significantly larger SC responses relative to HC participants and significantly higher orienting SC responses to initial stimulus presentation. During acquisition, despite a trend, there was no significant difference between youth with OCD and healthy controls in the acquisition of a fear-conditioned SCR and differential fear conditioning was observed for both groups as evidenced by larger SCRs to the CS+ compared to CS−. The absolute magnitude of the unconditioned fear response was significantly greater in youth with OCD compared to HC participants. During extinction, a three-way interaction test showed that youth with OCD had a different pattern of SCR over extinction trials, compared to the control group.

**Conclusions:** The present study is the first examination of fear conditioning and extinction in youth with OCD. During the acquisition phase, there were no significant group differences in fear-conditioned SCR; there was a trend towards overall larger SCRs in the OCD group, but this group difference did not reach statistical significance. During extinction, however, significant group difference were found such that healthy controls showed decreased SC over successive extinction trials, whereas youth with OCD showed no reduction in absolute SC responses at the end of the extinction phase. As hypothesized, these results indicate that youth with OCD and community controls are similar in their acquisition of a conditioned fear response, but differ in patterns of fear extinction. This poor extinction response suggests that youth with OCD may have deficits in inhibitory learning, which carries important implications for the success of ERP-CBT. While initial CBT models emphasize within-and-between session habituation as the central mechanism for therapeutic change, research suggests that inhibitory learning may be a key therapeutic component. Indeed, within-and-between session habituation in CBT has not been found to predict treatment outcome for youth with OCD. An inhibitory learning deficit may explain disparate exposure-based CBT outcomes among youth with OCD and offers preliminary support for consideration of CBT protocols that optimize inhibitory learning. Given these important clinical implications, it is crucial that research continue to explore the psychophysiological patterns of fear response among both youth and adults with OCD.

**Keywords:** OCD, Fear extinction, Fear conditioning, pediatric, CBT

**Disclosures:** Nothing to disclose.

**T23. Post-Traumatic Stress Avoidance is Attenuated by Corticosterone and Associated with Brain Levels of Steroid Receptor Co-Activator-1 in Rats**

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**Background:** Post-traumatic stress disorder (PTSD) manifests in a subset of individuals exposed to a traumatic stressor and is often accompanied by dysregulation of the hypothalamic-pituitary-adrenal (HPA) stress axis. Individuals with PTSD exhibit blunted HPA activity immediately after the traumatic event. Our laboratory has established a rodent model of stress that mimics the avoidance symptom cluster of PTSD. Animals are classified as ‘Avoiders’ or ‘Non-Avoiders’ post-stress based on avoidance of a predator-odor paired context. While the paraventricular nucleus (PVN) is essential for appropriate initiation and termination of the stress response, the limbic system can also influence the HPA axis. Limbic forebrain structures such as the central amygdala (CeA) and ventral hippocampus (VH) regulate HPA responses to emotional stress. Like the PVN, the CeA and VH are rich in glucocorticoid receptors (GR). The purpose of these studies was to 1) examine whether Avoider rats exhibited HPA hypofunction at the time of stress, 2) determine whether the HPA hypofunction is the result of inability to mount an HPA response or enhanced negative feedback, and 3) measure the expression of GR elements in the brains of Avoider rats because altered corticosterone levels may affect HPA feedback processes. We hypothesized that corticosterone levels are blunted in Avoider rats post-stress and administration of corticosterone prior to stress would increase HPA activity at the moment of the stressor and decrease the magnitude and incidence of avoidance of a predator odor-paired chamber. Furthermore, we hypothesized that predator odor stress would alter expression and/or phosphorylation of GR machinery such as FK506 binding protein (BP) 51 and steroid receptor coactivator (SRC)-1 in a brain region-specific manner in Avoiders relative to Non-Avoiders and unstressed Controls.

**Methods:** To test these hypotheses, male Wistar rats (300g) underwent a place conditioning procedure to assess avoidance of an odor-paired chamber. Rats were exposed to a neutral environment one day or to predator odor (bobcat urine) the next day paired with two distinct environments. Rats were indexed for avoidance of the predator-odor paired context. While the PVN, the CeA and VH are rich in glucocorticoid receptors (GR). The purpose of these studies was to 1) examine whether Avoider rats exhibited HPA hypofunction at the moment of the stressor and decrease the magnitude and incidence of avoidance of a predator odor-paired chamber. Furthermore, we hypothesized that predator odor stress would alter expression and/or phosphorylation of GR machinery such as FK506 binding protein (BP) 51 and steroid receptor coactivator (SRC)-1 in a brain region-specific manner in Avoiders relative to Non-Avoiders and unstressed Controls.

**Results:** During habituation, participants with OCD produced significantly larger SC responses relative to HC participants and significantly higher orienting SC responses to initial stimulus presentation. During acquisition, despite a trend, there was no significant difference between youth with OCD and healthy controls in the acquisition of a fear-conditioned SCR and differential fear conditioning was observed for both groups as evidenced by larger SCRs to the CS+ compared to CS−. The absolute magnitude of the unconditioned fear response was significantly greater in youth with OCD compared to HC participants. During extinction, a three-way interaction test showed that youth with OCD had a different pattern of SCR over extinction trials, compared to the control group.

**Conclusions:** The present study is the first examination of fear conditioning and extinction in youth with OCD. During the acquisition phase, there were no significant group differences in fear-conditioned SCR; there was a trend towards overall larger SCRs in the OCD group, but this group difference did not reach statistical significance. During extinction, however, significant group difference were found such that healthy controls showed decreased SC over successive extinction trials, whereas youth with OCD showed no reduction in absolute SC responses at the end of the extinction phase. As hypothesized, these results indicate that youth with OCD and community controls are similar in their acquisition of a conditioned fear response, but differ in patterns of fear extinction. This poor extinction response suggests that youth with OCD may have deficits in inhibitory learning, which carries important implications for the success of ERP-CBT. While initial CBT models emphasize within-and-between session habituation as the central mechanism for therapeutic change, research suggests that inhibitory learning may be a key therapeutic component. Indeed, within-and-between session habituation in CBT has not been found to predict treatment outcome for youth with OCD. An inhibitory learning deficit may explain disparate exposure-based CBT outcomes among youth with OCD and offers preliminary support for consideration of CBT protocols that optimize inhibitory learning. Given these important clinical implications, it is crucial that research continue to explore the psychophysiological patterns of fear response among both youth and adults with OCD.

**Keywords:** OCD, Fear extinction, Fear conditioning, pediatric, CBT

**Disclosures:** Nothing to disclose.
Results: Rats that exhibit persistent (> 6 weeks) avoidance of a predator odor-paired context exhibit significantly attenuated corticosterone levels immediately following traumatic stress and low corticosterone at the time of stress was highly predictive of later avoidance suggesting that HPA dysregulation may play a role in the subsequent emergence of PTSD symptoms. A lower percentage (25%) of rats pre-treated with corticosterone prior to stress were classified as Avoiders, relative to saline-treated rats (75% Avoiders). In the PVN, there were no differences in GR, pGR, or FKBP51 protein levels. There was also a trend (p = 0.06) toward a decrease in SRC-1 in Avoiders when compared to Non-Avoiders. PVN SRC-1 was strongly and negatively correlated with avoidance in all stressed rats. In the CeA, there were no differences in GR or pGR protein levels. There was a significant decrease in FKBP51 and SRC-1 protein content in Avoiders relative to Non-Avoiders. CeA SRC-1 content was trended to negatively correlated with avoidance in all stressed rats. In the VH, there were no differences in GR, pGR, or FKBP51 protein across groups post-stress. There was a significant increase in SRC-1 protein in Avoiders when compared to Control rats (p = 0.004) and Non-Avoiders (p = 0.02). VH SRC-1 content was strongly and positively correlated with avoidance in all stressed rats (r² = 0.54; p = 0.002).

Conclusions: These data suggest that low corticosterone levels in Avoiders post-stress reflect blunted HPA response to stress, although this does not preclude the possibility that negative feedback is also altered in Avoider rats. SRC-1, a protein involved in HPA negative feedback and corticosteroid releasing factor gene transcription was decreased in the PVN of Avoider rats. Changes in SRC-1, in particular, in the brain regions examined were highly predictive of later avoidance suggesting that the blunted HPA stress response in Avoider rats results from failure to mount and HPA response. One potential mechanism for this blunted HPA stress response is altered balance of excitatory and inhibitory inputs to parvocellular neurons in the PVN, a focus of ongoing studies.

Keywords: hypothalamic-pituitary-adrenal axis, posttraumatic stress, corticosterone

Disclosures: Dr. Gilpin is a consultant for Glauser Life Sciences, Inc.

T24. Psychostimulant-Induced Modulation of Prefrontal Cortical Activity, Early Sensory Signal Processing, and Behavioral Performance in Visually Guided Sustained Attention and Signal Detection Tasks

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Background: The prefrontal cortex (PFC) is responsible for multiple executive functions including the ability to attend to salient events. Catecholamine projections to the PFC play a prominent role in maintaining or shifting attention as dictated by changing behavioral contingencies. The psychostimulant drug methylphenidate (MPH-Ritalin) blocks reuptake of catecholamines and is used to treat ADHD as well as to improve attention and executive function in otherwise healthy individuals seeking the drug's cognitive enhancing effects. Prior work from this laboratory has shown that MPH improves rodent performance in a sustained attention task, a translational model for evaluating the potential efficacy of ADHD medications. The goal of the present study was to identify the cellular correlates of drug-induced and state dependent changes in performance of the aforementioned sustained attention task and a visually-guided signal detection task.

Methods: Fixed or driveable bundles of microwires were used to record the spike train activity of individual medial PFC neurons in rats performing a modified version of the McGaughy & Sarter (1995) sustained attention task. Other studies used the same approach to record local field potentials and multi-unit spike train activity in the dorsal lateral geniculate nucleus (dLGN) of anesthetized rats and rats engaged in a signal detection task. In all experiments neuronal activity was recorded before and after administration of MPH, 2.0 mg/kg i.p.

Results: In the sustained attention task single units (n = 104) recorded from PFC were classified as putative interneurons (n = 27, 26%), putative pyramidal neurons (n = 71, 68%), or unclassified (n = 6, 6%). 41% of putative interneurons and 58% of putative pyramidal neurons exhibited brief increases or decreases in firing rate associated with either sensory or motor events in the task. Neurons that displayed sensory responses (to signal light or lever extension) showed generally stronger responses in correct trials than in incorrect trials or omissions, consistent with the hypothesis that performance in this task is affected by the strength of representation of sensory information in the PFC. Following MPH administration basal firing rates for all recorded neurons were not systematically altered. However, MPH did have a consistent effect on sensory-responsive cells, causing a shift to shorter latencies and longer-duration responses. In a visual signal detection task, MPH enhanced the speed and strength of dLGN neuronal responses to light stimuli that were used as behavioral cues. MPH also improved reaction times of correct responses but the accuracy of signal detection in highly trained animals did not change. In anesthetized preparations analysis of LFP’s evoked by light stimuli revealed a decrease in both response latency and peak-to-peak amplitude. Cross correlations between multiple LFP channels within individual animals showed an increase in the strength of correlation following MPH. This increase in the correlation between locally summed potentials from multiple electrodes may be consistent with an increase in local synchrony of neural discharges. Lastly, segmented auto power spectral density analyses computed for progressive 5-minute intervals following injection of MPH revealed a shift from the prevalence of low frequency oscillations of 0-4 Hz to the clear emergence of two dominant bands at 4-6 Hz and 10-14 Hz.

Conclusions: The results of PFC experiments suggest that sustained attention task performance engages a subset of neurons distributed across the medial PFC, and that MPH...
effects on attention in the PFC are not represented by generalized excitability changes across the region, but rather by altered activity in cells displaying sensory cue related discharge. Overall, the results from dLGN experiments suggest a combination of single unit and local circuit mechanisms through which MPH can increase the efficacy of sub-cortical visual processing. Such effects on early sensory signal processing along with MPH-mediated facilitating effects in the PFC likely represent physiological substrates underlying the performance enhancing effects of MPH on sensory guided behaviors. Support provided by NIH NIDA R01 017960 to BDW and PhRMA Foundation Pre-Doctoral Award, NIDA F31DA037651 to RLN.

**Keywords:** prefrontal cortex, early sensory signal processing, methylphenidate, sustained attention, cognitive enhancement

**Disclosures:** Nothing to disclose.

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**T25. Brain Structure Changes in a 16p11.2 Deletion Mouse Model**

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**Background:** Animal models are powerful tools to study the molecular underpinnings of genes associated with psychiatric disorders. However, especially when studying psychiatric disorders, approaches that translate findings derived from these models to human brains become pivotal. Structural neuroimaging provides a viable path for translational research. We here report changes in grey matter and fiber-tract architecture in a 16p11.2 del/+ mouse model. 16p11.2 deletions are copy number variants (CNVs) exhibiting a high association with autism spectrum disorders (ASDs). Since ASDs show a strong sex-bias with males about four times more often affected than females, we also investigated sex-specific effects of the genotype.

**Methods:** We performed magnetic resonance imaging (MRI), including a diffusion weighed sequence, ex vivo. Mice were sacrificed at 70-days of age. Age/sex matched wild type and 16p11 del/+ mice (wildtype male = 7, del/+ male = 4, wildtype female = 5, del/+ female = 5). MR images were analyzed using Voxel-Based Morphometry for T1-weighted sequences and FSL tools (http://www.fmri-box.ac.uk/fsl/) for the DTI data sets. We used a modified version for the post-mortem mouse brain of these standard protocols.

**Results:** We did not find any significant changes in Grey Matter. DTI revealed pronounced decreases in fractional anisotropy, indicative of decreased white matter integrity, in fiber tracts that comprise the corpus callosum and external capsule in del/+ animals regardless of sex. Notably, male del/+ animals also showed a pattern of increased white matter integrity in fiber tracts medial and ventral to the dorsal striatum adjacent to the lateral ventricles, including the internal capsule and early fornix.

**Conclusions:** Changes of striatal neuroanatomy have been previously described in 16p11 del/+ mice, while sex-specific effects have not yet been elucidated. Our findings of sex-specific fiber tract changes point to striatal deficits and match behavioral and molecular changes found specifically in male 16p11 del/+ mice.

**Keywords:** 16p11 del/+, autism, CNV, endophenotype, genetic mouse model, MRI

**Disclosures:** Nothing to disclose.

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**T26. The Brain-Derived Neurotrophic Factor Val66Met Polymorphism is Associated with Altered Amygdala-Cortical Structural Covariance in Adolescence**

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**Background:** The brain-derived neurotrophic factor (BDNF) Val66Met polymorphism effects plasticity related processes in the brain and may predict risk for mood and anxiety disorders. The purpose of this study was to investigate whether variation in this gene is related to sex-specific development of amygdala-based networks during childhood and adolescence.

**Methods:** Magnetic resonance images were analyzed in 339 Caucasian youths ages 8-21 studied as part of the Philadelphia Neurodevelopmental Cohort. Amygdala volumes and cortical thickness were computed with MAGeT and CIVET automated processing pipelines respectively. Amygdala-cortical interactions were assessed by examining covariance of amygdala volumes with thickness throughout the cortex. BDNF genotype specific differences in resting state functional connectivity were examined where significantly different structural relationships were found.

**Results:** In adolescents ages 12-17 amygdala-cortical covariance was significantly different between Met allele carrier and individuals homozygous for the Val allele in the insula, subgenual cingulate, cuneus, middle temporal gyrus and precuneus (Ps < 0.05, FDR corrected). In Met allele carriers amygdala volume was positively associated with cortical thickness whereas negative associations were found in Val allele homozygotes. These differences were driven by females, where amygdala-cortical covariance was significantly different between genotypes in similar cortical regions as well as in the association between the amygdala and dorsal lateral frontal cortex (Ps < 0.05, FDR corrected). In Met allele carriers stronger corresponding resting state functional connectivity between the amygdala and the subgenual cingulate, the insula and the middle temporal gyrus was found.

**Conclusions:** The timing of a sex-specific influence of the BDNF Val66Met polymorphism on amygdala-cortical networks in adolescence coincides with the high-risk phase of development, when onset of depression occurs, more commonly in females. These findings suggest that coordinated development of the amygdala with the cortex is influenced by BDNF genotype in a sex-specific manner, and provides a potential genetic mechanism of neural
susceptibility for depression and related illnesses during adolescence.

**Keywords:** adolescence, imaging genetics, amygdala-based networks, resting state functional connectivity, structural covariance

**Disclosures:** Nothing to disclose.

T27. Cortical Inhibitory Deficits and Suicidality in Children and Adolescents

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**Background:** Suicide is a significant public health problem and is associated with many major mental illnesses, including major depressive disorder (MDD). In particular, suicide is a leading cause of death in adolescents and young adults. Abnormalities in gamma-aminobutyric acid (GABA), the brain’s major inhibitory neurotransmitter, have been shown in previous studies of suicidal adults. The assessment of suicidal risk is challenging, and objective biological markers have the potential to help identify individuals at high risk and to inform clinical decision-making. The current study aimed to examine the relationship between a transcranial magnetic stimulation (TMS) index of cortical inhibition and suicidality in a child and adolescent population. To our knowledge, no prior studies have examined TMS correlates of suicidality.

**Methods:** Forty-four medication-naïve children and adolescents (22 with MDD and 22 healthy controls) completed clinical assessments and TMS testing. Measures of suicidality were drawn from items on the clinician-rated Children’s Depression Rating Scale-Revised (CDRS-R) and adolescent self-report Quick Inventory of Depressive Symptomatology (QIDS-A17-SR). Two composite scores were created: a suicidal ideation score (a scaled mean of CDRS-R and QIDS-A17-SR items pertaining to suicidal thoughts) and an overall suicidality score (a scaled mean of items rating both suicidal and morbid ideation). Single-pulse TMS was applied to the primary motor cortex in both hemispheres at 140% of the resting motor threshold. The cortical silent period (CSP), a TMS index of GABA-B-mediated inhibitory neurotransmission, was measured via surface electromyography in the contralateral abductor pollicis brevis. Relationships between CSP duration and suicidality measures were assessed with the Spearman correlation coefficient (ρ).

**Results:** Inverse relationships were observed between CSP duration and suicidal ideation score, which reached significance in the right hemisphere (ρ = -0.382, p = 0.010) but not in the left hemisphere. The CSP duration also showed a significant negative correlation with the overall suicidality score (ρ = -0.435, p = 0.003) in the right hemisphere but not in the left hemisphere.

**Conclusions:** In this exploratory analysis of a pediatric sample, measures of suicidality were associated with TMS-measured GABAergic dysfunction in the right hemisphere. TMS measures of cortical inhibition show promise as potential biomarkers of suicidal risk.


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**Background:** Growing evidence suggests that early life stress (ELS) in humans leads to life-long changes in connectivity between limbic and cortical regions, with consequential emotional and cognitive deficits. Specifically, ELS-exposed children display a premature emergence of negative functional connectivity between the amygdala and the prefrontal cortex. In the rat, maternal separation ELS reportedly results in accelerated amygdaloid structural maturation. Therefore, it is likely that anxiety-associated dysfunction after ELS is due to aberrant maturation of...
corticolimbic circuitry. However, this hypothesis has not yet been directly tested in an animal model. Manganese-enhanced MRI (MEMRI) utilizes manganese (Mn), a metal that, when injected systemically, enters only excitable neurons through voltage-gated calcium channels to then be transported trans-synaptically. MEMRI demonstrates excellent agreement with fMRI maps of neural representations and has emerged as an effective technique to assess differential circuit activity in response to stimuli in freely moving animals. Here, we compared Mn uptake in ELS-exposed versus control-reared rats to measure the differential effect of stress exposure on corticolimbic activity during early adolescence (i.e., puberty). Because Mn is only taken up in excitable neurons, we measured stress-induced activation in freely moving animals by subjecting animals to a stressful stimulus after Mn administration, and later imaging with MRI.

Methods: Male Sprague-Dawley rats were separated from their mother and littermates for 4-h per day between postnatal days 2-20. Control subjects were left undisturbed except for cage cleaning. On postnatal day 30, variable TR images were acquired under isofluorane anesthesia using RARE pulse sequence (TE=12.5 and TR: 460, 900, 1400, 2800, 6000 msec.) Images were acquired with a 3 cm2 field of view, data matrix = 128 x 128 x 20 slices, thickness = 1 mm. T1 measurements were computed by fitting absolute signal at particular TR. Rats were then administered two daily subcutaneous injections (10 mg/mL) of 75mg/kg MnCl2 tetrahydrate. Beginning the following day, subjects were exposed to four daily stressors, alternating between 15-min exposure to ferret odor (days 1 and 3) and 30-min restraint (days 2 and 4). Rats were recorded during ferret odor exposure for subsequent analysis of fear and defensive behaviors. Twenty-four hours following the last stressor, all subjects underwent a post-stress scan. Each subject was registered to a 3D segmented and annotated rat brain atlas. Differences between pre- and post-stress T1 relaxation times for each region were calculated and compared between groups using unpaired t-tests.

Results: All subjects reached puberty as assessed by preputial separation between postnatal days 30-33, signifying that the age of our subjects correlated with early adolescence. Compared to control-reared subjects, early adolescent rats exposed to maternal separation displayed lower stress-induced Mn uptake in the orbitofrontal cortex (t(8)=2.650; p = 0.029), and higher stress-induced uptake in the intercalated amygdaloid nucleus (t(10)=2.68; p = 0.023), the lateral posterior thalamic nucleus (t(9)=2.43; p = 0.038), and the raphe obscurus nucleus (t(9)=2.87; p = 0.019). ELS-exposed subjects also displayed significantly less burying behavior during their first ferret odor exposure, compared to control-reared adolescents (t(8)=3.56; p = 0.007).

Conclusions: These data illustrated for the first time in an animal model that maternal separation ELS leads to stress-induced activity changes throughout a corticolimbic circuit. Findings of opposing activity changes in the amygdala and prefrontal cortex in response to stress exposure during early adolescence support clinical evidence that ELS causes a prematurely negative functional connectivity between the amygdala and the PFC. The unexpected observation of higher Mn uptake in the raphe obscurus of ELS subjects also may underscore the likelihood that ELS alters stress effects on gastrointestinal motility. Together, these findings point to circuit-wide changes that are evident in adolescence and corroborate clinical reports of aberrant corticolimbic maturation and stress responsivity after ELS. Further investigation into the developmental trajectory of these differences is currently underway.

Keywords: maternal separation, MRI, amygdala, prefrontal cortex

Disclosures: Dr. Craig Ferris has a financial interest in Animal Imaging Research, the company that manufactures the rat RF coil system used in this study.

T29. Preliminary Evidence for Computer-based Training Targeting Hostile Interpretation Bias as a Treatment for DMDD

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Background: Our objective was to assess the potential of treating Disruptive Mood Dysregulation Disorder (DMDD) in children by targeting hostile interpretation bias (HIB) with computer-based training. HIB refers to a bias towards interpreting ambiguous social cues, such as face-emotions, as hostile. Relative to youths without psychopathology, youths with DMDD misjudge face-emotions, have an attentional bias towards threatening facial expressions, and may also be biased towards judging ambiguous facial expressions as hostile. The latter is consistent with HIB and may contribute to irritability in youths with DMDD by encouraging in-kind reactions to presumed hostility.

A recent report suggests that youths with conduct problems become less aggressive in response to a computer-based treatment targeting a tendency to judge ambiguous faces as angry rather than happy. We conducted three experiments to explore the application of this treatment to DMDD. We tested: 1) whether youths with DMDD express a HIB towards ambiguous face-emotions; 2) whether judgment of ambiguous faces can be altered in healthy youths by training; and 3) whether such training in youth with DMDD is associated with reduced irritability and associated changes in brain function.

Methods: In all experiments, participants made forced-choice happy vs. angry judgments of 15 randomly presented face-emotion stimuli that varied on a morph continuum of happy to angry. These judgments were used to calculate a “balance point,” the facial expression at which a participant’s judgment switches from predominantly happy to predominantly angry. For the first experiment, we compared balance point in youth with DMDD (n = 63) vs. healthy youths (n = 26). For the second experiment, this assessment task was converted to a 20 minute training task by adding feedback designed to shift the balance point towards more happy judgments of ambiguous faces. The training program was completion of four once-daily training tasks over 4 to 5 days. We then conducted a
double-blind, randomized controlled trial of active versus sham balance-point training in 19 healthy youths. For the third experiment, we piloted four sessions of open, active balance-point training in 14 youths with DMDD. Outcome measures were: 1) improvement in DMDD symptoms measured by the Clinical Global Impressions-Improvement (CGI-I) and 2) irritability measured by the Affective Reactivity Index (ARI).

Ten youths in the open pilot trial also completed an implicit fMRI face-emotion processing task before their first training session and after their last training session. During fMRI, they judged the gender of face stimuli with subtle face-emotions. Stimuli were 30 morphs each of 50% angry, happy or fearful face-emotions morphed with 50% neutral face-emotion. These were randomly presented for 2s each with a jittered fixation cross between them. Images were processed with standard AFNI procedures to estimate subtle face-emotion-associated neural activity in lateral orbito-frontal and amygdala regions of interest.

Results: Relative to healthy youths, DMDD youths tend to classify ambiguous faces as angry rather than happy, reflected in a balance point shift (t(87) = 2.39, p = 0.02, Cohen’s d = 0.51). In both healthy and DMDD youths, four days of active training is associated with a shift in balance point towards more happy judgments (p’s < 0.001). These shifts persisted for two weeks without further training (p’s < 0.003). In the open trial, DMDD-symptoms were slightly improved immediately post training (CGI-I M(SD) = 4.4(1.1), t(13) = 2.2, p = 0.044, d = 0.59) and improved one week after training (CGI-I M(SD) = 3.5(1.3), t(13) = 4.4, p < 0.001, d = 1.17). Parent’s ratings of the child’s irritability on the ARI decreased immediately after training by b(SE) = -1.57(0.64) ARI points, p = 0.017. These reductions persisted 1 and 2 weeks after training, b(SE) = -1.50(0.64) and -2.41(0.65) points, respectively, p’s ≤ 0.023.

After training, neural activation to subtle (i.e., 50%) expressions of happiness increased relative to subtle expressions of anger in right lateral OFC, b(SE) = 0.19(0.08), p = 0.021, and left lateral OFC, b(SE) = 0.21(0.08), p = 0.009, with a trend in the left amygdala b(SE) = 0.15(0.08), p = 0.072.

Conclusions: Three experiments provide the foundation for a controlled trial of interpretation-bias training treatment in irritable youths. Experiment 1 shows that DMDD youths rate ambiguous face emotions in a way that is consistent with a hostile interpretation bias. Experiments 2 and 3 show that daily computer-based training can shift such ratings toward happy judgments and away from angry judgments, and that this shift persists for two weeks without further training. Finally, in Experiment 3, four sessions of daily training in youth with DMDD was associated with reduced irritability and possibly altered lateral OFC function in response to subtle expressions of happiness vs. anger. These results support the need for further research on computer-based treatment targeting interpretation bias of angry faces in DMDD. Such treatment may decrease irritability and change neural systems underlying the evaluation of hostile intent.

Keywords: disruptive mood dysregulation disorder, interpretation bias, irritability

Disclosures: MRM and IPV are co-directors of Jericoe Ltd, which produces software for the assessment and modification of emotion recognition. All other authors have no conflicts of interest or outside financial support to report.
to collect 50 rewards within 30 minutes. Following completion of the FR1 training, required response number was increased to determine breakpoints. Mice performed PR twice under two conditions, a valued state (the reward had incentive value) and a devalued state (mice were allowed to free feed on reward for an hour before starting the progressive ratio task).

**Results:** In the 5-CSRTT, the DAT Val559 mice acquired the task faster than their WT counterparts (Genotype $P = 0.014$, Time $P = 0.0001$, Interaction $P = 0.0001$; Two-Way RM-ANOVA; $WT = 18$, HOM = 19). At baseline, performance of DAT Val559 mice and WT in the task was not statistically different. Increasing the delay time, however, induced more premature responses in the DAT Val559 mice ($P = 0.029$, two-tailed Student’s t-test, $WT = 15$, HOM = 19). Interestingly, under the variable delay, the opposite phenotype was observed; WT mice made more premature responses ($P = 0.044$; two-tailed Student’s t-test; $WT = 15$, HOM = 19). On the PR task in the valued state, DAT Val559 mice had a significantly higher breakpoint ($P = 0.048$; two-tailed Student’s t-test; $WT = 11$, HOM = 9). When in the devalued state, DAT Val559 trended towards having a higher breakpoint with significantly more head entries into the reward magazine ($P = 0.024$; two-tailed Student’s t-test; $WT = 11$, HOM = 9).

**Conclusions:** The alteration in performance on the 5-CSRTT arising with changes in temporal delays suggest that the DAT Val559 mice may possess an inability to appropriately time the length of delays and that under conditions where time is not a key determinant of successful outcome, these animals are actually at an advantage, whereas when the ability to accurately time a response is critical, this trait manifests as impulsivity. Our results track with several recent studies indicating both the importance of the DA system in interval timing (Hayton et al. 2012; Bussi et al. 2014) and the hypothesis that subjects with ADHD may possess a timing deficit (Rommelse et al. 2007). DAT Val559 mice also demonstrated an increased breakpoint on the PR task, suggesting higher motivation for an extrinsic reward. This finding may explain the faster rate of learning seen with DAT Val559 mice in the 5-CSRTT, referencing a stronger state of motivation or reward-driven hyperfocus, possibly also relevant to the continued effort exerted by DAT Val559 mice in the PR task after rewards were devalued. Future studies will explore the ability of these animals to integrate negative reinforcers into their decision-making. Together, these studies add to prior findings demonstrating that DAT-mediated DA efflux can alter behavior and psychostimulant responses, supporting altered control of DAT function as a risk factor for ADHD.

**Keywords:** Dopamine, ADHD, impulsivity, reinforcement, dopamine transporter

**Disclosures:** Nothing to disclose.

**Background:** Children with neurodevelopmental disorders (NDDs), including attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD) and pediatric obsessive compulsive disorder (OCD) exhibit considerable phenotypic and genotypic overlap and common alterations in brain structure and function, relative to controls. However, most studies compare only one of these NDD groups with healthy controls. Here, we expand on the conventional case-control neuroimaging approach to examine common and disease-specific white matter markers present in a large sample of children with ADHD, OCD, or ASD.

**Methods:** Diffusion imaging and behavioural measures were acquired in 234 children (ADHD: $n = 47$, Mean age $10.3 \pm 1.8$; OCD: $n = 39$, Mean age $12.6 \pm 2.6$; ASD: $n = 84$, Mean age $11.4 \pm 3.4$; controls, $n = 64$, Mean age $10.8 \pm 2.8$). Voxel-wise comparisons of white matter fractional anisotropy (FA) were conducted: 1) between each NDD subgroup versus healthy controls, and 2) between pairs of NDD subgroups. Brain-behavior analyses examining relationships between white matter FA and measures of continuous behavioural symptoms exhibited across children with ADHD, OCD and ASD (measured with the Child Behavior Checklist Attention Problems Subscale, The Social Communication Questionnaire, Toronto Obsessive Compulsive Scale and Adaptive Behavior Assessment System-II General Adaptive Composite) were carried out in our overall NDD sample. Results significant following multiple comparison correction are reported.

**Results:** We found: 1) diffuse reductions in white matter FA in children with ADHD and ASD versus controls and in OCD versus ASD, 2) no white matter differences in OCD versus controls or ADHD versus ASD, and 3) reductions in frontal, striatal and thalamic white matter FA in ADHD versus OCD that largely corresponded to white matter tracts linking frontal-striato-thalamic circuitry. On brain-behavior analyses we found: 1) positive relationships between white matter FA and general adaptive functioning across children with ADHD and ASD versus controls or ADHD versus ASD, and 2) reduced positive relationships between white matter FA and attention problems and obsessive compulsive symptoms across children with either ADHD or OCD.

**Conclusions:** Our findings identify significant differences in white matter structure in children with ADHD versus OCD within a fronto-striato-thalamic circuit that has been implicated in both disorders. Follow-up analyses indicate that opposing structural white matter properties within this fronto-striato-thalamic circuit predicts levels of attention problems and obsessive compulsive symptoms across children with ADHD and OCD. The transdiagnostic value of our work was additionally confirmed based on relationships between brain white matter FA with general everyday functioning that cut across...
diagnostic boundaries. Taken together, our results point to novel white matter markers underlying distinct and common aspects of brain connectivity in children with NDDs and may be useful for biologically informed subtyping in NDDs and monitoring biological markers of treatment response.

**Keywords:** Diffusion tensor imaging, Neurodevelopmental Disorders, brain connectivity

**Disclosures:** Nothing to disclose.

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T33. Male-Specific Reward Learning Deficits in a Mouse Model of Autism

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**Background:** Autism spectrum disorders (ASD) are highly sex biased, presenting in 4.5 males for every female. The reasons for this sex bias are completely unknown, but epidemiological findings indicate that this male vulnerability is related to an increased risk of developing ASD in response to genetic lesions. Autism spectrum disorders (ASD) are characterized by disruptions in behavior including restricted interests, repetitive behaviors, and difficulties performing appropriate social interactions. Several theories integrating these disparate systems have implicated mechanisms of reward, motivation, and action-outcome prediction. Dysfunction in the structure and activity of striatal circuitry, which regulates these cognitive domains, have been strongly implicated in the pathophysiology of autism. Striatal function is known to be regulated differently in males and females, suggesting that sex differences in reward or motivation may underlie differential susceptibility to ASD by genetic lesions. To test these ideas, we have tested motivation and reward-based learning in 16p11.2del/+ mice, which model one of the most common copy number variations associated with ASD.

**Methods:** A mouse model of 16p11.2 hemideletion on a mixed 129/b6 background (the chr7qF3 deficient mouse) has previously been generated (Horev et al, PNAS 2011). Adult male and female 16p11.2del/+ mice were tested for operant learning and motivation using mouse operant 9-hole chambers with a sweetened liquid reinforcer. Additional cohorts of animals were sacrificed to examine gene expression and protein phosphorylation in the striatum.

**Results:** Compared to wildtype mice, 16p11.2del/+ male, but not female, mice have early impairments in the ability to associate a response with a reward as measured by Fixed Ratio responding. Once fully trained on the fixed ratio task, animals were probed with a Progressive Ratio schedule, which assesses motivation to continue responding as task demands become more challenging. 16p11.2del/+ males demonstrated significantly reduced progressive ratio responding, indicating deficits in motivation to seek reward, while female 16p11.2del/+ were unaffected. Importantly, sucrose preference when given freely in home cage was unaltered in 16p11.2del/+ mice, indicating that mechanisms of reward preference are unaffected. Gene expression analysis in male and female del/+ revealed that nearly all genes in the 16p11.2 homolog region are expressed in the striatum, and all these genes are reduced in expression 50% in both sexes. However, male del/+ animals showed selective upregulation of dopamine receptor 2 and adenosine receptor 2a in striatum, while female del/+ did not differ from wildtypes, suggesting that signaling mechanisms regulating medium spiny neuron development are more impacted in del/+ males. The 16p11.2 region contains the essential neuronal kinase ERK1 (mapk3), which has been shown to specifically reduce neuronal plasticity in the striatum. In response to a brief consumption of sucrose, del/+ males show hyperphosphorylation of ERK1 in the striatum, relative to smaller increases in ERK1 phosphorylation in wildtype males following sucrose, while females of either genotype do not show any activation of ERK1 following sucrose.

**Conclusions:** These data indicate that fundamental mechanisms of goal-directed behavior are altered in 16p11.2del/+ male mice while sparing females. These findings provide strong support for the idea that sex differences in reward and motivation are involved in the pathophysiology of ASD.

**Keywords:** sex differences, reward system, striatum, autism Spectrum Disorders

**Disclosures:** Nothing to disclose.

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T34. State Matters? Intrinsic Brain Function in Children with Autism Awake and Asleep

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**Background:** Increasingly, functional magnetic resonance imaging (fMRI) studies in autism spectrum disorders (ASD) converge on aberrant brain connectivity. Understanding the pathophysiology of such abnormalities in an early onset disorder like ASD requires investigations at the earliest possible ages. Nevertheless, for pragmatic reasons, most fMRI studies have been conducted in adults and late school-age children. Recently, a few groups have begun to use fMRI during natural sleep in typical children and in children with ASD. The underlying assumption of these sleep fMRI studies is that findings obtained during sleep will generalize to wakefulness, but this has not been tested in typical children or in children with ASD. Addressing this gap will enable leveraging the power of imaging methods to study early development of brain function. Thus, as a first step, we aimed to provide an initial characterization of whole-brain intrinsic functional connectivity (fIC) across awake and sleep states in children with ASD.

**Methods:** Twenty-two well-characterized children with DSM-5 ASD diagnosis (18 males, age: 6.8 ± 0.8 years) completed two separate resting state fMRI scan sessions (~15 days apart): one while awake, the other during natural sleep. Following standard preprocessing, R-fMRI data were registered to a 2mm standard template. Nuisance regression included the 24-Friston motion parameters, CSF and white matter. We assessed four whole-brain R-fMRI measures that
capture distinct complementary aspects of the functional connectome. All have been previously found abnormal in ASD. These include fractional amplitude of low frequency (fALFF), regional homogeneity (ReHo), voxel mirror homotopic connectivity, and functional connectivity of nine independent components (IC) obtained with independent component analysis (ICA) and dual regression. For these measures we first quantified between-state differences (paired t-test, covariates: age, mean framewise displacement and subject means for each R-fMRI index). We then computed test-retest reliability between states (intraclass correlation coefficient, ICC) using linear mixed effects model covarying for age, meanFD, and subject means for each R-fMRI index within subjects; and for sex between subjects. All analyses were statistically corrected for multiple comparisons.

Results: State (i.e., sleep/awake) affects functional brain architecture in a regionally specific pattern. Consistent with studies in typical adults, state affected mostly subcortical areas (thalamus), as well as motor and sensory (parietal and occipital) cortex. For example, greater cortico-cortical and lower cortico-subcortical iFC characterized wakefulness. Higher order regions such as those included in the default mode and fronto-parietal networks did not differ significantly across states. Between state reliability varied from moderate to high; with higher ICC in default mode network regions, occipital pole and medial occipital cortex. ICC varied also as a function of R-fMRI index, being greater for ReHo and lower for fALFF.

Conclusions: This is the first analysis of intrinsic brain function measured in young children with ASD during natural sleep as well as while awake. Despite expected state differences, numerous intrinsic functional properties remain stable across states in children with ASD. Overall, our findings are encouraging for future efforts to examine individuals longitudinally from early life (during natural sleep) to late childhood and adulthood (when scanning while awake is more practical).

Keywords: Autism, resting state fMRI, sleep, Large Scale Networks

Disclosures: Nothing to disclose.

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T35. Identifying Neural Targets of Antidepressant Treatment in Adolescent Depression

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Background: Depressive disorders are common in adolescents, and are associated with high risk of recurrence, comorbidity, future disability, and suicide. Because significant maturational change takes place during adolescence, neural mechanisms of depression and treatment response may differ from adults. Currently, only 60% of adolescents with depression respond to standard antidepressant treatments. It is very challenging to conceptualize and test new treatments for adolescent non-responders to these first-line treatments because we currently do not know the specific brain changes that are required to relieve depression. Research is critically needed to identify the neural targets of standard treatments (the specific treatment-induced changes underlying the response to these treatments) for adolescent depression that could be used in testing of new interventions for treatment-resistant depression.

Methods: Fourteen adolescents with major depressive disorder aged 12-19 participated in a study in which neuroimaging and clinical data were collected before and after the adolescents received 8 weeks of antidepressant treatment from their doctor. Baseline clinical assessment included a comprehensive clinical interview by a trained clinician with the adolescent and parent, and adolescents completed the Beck Depression Inventory-II (BDI-II). The neuroimaging protocol included resting-state fMRI and an fMRI task in which participants matched either faces with negative emotional expressions or neutral shapes. Adolescents repeated the BDI-II at the second, post-treatment scan. A dimensional treatment response variable was calculated by subtracting the pre-treatment BDI-II score from the post-treatment BDI-II score. Resting-state fMRI analyses focused on resting-state functional connectivity (RSFC) of the amygdala. We conducted a whole-brain regression at each voxel using the average time series within anatomically-based amygdala regions of interest for each person, yielding individual-level amygdala RSFC maps for each scan. For the emotion task, whole-brain regression analyses were conducted for each individual using the emotion and shape regressors. Neural change was calculated by conducting a whole-brain subtraction of the baseline neural circuitry data (amygdala RSFC maps and brain activation to negative emotion) from the post-treatment results. Finally, we conducted whole-brain regression analyses to identify the relationship between treatment response and the indices of neural change (separate analyses for amygdala RSFC and emotion task change results). Whole-brain correction for multiple comparisons was conducted using AlphaSim using individual voxel p-threshold 0.005 to identify the cluster size to achieve a whole-brain cluster significance of $\alpha = 0.05$.

Results: Treatment response (the degree of reduction of depression symptoms) was related to decrease in amygdala RSFC with the precuneus and with the mid-cingulate cortex, and an increase in amygdala RSFC with the right inferior frontal cortex. For the task fMRI data, a greater treatment response was significantly related to a greater decrease in activation in response to negative emotion over time within the anterior cingulate cortex, the precuneus, and the caudate nucleus.

Conclusions: This pilot neuroimaging study suggested that change in connectivity and activation of key neural circuits involving amygdala, anterior cingulate and precuneus are strongly related to the degree of treatment response. Therefore, these measures could potentially serve as neural targets for treatment response. Research in larger samples is required to confirm these findings. Once this information is established, the next step is to test whether other treatments (either other protocols of existing treatments, combined treatments, or novel interventions) can impact the targets and make the necessary changes to achieve a treatment response in adolescents who are resistant to standard treatments.
**Keywords:** Adolescent Depression, neural circuitry, Anti-depressant, amygdala, precuneus

**Disclosures:** Nothing to disclose.

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**T36. Atypical Neuroanatomy and Intrinsic Functional Connectivity as an Intermediate Imaging Phenotype for Autism Spectrum Disorder**

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**Background:** Autism spectrum disorder (ASD) is a highly heritable neurodevelopmental disorder, yet the search for genes with a definitive role in its etiology has remained elusive. Deconstructing the disorder with endophenotypic approach should boost the statistical power of genetic studies and clarify the pathophysiology of autism. We aimed to test for intermediate imaging phenotypes of neuroanatomy and its intrinsic functional connectivity (iFC) in males with ASD and their unaffected brothers, as compared to typically developing (TD) males.

**Methods:** We assessed 20 males with ASD, 20 unaffected brothers, and 54 TD males (94, aged 9-19 years, in total) with clinical evaluation, and undertook structural and resting-state functional MRI scans. Voxel-based morphometry was performed to obtain regional gray and white matter volumes. General linear analyses of the volumes of brain regions, adjusting for age, full-scale IQ and tissue specific volume, were formed to obtain regional gray and white matter volumes. Functional MRI scans. Voxel-based morphometry was performed to test for intermediate imaging phenotypes of neuroanatomy and its intrinsic functional connectivity (iFC) in males with ASD and their unaffected brothers, as compared to typically developing (TD) males.

**Results:** We found abnormal neuroanatomy in gray matter volume in the mid-cingulum, and white matter volume in the left superior corona radiata were shared between males with ASD and their unaffected brothers as compared to the TD males. Moreover, reduced connectivity between the mid-cingulum and right inferior frontal gyrus, and increased iFC between the mid-cingulum and bilateral medial occipital gyrus were shared among males with autism and their unaffected brothers.

**Conclusions:** Our findings suggest that atypical neuroanatomy and its associated iFC may be an intermediate imaging phenotype for ASD.

**Keywords:** autism spectrum disorder, neuroanatomy, Resting State Functional Connectivity, endophenotype

**Disclosures:** Nothing to disclose.

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**T37. Changes in Clinical Severity, Social and Cognitive Abilities of Ten Patients with Rett Syndrome Treated with IGF-1 in an Open Label Trial**

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**Background:** Rett syndrome (RTT) is a devastating neurodevelopmental disorder that has no cure. After 6-18 months patients show regression of acquired skills, motor and speech impairment, cardio-respiratory distress, microcephaly and stereotyped hand movements. The majority of RTT patients display mutations in the gene that codes for the Methyl-CpG binding protein 2 (MeCP2). Clinical observations and neurobiological analysis of mouse models suggest that defects in the expression of MeCP2 protein compromise the development of the central nervous system, especially synaptic and circuit maturation. Thus, agents that promote brain development and synaptic function are good candidates for ameliorating the symptoms of RTT. In particular, Insulin-like growth Factor 1 (IGF1) and its active peptide (1-3)IGF1 cross the Blood Brain Barrier, and therefore are ideal treatments for neurodevelopmental disorders, including RTT (Tropea et al., 2009). Indeed, both (1-3)IGF1 and IGF1 treatment significantly ameliorates RTT symptoms in a mouse model of the disease (Tropea et al., 2009; Castro et al., 2014). In a previous study we established that IGF1 is safe and well tolerated in Rett patients (Pini et al., 2012). In this study we examine the effects of IGF1 treatment on clinical, physiological and behavioural parameters.

**Methods:** Methods: In this study we assessed the effects of IGF1 administration on International Severity Score (ISS), Brain Activity, and social and cognitive abilities of 10 RTT patients treated with IGF1 and 10 untreated controls. Clinical, physiological and behavioural parameters were measured before administration (T0) and at the end of the administration (T1). Patients’ selection and drug administration was performed as described in Pini et al., 2012. The International Severity Scale (ISS) was used to evaluate the severity of disease in each of the patients, with a lower score indicating a lower disease severity. EEG measurements were carried out using the widely accepted 10-20 layout and lasted 1-2 hours. The analysis was performed for a minimum duration of 28 minutes each, sampled at a frequency of 128 Hz. Analysis was conducted using a custom-made script in MATLAB. For the analysis of social and cognitive abilities two independent observers blind to the identity of treated individuals observed footages of patients recorded in identical conditions at two different time points: T0 and T1-which corresponds to the pre-treatment and post-treatment phases for the treated patients-and comparable time for untreated controls. They evaluated the ability of the patients to interact with people and environment as well as the negative features of RTT and assigned a score from 1 to 5, where 1 represents heavy RTT and 5 absence of RTT.

**Results:** The clinical assessment showed significant improvement after IGF1 treatment: Mean Change in ISS in IGF1 Treated versus Untreated Patients (p = 0.0106, Mann-Whitney U Test, two-tailed), while no significant changes were observed in brain activity. Analysis of Combined Features Social and Cognitive Scores Before Treatment for IGF 1 patients and controls shows no differences between the groups, while the change was highly significant (p = 0.009) after the treatment as analyzed by Wilcoxon Matched-Pairs Signed Ranks Test. **p < 0.01. No differences were observed between patients with seizures versus non-epileptic ones.

**Conclusions:** Conclusions: Even considering that this was an open labeled study, we find a significant improvement in...
the ISS and cognitive and social abilities of RTT patients treated with IGF1 but not of the untreated controls. We find no differences in the performances of patients treated with antiepileptic medications. These results have applications to other pathologies considering that IGF1 has been shown to be effective in other disorders of the autism spectrum.

**Keywords:** Rett Syndrome, Insulin-like growth factor 1, autism spectrum disorders

**Disclosures:** DT is an inventor on a patent for the use of IGF1 in Rett Syndrome

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**T38. WITHDRAWN**

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**T39. Oxidative Damage and Antioxidant Defenses in Healthy Adolescents with a History of Childhood Abuse and Neglect**

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**Background:** Early life stress (ELS) has been associated with biological and psychosocial alterations due to developmental reprogramming. Childhood abuse and neglect can alter brain development, increasing stress-reactivity and vulnerability to depression, post-traumatic stress disorder (PTSD), drug abuse, and schizophrenia. Oxidative stress (OS), including higher production of reactive oxygen and nitrogen species (ROS and RNS), have been extensively implicated in the progression of mood disorders and schizophrenia, due to the high vulnerability of brain to increased oxidative load. We investigated whether childhood maltreatment is associated with an imbalance between the production of oxidative markers and antioxidant defenses.

**Methods:** Thirty strictly healthy adolescents with history of childhood maltreatment and twenty-seven adolescents without a history of ELS were recruited for the study. History of CM was assessed by Childhood Trauma Questionnaire (CTQ), a self-report instrument that evaluates sexual, physical and emotional abuse, as well as physical and emotional neglect. Exclusion criteria to both groups included: a) presence of major axis I mental disorder such as psychotic disorder, mood disorder and posttraumatic stress disorder (PTSD), b) intellectual impairments, c) presence of systemic diseases (including hypertension, inflammatory diseases, such as rheumatoid arthritis or infection) or neurological disorder, d) neoplasias and e) use of any substance that may induce immune or endocrine changes. Exclusion criteria were determined by interviews and by the Schedule for Affective Disorders and Schizophrenia for School Aged Children - Present and Lifetime Version (K-SADS-PL) and the Wechsler Abbreviated Scale of Intelligence (WASI) inventories. Ten milliliters of peripheral blood were collected by venipuncture in EDTA tubes and the plasma samples were stored at -80°C until analysis. Redox state was estimated by plasma levels of protein carboxylation, total thiol content (SH), superoxide dismutase (SOD), glutathione peroxidase (GPx), as well as total reactive antioxidant potential (TRAP).

**Results:** Childhood maltreatment was associated with oxidative stress. We found an increased protein carboxylation (p = 0.037) within adolescents reporting childhood abuse and neglect. In addition, circulating levels of SOD were higher in adolescents exposed to childhood maltreatment (p = 0.012). Conversely GPx levels displayed a significant reduction when compared to controls (p < 0.001). We investigated the SOD/GPx ratio to evaluate susceptibility to oxidative damage. Adolescents exposed to maltreatment showed a higher SOD/GPx ratio (p = 0.001). In addition, we identified a significant increase in the TRAP antioxidant capacity in the childhood maltreatment group (p = 0.020).

**Conclusions:** The presence of higher ROS and RNS can be detrimental if endogenous antioxidant mechanisms account for the ROS/RNS. Our data suggests an important imbalance between oxidative molecules/antioxidant defenses in participants who have undergone childhood maltreatment. Specially, we observed an increased SOD/GPx ratio in adolescents exposed to ELS. Although this work did not establish a causal link between the childhood maltreatment and oxidative stress markers, the data may indicate a pivotal role for ELS in the modulation of biological functions during adolescence prior to the expression of psychiatric disorders later in life.

**Keywords:** Early life stress, Child abuse and neglect, oxidative stress, Redox modulation, Adolescents

**Disclosures:** Nothing to disclose.

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**T40. Prenatal Stress Alters Intrauterine Microbiome and Contributes to Adult Female Behavioral Changes**

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**Background:** Infants develop in the relative sterility of the uterus, but upon birth quickly become colonized by billions of bacteria. Commensal microbes from the maternal gastrointestinal and reproductive tracts are the first to colonize the newborn, thus raising the possibility that commensal microbes are involved with the transgenerational transmission of the maternal environment. Recent studies demonstrate that exposure to stress changes the composition of the intestinal microbiota, which is associated with development of stress-induced changes to social behavior, anxiety, and depression. Psychiatric disorders have been associated with in utero and early neonatal exposure to maternal stress, though the underlying mechanisms are not fully understood. In this study we address the contribution of maternal stress and commensal microbes to the development of adult psychopathology.

**Methods:** Pregnant C57/BL6 females were randomly assigned to either the stressed experimental group or non-stressed control group. Mice were restrained between embryonic day (E) 10-E16, for a period of two hours using
a well-validated restraint stress paradigm, or they were left undisturbed throughout pregnancy as a control. Placental tissue and amniotic fluid were collected from pregnant females and fetuses at E17.5 in one cohort of mice. Fetus gender was determined by microscopic inspection of existing reproductive structures through microdissection of the fetus. Microbial diversity was assessed using the Illumina MiSeq platform, for targeted 16S ribosomal RNA gene sequencing. RT-PCR was used to examine gene expression. In a second cohort of mice, behavior was assessed with the elevated plus maze (EPM), the tail suspension test (TST) and the novel object recognition test when the offspring were fully grown at 6 weeks of age.

**Results:** Here, utilizing a rodent model, we demonstrate that prenatal stress leads to alterations in the maternal and female offspring intestinal microbial populations, as well as alterations in the placental microbiota. We show alterations in female, but not male, placental gene expression in response to stress, including IL-6, MAO-A and OGT. There are also elevations of cytokines in utero in response to stress. Furthermore, we show that these changes are associated with alterations in cognition and anxiety, as well as neural gene expression, in the adult female offspring.

**Conclusions:** Utilizing a translational rodent mouse model of prenatal stress, we demonstrate alterations in the structure of the microbiome, increased cytokines in utero, and changes in placental gene expression. Furthermore, there are long-lasting behavioral and microbial changes in female, but not male offspring. This suggests that gestation is a critical window in contributing to the development of adult psychopathology, and that the microbiome may be a key link between early life environment and later life behavioral changes. Understanding how gut microbes impact neurodevelopment will have important implications for the rationale design and use of therapeutics targeted to alter microbial populations, such as probiotics, which can be safely used during pregnancy.

**Keywords:** Gut Microbiome, prenatal stress, transgenerational

**Disclosures:** Nothing to disclose.

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**T41. Progressive Abnormalities of Structural Brain Maturation in Youth with Psychosis-Spectrum Symptoms**

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**Background:** Structural brain abnormalities are a prominent finding in psychotic disorders including schizophrenia. However, it is unclear when such deficits emerge in the disease process and if such deficits are present in association with less severe psychosis-spectrum (PS) symptoms. As psychotic disorders including schizophrenia typically begin in youth and are increasingly conceptualized as aberrations of neurodevelopment, it is critical to understand how abnormalities of structural brain development evolve. Here, we investigated the presence and progression of structural brain abnormalities in youth with PS symptoms studied as part of the Philadelphia Neurodevelopmental Cohort (PNC).

**Methods:** Subjects included 721 youth ages 8-22 imaged as part of the PNC (mean age 15.01 years, SD = 3.7; 362 females), including 393 youth with PS symptoms and 328 typically developing (TD) youth. Dimensional severity of psychosis-spectrum symptoms was summarized in a single factor using a previously reported factor analysis. All subjects completed a T1-weighted structural scan (1mm isotropic voxels) on the same scanner (Siemens Tim Trio 3 Tesla) and had adequate data quality. Images were preprocessed using multi-atlas skull stripping (MASS) followed by bias correction and tissue segmentation using multiplicative intrinsic component optimization (MICO). Regional volumes were assessed using a multi-atlas segmentation procedure that employs a deformable registration with attribute matching and mutual-saliency weighting (DRAMMS). Voxelwise analyses were performed in a common study-specific template space, and used volumetric RAVENS maps that perform better than other typical VBM-based approaches. To flexibly account for nonlinear patterns in brain development, group level analyses employed penalized splines within a general additive model (GAM). Type I error was controlled using Gaussian random field theory (cluster z > 2.3, corrected p < 0.01). As a final step, the complex multivariate pattern of structural maturation was summarized as a unitary “brain age” using machine learning methods. Specifically, all regional volumes were entered into a 10-fold cross-validated linear support vector regression (SVR) trained on the chronological age of each subject. All analyses covaried for sex, race, and intracranial volume. In order to examine specificity of structural deficits seen in psychosis-spectrum symptoms, we additionally repeated all analyses in a sample of 807 youth imaged as part of the PNC with other psychopathology (predominantly mood and anxiety disorders) who lacked psychosis-spectrum symptoms.

**Results:** Compared to TD youth, youth with PS had diminished gray matter (GM) volume (p = 4.2x10^-8), and a relative expansion of white matter (WM) volume (p = 1.3x10^-8). Notably, these deficits were progressive and worsened as adolescence advanced, as evinced by a significant nonlinear age by group interaction (GM: p = 6.6x10^-7; white matter: p = 8.2x10^-7). Dimensional severity of PS symptoms as summarized by the factor analysis was similarly associated with loss of GM (p = 0.02) and expanded WM (p = 4.1x10^-3). Lobar analyses of regional volumes revealed that progressive GM loss and WM expansion was maximal in frontal and temporal cortex. Similarly, voxelwise analyses revealed multiple, distributed clusters of significant gray matter volume loss in frontal, temporal, and parietal cortex. Nonlinear analyses of voxelwise data using penalized splines revealed clusters of progressive volume loss (significant age by group interactions) that were maximal in the medial temporal lobe bilaterally. When multivariate patterns of brain development were summarized as a unitary “brain age” measure using machine learning tools, PS youth had significantly accelerated brain aging that worsened significantly as adolescence progressed. Youth with
other psychopathology (OP) did not display any of the structural brain deficits seen in PS participants.

**Conclusions:** Using a large sample of PS youth, we delineate widespread abnormalities of structural brain development. These abnormalities include progressive, premature GM loss and WM expansion. Structural abnormalities scaled with severity and were specific to the presence of PS symptoms. Concordant with brain regions impacted in clinical samples of psychotic adults, both regional and voxelwise analyses revealed that deficits were maximal in frontal and temporal cortex. This complex constellation of structural deficits could be summarized as a pattern of premature brain development. Our data provides novel evidence that structural brain abnormalities are present early on in youth with PS symptoms and progress as adolescence unfolds. Future studies could use the presence of such abnormalities in conjunction with clinical presentation to predict risk and aid in stratification to treatment interventions.

**Keywords:** MRI, Brain development, Subclinical psychosis, Structural MRI

**Disclosures:** Nothing to disclose.

### T42. Targeting PDE4 Isoforms for Alcoholism and Alzheimer’s Disease: An Implication in Alcohol-related Dementia

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**Background:** Chronic alcohol consumption can cause alcohol-related dementia and 50-75% of detoxified alcoholics have memory or cognition impairment. Phosphodiesterase 4 (PDE4), one of the 11 PDE enzyme families that hydrolyze cyclic nucleotides, is critical for controlling intracellular cyclic AMP (cAMP) concentrations and plays an important role in regulating alcohol consumption and mediating memory associated with Alzheimer’s disease. However, the contributions of individual PDE4 isoforms (PDE4A-D) remain unclear.

**Methods:** Mice deficient in PDE4A, PDE4B, or PDE4D (4AKO, 4BKO, and 4DKO, respectively) and their wild type (WT) controls were tested for alcohol consumption and preference using the two-bottle choice test and memory using passive avoidance and water-maze tests; memory performance was measured in the absence or presence of beta-amyloid peptide 1-42 (Abeta42) infused into the dorsal hippocampus.

**Results:** Compared to the WT controls, 4AKO mice displayed significant decreases in alcohol intake and preference and reversal of Abeta42-induced memory deficits. In contrast, 4BKO mice only mimicked the ability of 4AKO mice to reduce alcohol consumption while 4DKO mice only to reverse Abeta42-induced memory deficits. In addition, levels of cAMP and phospho-CREB (pCREB) were increased in the hippocampus of 4AKO or 4DKO mice, which also showed reversal of Abeta42-induced decreases in pCREB.

**Conclusions:** These data suggest that PDE4 isoforms have different roles in mediating alcohol-drinking behavior and memory in Alzheimer’s disease, which are mediated by cAMP/CREB signaling. The results indicate PDE4A as a potential new target for alcohol-related dementia, although studies with animal models of alcohol-related dementia are needed to clarify this.

**Keywords:** Phosphodiesterase-4 (PDE4), Alcohol consumption, beta-amyloid peptide 1-42, Memory, CREB

**Disclosures:** Nothing to disclose.
Conclusions: Our data indicates that resilient inhibitory synapses in Alzheimer’s disease undergo significant increase in function which is accompanied by abnormal deposition of inhibitory postsynaptic density proteins with APP and Abeta amyloid peptides. Abnormal organization and distribution of inhibitory synapses in the entorhinal cortex in patients with prodromal AD may be partially responsible for the E/I imbalance and subsequent aberrant electrical activity which is highly correlated with cognitive decline in the continuum of AD.

Keywords: synapses, glutamate GABA, electrophysiology, Postmortem Brain Tissue, Alzheimer’s disease

Disclosures: Nothing to disclose.

T44. Local Changes in Sleep EEG Activity in Obstructive Sleep Apnea: Link to Alzheimer’s Disease?

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Background: Sleep disturbance and obstructive sleep apnea (OSA) increase with aging, are prevalent in patients with Alzheimer’s disease (AD), and are associated with greater risk for developing AD. OSA consists of frequent breathing pauses leading to repeated episodes of hypoxia and sleep fragmentation. While the mechanism by which OSA increases risk for AD is unknown, recent work suggests that decrements in sleep slow wave activity (SWA) may be mechanistically linked with amyloid deposition. It is now widely recognized that sleep is not a global phenomenon, but can occur on a local level in an otherwise awake brain. Similarly, sleep disruption might also occur in local cortical areas in an otherwise sleeping brain, a phenomenon that can be termed local sleep deprivation. In support of this, we have recently demonstrated a regionally-specific local sleep deficit (EEG power reductions) in posterior brain regions of asymptomatic, healthy middle-aged subjects with OSA relative to control subjects. Here we sought to extend this finding by localizing the neural sources contributing to this posterior scalp deficit and to further explore the mechanisms by which alterations in the regional distribution of sleep may relate to known patterns of amyloid deposition.

Methods: Healthy adults (n = 16, ages 35-66) with OSA (apnea hypopnea index (AHI) > 10) and controls without OSA (AHI < 5) underwent high-density (256 channel) polysomnographic recordings. Five minute segments of continuous sleep free from respiratory events and arousals were taken from the all-night sleep data of subjects in each group for spectral analysis and cortical source imaging (sLORETA). A within-subject analysis of sleep segments with and without respiratory events in the OSA subjects was also conducted to determine the cortical changes that resulted directly from the presence of apneic events themselves when compared to high-quality sleep in the same individual. Differences in spectral density were assessed using unpaired t-tests and statistical non-parametric mapping (SnPM) cluster testing was used to determine significantly different cortical sources of electrical activity.

Results: Between-group analyses of respiratory event-free sleep fragments confirmed, in ostensibly normal sleep, the presence of the posterior reduction in SWA previously reported in the OSA group compared to controls. Source modeling of these sleep segments localized this reduction to the cingulate, particularly posterior regions bilaterally. A within-subject analysis of sleep segments with and without respiratory events in the OSA subjects revealed that, despite the unsurprising occurrence of more high frequency activity (> 14 Hz) in all cortical areas suggestive of disrupted sleep during respiratory events, slow waves were preserved in a circumscribed region of the fronto-medial cortex.

Conclusions: Our data at the group level indicate that sleep in OSA subjects fails to adequately involve the posterior cingulate, a peak region for amyloid deposition in preclinical AD. In an effort to understand how this local deficit might occur, we examined ideal sleep in OSA subjects and noted a marked reduction in SWA in all but a restricted region of the frontal cortex. These data suggest that the typical spatial and temporal progression of sleep is likely disrupted in OSA. In our studies using serial awakenings in normal subjects, we have found that slow waves follow two spatially and temporally distinct processes during the transition from wakefulness to sleep. The early process is characterized by isolated, large amplitude slow waves, Type 1 slow waves, which broadly involve fronto-medial brain regions and may be organized subcortically. Later in the falling asleep process, smaller, Type 2 slow waves originate from local cortical regions and engage many more circumscribed cortical areas. Sleep in OSA subjects appears to be characterized by large amplitude slow-waves (Type 1), typical of early sleep. In contrast, Type 2 slow waves, those that occur later in the process of falling asleep develop sub-optimally, reflected by a marked reduction in SWA in all other cortical regions. A failure of the typical progression of sleep offers a mechanistic explanation for the circumscribed loss of sleep in the posterior cingulate present in OSA subjects relative to controls. Importantly, the posterior cingulate is a peak region for amyloid deposition in preclinical AD, and sleep plays a critical role in the regulation of amyloid. Local sleep deprivation resulting from OSA may therefore be directly linked with amyloid deposition in sensitive cortical regions. These data suggest a mechanism by which sleep disruption in OSA may contribute to AD progression and provide a target for early treatment during a potentially reversible stage of AD neuropathology. They also suggest a process by which OSA may lead to exacerbation of other neuropsychiatric disorders.

Keywords: Alzheimer’s disease, sleep, slow waves

Disclosures: Ruth Benca: Consultant for Janssen, Jazz, Merck and grant support from Merck Giulio Tononi: Consultant and grant support from Philips Healthcare. Holds David P. White Chair in Sleep Medicine, endowed by Philips Healthcare.
T45. Familiarity of Sensorimotor Alterations in Autism Spectrum Disorder

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Background: Individuals with autism spectrum disorder (ASD) frequently demonstrate sensorimotor abnormalities. These deficits often emerge early in development before social-communication symptoms. We previously found that that they may be present in unaffected first-degree relatives of individuals with ASD. Sensorimotor abnormalities thus represent promising intermediate phenotypes that are expressed in both affected individuals and their unaffected first-degree biological relatives. The extents to which sensorimotor abnormalities in ASD are familial and evident across different types of motor behaviors have yet to be determined.

Methods: Thirty family trios (proband with ASD, biological mother, biological father) and 70 age-, IQ- and gender-matched healthy controls (42 proband controls, 28 parent controls) completed eye movement and manual motor testing. Participants with ASD were between ages 6-20 years and had a Performance IQ >70. Individuals >50 years of age were excluded to limit variable age-related effects on motor functioning.

Participants completed a visually guided saccade test to measure oculomotor control, and a test of precision grip force to examine manual motor abilities. During oculomotor testing, participants received 60 trials in which they made saccades to peripheral targets that appeared unpredictably but with equal probability at 12 or 24 deg to the left or right of center. The latency, accuracy, velocity and acceleration of each saccade were examined. During manual motor testing, participants completed 36 8 sec trials in which they used their thumb and index fingers to press against opposing precision load cells secured to a custom-grip device attached to an arm brace. They viewed two horizontal bars: a red/green target bar and a white force bar. The force bar moved upwards with increased force, and participants were instructed to press on the load cells as quickly as possible when the target bar turned green so that the force bar reached the height of the target bar. The target was set to 15, 45, or 85% of the participant’s maximum voluntary contraction (MVC); the order of force levels and hand tested were randomized. Accuracy and rate of force increase during the initial force pulse were examined. We also compared the mean and variability (coefficient of variation, or CoV) of participants’ sustained force for each trial. Last, the rate of force decrease at the end of each trial was examined. All parents of probands also completed the Broad Autism Phenotype-Questionnaire (BAP-Q) to determine the relationship between sensorimotor performance and subclinical psychological characteristics associated with ASD.

Results: Individuals with ASD showed reduced saccade accuracy, reduced accuracy of their initial grip force, increased variability of their sustained grip force, and a reduced rate of grip force relaxation at the end of trials. Unaffected mothers of individuals with ASD also showed reduced saccade accuracy and reduced accuracy of their initial grip force. Unaffected fathers did not show any differences in oculomotor or grip force performance relative to controls. Reductions in saccade accuracy as well as reduced velocities and rates of acceleration of saccades co-segregated in families of children with ASD. Manual motor deficits were not intercorrelated among family members. Reduced saccade accuracy in fathers of children with ASD was associated with their greater social aloofness, pragmatic language deficits, and behavioral rigidity as measured by the BAP-Q. Reduced rates of grip force relaxation in fathers of individuals with ASD were related to their pragmatic language deficits.

Conclusions: In the present study, we found that individuals with ASD show multiple forms of sensorimotor impairment including reduced accuracy of saccadic eye movements and initial precision gripping force, as well as increased variability of sustained gripping force and reduced rates of force relaxation. Reduced accuracy of saccades and initial grip force also were seen in mothers of children with ASD, and deficits in saccade control were familial. These findings indicate that the ability to make accurate, rapid sensorimotor responses based on feedforward control processes is compromised in ASD and in unaffected mothers of children with ASD. Feedforward motor control processes are guided by motor cortices and anterior cerebellar circuits that previously have been implicated in MRI and postmortem studies of ASD. Our results thus suggest that fronto-cerebellar dysfunctions may be differentially expressed in mothers compared to fathers of children with ASD and their study thus may provide key insights into maternally associated etiopathological mechanisms. Paternal sensorimotor features were associated with broader autism phenotypic characteristics indicating that the severity of fathers’ subclinical deficits may covary across different domains. Overall, our results indicate that sensorimotor impairments may serve as useful biological intermediate phenotypes whose study may help determine pathophysiological mechanisms associated with ASD.

Keywords: autism spectrum disorder, endophenotypes, sensorimotor

Disclosures: Nothing to disclose.

T46. Targeting the Reward Circuit Using DBS for Binge Eating in Rats: No One Size Fits All

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Background: Binge eating is a difficult to treat behavior that complicates the treatment of several psychiatric disorders in addition to obesity. Emerging pre-clinical and clinical evidence has demonstrated the capacity of neuro-modulatory interventions to modify target symptoms in disorders of appetitive behavior. Our work explores the capacity of deep brain stimulation (DBS) targeted to the brain reward circuit to modify binge eating behavior in rats. We first tested the hypothesis that DBS targeted to the nucleus accumbens (NAc) core would result in
Conclusions: Our findings highlight the potential of size within the same animal. However, significant binge reductions resulted selectively from stimulation of either the NAc core or shell for each animal, but rarely did stimulation in both sites reduce binge eating behavior. In subsequent studies we directly compared effectiveness of the core and shell sub-regions of the NAc as targets for DBS.

Methods: All experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Dartmouth College. For the initial core stimulation experiments, male Sprague-Dawley rats (250-300g) were implanted bilaterally with stimulating electrodes in the NAc core sub-region (N = 18). Following recovery from surgery, rats were started on an established binge eating paradigm. Briefly, rats received limited access (2 hours/day) to a palatable high sugar, high fat diet with ad libitum access to house chow and water until a stable binge size was achieved. For stimulation, a current-controlled stimulator was used to generate a continuous train of high frequency pulses that were verified by oscilloscope. The experimental design was a group (stimulation and sham intervention) X session (3 intervention and 3 post-intervention sessions) design with simple randomization. The data were analyzed using a repeated-measures ANOVA. For experiments comparing NAc sub-regions, rats were implanted bilaterally in both the nucleus accumbens core and shell (N = 16) using custom-built electrode arrays that allowed for bilateral stimulation of either NAc sub-region within the same animal. Similar to clinical DBS treatment, stimulation parameters were titrated for each animal and stimulation site by manipulating the type of stimulation (monopolar or bipolar) and current strength (150 - 500 μA) to achieve maximum effect on binge eating behavior.

Results: The primary finding in the initial core DBS experiment was a reduction in binge size with bilateral nucleus accumbens stimulation compared to sham stimulation with a main effect for group (F(1,17) = 8.12, p = 0.012) and a main effect for session (F(1,16) = 15.35, p = 0.001). Despite the main effect, however, nearly half of the rats in the DBS intervention group failed to show any reduction in binge size with stimulation. To explore this unexpected variation in outcome further we performed secondary regression analyses to investigate the relationship between observed variations in DBS induced binge reductions and either: 1) each animal’s pre-intervention average binge size; or 2) variation in electrode location within the NAc along the anterior-posterior axis. Neither regression showed a significant correlation. Subsequent investigation comparing the effect of either NAc core or shell DBS within each animal produced significant reductions in binge size in nearly all animals. Interestingly, however, significant binge reductions resulted selectively from stimulation of either the NAc core or shell for each animal, but rarely did stimulation in both sites reduce binge size within the same animal.

Conclusions: Our findings highlight the potential of neuromodulatory interventions (DBS) targeted to the reward circuit (NAc) in the treatment of disorders of appetitive behavior (binge eating). The data suggest that DBS in either the NAc core or shell has the capacity to reduce binge size. However, these findings also suggest that a subset of animals respond only to core stimulation while others respond to shell DBS. We postulate that an unknown heterogeneity in the brain circuitry of binge eating behavior underlies the observed variation in DBS treatment outcomes. These findings underscore an ongoing need to identify relevant markers of such individual heterogeneity that underpins treatment outcome variability. Once identified, such biomarkers could be used to individualize target selection and maximize therapeutic efficacy.

Keywords: DBS, Binge eating, reward neural circuitry

Disclosures: By way of disclosure, in the past three years, Dr. Green has received research grants from Novartis and Janssen to support research studies, and has served as an uncompensated consultant to Otsuka and Alkermes and as an (uncompensated) member of a data safety monitoring group for Eli Lilly. Moreover, he is the inventor or patent application related to treatment of substance abuse. None of the other authors have any potential conflicts to disclose.

T47. Fear of Food in Anorexia Nervosa: Harm Avoidance is Linked to Diminished Neural Response to Taste Reward

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Background: Anorexia nervosa (AN) is a disorder characterized by extremely restricted intake resulting in low body weight. Healthy individuals tend to report greater discomfort when hungry and a higher subjective value of food, whereas individuals with AN describe elevated anxiety with satiety, and a reduction in dysphoric mood states with food refusal. This study investigated brain reward response in insula–striatal pathways to sweet taste and water in relation to anxiety and inhibition during hungry and satiated states to examine response to salient stimuli in individuals remitted from AN (RAN) to avoid the confound effects of malnutrition.

Methods: Twenty-three RAN women (16 pure restricting subtype, 7 restricting-purging subtype) were compared to 17 age- and weight-matched healthy women (CW). Participants completed two counterbalanced study visits 24 hours apart, following either a 16 hour fast or a standardized breakfast. The sucrose task paradigm consisted of four blocks in which individual trials of 1.0 cc of ionic water or a10% sucrose solution were pseudorandomly delivered every 20 seconds for a total of 40 stimuli presentations for each condition. Data acquisition took place using 3 Tesla GE MR Scanners. To test our hypothesis that satiety modulates response to sweet taste, we employed a Group (RAN, CW) x Visit (Hungry, Satiated) x Taste (Sucrose, Water) linear mixed effects analysis within four regions of interest (ROIs) based on prior research: bilateral insula, caudate, ventral striatum and putamen. Secondary exploratory Huber robust regressions conducted within group examined the relationships between percent signal change...
Anorexia nervosa (AN) is characterized by severe restrictive eating and emaciation, with high rates of morbidity, chronicity and mortality. Current treatments have high rates of relapse and recurrence, thus developing improved therapies is a high priority in mental and public health. A limiting factor in developing improved treatments is a lack of adequate knowledge on how molecular mechanisms of susceptibility genes affect disease course. We have previously discovered several lipidomic biomarkers for AN and revealed that soluble epoxide hydrolase (sEH) as a mechanism of the disease association between EPHX2 gene and AN through an integrative multi-domain Omics strategy. The purpose of this study was to investigate if postprandial oxylipin responses in subjects with eating disorders differ from healthy control subjects.

**Methods:** EPHX2 – AN association was previously identified through an exon sequencing and replication study. Lipidomic and metabolomic assays were conducted using the GC/MS and LC/MS/MS systems. The pilot postprandial oxylipin response study was conducted in 6 weight-restored patients with eating disorders (5 AN and 1 bulimic nervosa) and 5 healthy control women. Intra-individual change (before and after a meal) of oxylipin responses were calculated and compared in patients versus controls. All statistical analyses were performed in R 3.1.3.

**Results:** Previously, EPHX2 was identified as an AN susceptibility gene. Dysregulation in plasma polyunsaturated fatty acids (PUFAs) and associated Cytochrome P450 PUFAs were observed in both ill AN and weight-restored AN compared to controls. Elevated soluble epoxide hydrolase level was identified in AN via in vivo markers and ex vivo assay. In this pilot study, among the ω-3 PUFAs and ω-6 PUFAs derived oxylipins, postprandial shift of ω-6 arachidonic acid’s pro-inflammatory diol-fatty acid (5,6-DiHET) was found increased in patients but decreased in controls (1.51 versus -4.09, p = 0.09) after accounting for the variance of pre-catalyst epoxy-fatty acid level (5,6.EET) and age. By contrast, none of the postprandial diol-fatty acid shift from ω-3 PUFAs had as big of a change between patients and controls.

**Conclusions:** While the combined use of enzymatic activity assessment and multi-domain EPHX2 omics markers revealed upregulated sEH and oxylipin dysregulation to play significant roles in AN, the PUFA dysregulation and the increase of postprandial ω-6 pro-inflammatory oxylipin observed in patients suggest that specific dietary factor may modulate the effect sEH has on AN. Our pilot data supports the emergence of evidence pointing at gene-diet interaction as a key element that moderates genetic susceptibility and illness course. This study is timely for AN treatment development because both enzyme inhibition and dietary modulation are accessible for research and development. Furthermore, this study offers a proof of principle for an approach to psychiatric nutrigenomic studies and paves the way to effective dietary-intervention strategies for genetically at-risk individuals.

**Keywords:** Gene–environment interaction, Nutrigenomics, Eating Disorders

**Disclosures:** Nothing to disclose.
Conclusions: the withdrawal-induced hypophagia in food cycling rats. as well as to reduce the excessive intake of palatable diet and able to block the phase-specific learning and memory deficits proliferating cells. Importantly, memantine treatment was hippocampus as well as a withdrawal-dependent decrease of expression of immature neurons in the dentate gyrus of the animals. In addition, diet cycled rats showed reduced over a ''novel place'', compared to control Chow/Chow processing, and a bias in their preference for a ''novel cue'' palatable food showed both a weakened contextual spatial processing, and a bias in their preference for a “novel cue” over a “novel place”, compared to control Chow/Chow animals. In addition, diet cycled rats showed reduced expression of immature neurons in the dentate gyrus of the hippocampus as well as a withdrawal-dependent decrease of proliferating cells. Importantly, memantine treatment was able to block the phase-specific learning and memory deficits as well as to reduce the excessive intake of palatable diet and the withdrawal-induced hypophagia in food cycling rats.

Results: We found that Chow/Palatable rats withdrawn from palatable food showed both a weakened contextual spatial processing, and a bias in their preference for a “novel cue” over a “novel place”, compared to control Chow/Chow animals. In addition, diet cycled rats showed reduced expression of immature neurons in the dentate gyrus of the hippocampus as well as a withdrawal-dependent decrease of proliferating cells. Importantly, memantine treatment was able to block the phase-specific learning and memory deficits as well as to reduce the excessive intake of palatable diet and the withdrawal-induced hypophagia in food cycling rats.

Conclusions: In summary, our results provide evidence that withdrawal from intermittent access to palatable food on hippocampal function and hippocampal neurogenesis, a pivotal mechanism of plasticity.

Methods: Male Wistar rats were either fed Chow continuously for 7 days/week (Chow/Chow group), or fed Chow intermittently 5 days/week, followed by a high-sucrose, palatable diet 2 days/week (Chow/Palatable group). Following diet cycling, hippocampal function and neurogenesis were assessed either during acute withdrawal or following renewed access to palatable food. Furthermore, the ability of the uncompetitive N-methyl-D-aspartate receptor antagonist, memantine, to prevent the diet-induced memory deficits as well as to block maladaptive eating behavior was tested.

Results: We found that Chow/Palatable rats withdrawn from palatable food showed both a weakened contextual spatial processing, and a bias in their preference for a “novel cue” over a “novel place”, compared to control Chow/Chow animals. In addition, diet cycled rats showed reduced expression of immature neurons in the dentate gyrus of the hippocampus as well as a withdrawal-dependent decrease of proliferating cells. Importantly, memantine treatment was able to block the phase-specific learning and memory deficits as well as to reduce the excessive intake of palatable diet and the withdrawal-induced hypophagia in food cycling rats.

Conclusions: In summary, our results provide evidence that withdrawal from intermittent access to palatable food on hippocampal function and hippocampal neurogenesis, a pivotal mechanism of plasticity.

Keywords: Compulsive eating, NMDA Antagonists, Neurogenesis, Palatable food, learning and memory

Disclosures: Nothing to disclose.
and-taking that are characteristic of substance dependence. Although chronic exposure to drugs of abuse can result in inflexible decision-making, recent evidence has suggested that pre-existing decision-making impairments might influence future drug-taking behaviors. Therefore, inflexible decision-making may be both an antecedent as well as a consequence of addiction. However, it is unclear if the aspects of flexible decision-making that influence future drug taking behaviors are the same ones that are affected by chronic exposure to drugs of abuse.

**Methods:** To directly investigate this, we measured decision making in rats (N = 24) using a novel three-choice probabilistic discrimination and reversal task (PDRL) before and after rats were allowed to self-administer cocaine for 14 days.

**Results:** Performance in the reversal, but not the discrimination, phase of the PDRL predicted cocaine self-administration (R = 0.71; p = 0.009) and was impaired following cocaine self-administration (p = 0.002). We also analyzed the choice behavior of rats in the PDRL using a computational model to determine if different aspects of choice behavior were mediating the relationship between reversal performance and cocaine self-administration. We found that individual differences in the sensitivity of rats to positive, but not negative, outcomes predicted future cocaine self-administration (R = -0.78; p = 0.003). In contrast, sensitivity to negative, but not positive, outcomes was significantly disrupted following cocaine self-administration (p = 0.02).

**Conclusions:** These results provide a novel demonstration that dissociable components of flexible decision-making are key behavioral biomarkers uniquely predictive of or affected by cocaine self-administration. Ongoing pharmacological experiments will identify the neurochemical mechanisms underlying these behavioral processes that contribute to either addiction vulnerability or pathophysiology, to identify unique pharmacological targets for the prevention and treatment of addiction.

**Keywords:** addiction, Decision Making, computational modeling

**Disclosures:** Nothing to disclose.
lincRNAs contribute to gene-expression via epigenetic regulation, our results are consistent with the concept that molecular alteration underlying complex phenotypes like suicide does not rest in the perturbation of single genes, but in mechanisms modulating networks of genes, likely in interaction with environmental factors such as stressful events.  

**Keywords:** suicide, aggression, long noncoding RNA, fMRI faces paradigm  

**Disclosures:** Nothing to disclose.

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**T53. Biological and Behavioral Dissection of the Role of the Serotonin 1B Receptor in Impulsivity**

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**Background:** Impulsivity is a core feature of many psychiatric disorders including substance use disorder, pathological gambling, attention deficit hyperactivity disorder, and personality disorders. There are limited efficacious pharmacological treatments for maladaptive impulsive behavior. Furthermore, impulsivity is a multi-dimensional construct, including components of impulsive action (motoric impulsivity/response inhibition) and impulsive choice (cognitive impulsivity/intolerance to delays). A better understanding of the neural circuits underlying impulsivity, including dissecting its different subcomponents, would greatly advance the development of more targeted and effective pharmacological treatments for psychiatric disorders in which impulsive behavior plays a key role.  

**Methods:** Given that polymorphisms in the serotonin 1B receptor (5-HT1BR) are associated with impulsive behavior, and 5-HT1BR knockout (KO) mice display increased impulsivity, we generated a transgenic mouse line which allows for inducible and tissue-specific knockout of 5-HT1BRs. The line was created by replacing the endogenous htr1b coding region with a cassette containing a floxed tetracycline operator and htr1b cDNA (tetO1B). Crossing tetO1B mice to mouse lines expressing the tetracycline-dependent transcriptional silencer (tTS) or Cre transgenes under the control of various promoters, allowed for tissue specific knock-down of 5-HT1BR, the former of which could be rescued by treatment with doxycycline. Using this model, we have begun dissecting the circuits through which 5-HT1BRs modulate impulsive behavior. In order to test the hypothesis that impulsive action and impulsive choice represent dissociable behavioral constructs with distinct neural circuits, we also collected a number of behavioral measures of impulsivity in a single cohort of mice including whole-brain knockdown of 5-HT1BR and controls for factor analysis.  

**Results:** The absence of 5-HT1BR expression caused increased impulsivity in the differential reinforcement of low-rate responding (DRL) and Go/No-Go operant paradigms, representing a deficit in impulsive action. Interestingly, this was reversed with adult rescue of the receptor, suggesting an adult mechanism of action. This phenotype was also seen with selective knockdown of 5-HT1BR in GABAergic cells throughout the brain suggesting that the 5-HT1BR effects on impulsivity are mediated through modulation of inhibitory tone. Impulsive choice was also assessed in mice that lacked 5-HT1BRs. Interestingly, there was no significant effect of 5-HT1BR expression on the discounting rate in the delayed discounting (DD) or probabilistic discounting (PD) operant tasks. Further analysis using an exploratory factor analysis (EFA) revealed a good-fitting two-factor model for behavioral data with the latent factors representing impulsive action and impulsive choice (RMSEA < 0.001, CFI = 1.0, TLI = 1.0). Specifically, DRL and Go/No-Go measures loaded onto one factor, while DD and PD loaded strongly onto the other. Loadings of all indicators were > 0.4 and the two factors were not significantly correlated. Finally, a multiple indicator multiple causes (MIMIC) analysis revealed that 5-HT1BR expression and sex were significant predictors in the two-factor model. Male mice had significantly higher levels of impulsivity on both factors regardless of genotype, while 5-HT1BR knockdown resulted in significantly increased impulsivity in the impulsive action factor only.  

**Conclusions:** Overall, our results point to a role for 5-HT1BR modulation of GABAergic signaling in the modulation of impulsive action. Furthermore, our data support the conclusion that impulsive action and impulsive choice are distinct behavioral phenotypes with dissociable biological influences.  

**Keywords:** impulsivity, Serotonin 1b receptor, addiction, behavioral inhibition, Attention Deficit Hyperactivity Disorder  

**Disclosures:** Nothing to disclose.

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**T54. Brain Activity and Transcriptional Programs in Frontal Lobe and Hippocampus: Uncoupling and Recoupling Following Anesthesia**

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**Background:** Neuronal activity can modify gene regulation in the central nervous system through the release of signaling molecules which feed back to the nucleus. Altered neuronal communication which occurs in processes such as exposure to drugs of abuse, or activation of nociceptive afferents can lead to long-term plastic changes mediated by stimulus-coupled transcriptional events. This synchronically driven coupling modifies transcription factor expression in the cell’s nucleus ultimately leading to changes that feed back to the synapse. Functional brain imaging and electrical recordings have described basal activity patterns that are present in the brain during wakefulness. The present study explores the question of whether there is a basal “set point” for the transcription coupled to this basal activity by chronically modulating GABA-A receptors, the major inhibitory ionotropic receptors in brain using isoflurane anesthesia. If neural activity is depressed, what happens to the expression of activity regulated genes required for neural plasticity? Additionally, despite the widespread use
of general anesthesia its effects on neural circuits at the molecular level remain poorly characterized. This gap in knowledge is problematic given growing evidence that in some people cognition is altered for days or months after surgery, potentially due to a plastic event(s) triggered by decreased neuronal activity.

**Methods:** Modulation of gene expression was examined in frontal cortex and hippocampus, two regions involved with executive function, working and long-term memory using massively parallel RNA sequencing (RNA-Seq). This method quantitatively measures every transcript in a given tissue, and the aggregate gene expression changes can be used to describe all major biological processes occurring in that tissue. Transcriptional changes were assessed in rats anesthetized with isoflurane for 1h, 10h, or 10h with a 24h recovery period (n = 4 per time point, >40 million reads per sample).

**Results:** At the three time points in both tissues, upregulation and downregulation of expression were noted at approximately equal frequency. Transcriptional alterations in the frontal lobe and hippocampus follows a multi-phase progression, with an initial sharp decrease in several transcription factors associated with neuronal activity, followed by rebound and recovery. In addition, we describe several other novel responses to anesthetics, including long-term alterations in the expression of several ionotropic receptors. In general, effects were more accentuated in the neocortical sample compared to archicortex (hippocampus), and the genes affected are not fully identical between the two regions. Interestingly, at the 10 hour anesthesia time point the hippocampus appears more resistant to anesthesia-induced transcriptional changes. Eighty-four percent fewer genes showed statistically significant alterations in hippocampus compared to frontal cortex. It is to be expected that several genes which change in response to anesthesia are expressed in distinct subsets of brain cells.

**Conclusions:** In aggregate, our results suggest that some circuits are more affected than others, and recover and rebound differentially. This hypothesis is being addressed with immunocytochemical localization. The present findings describe a program of baseline transcriptional activity in the awake brain, which undergoes a form of long-term plasticity after exposure to anesthesia. The basal transcriptional activity is hypothesized to be the molecular equivalent of the default mode network seen with functional imaging. While the genes that decrease may reflect the activity-coupled default transcriptional network, the role of the genes that are elevated when activity is switched off is less clear. Overall, states of consciousness or unconsciousness may exhibit characteristic programs of gene expression that can vary on a region-specific basis. Understanding these processes could have an impact on what the molecular requirements are for optimal maintenance and modulation of neural circuits in normal function and which may be disordered in neuropsychiatric conditions.

**Keywords:** Anesthetic, Cognitive Resilience, Transcriptomics, RNA-seq, neural plasticity

**Disclosures:** Nothing to disclose.

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**T55. Genetic and Optogenetic Strategies for Probing the Complex Output Pathways of the Lateral Habenula**

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**Background:** The habenula is a paired nucleus residing just dorsal to the thalamus. It consists of medial and lateral subnuclei (MHB, LHb), whose projections initially share a common descending output tract, then diverge to innervate distinct brainstem structures. Recent studies of LHb function have focused on the inhibition of dopamine-mediated reward signals. LHb neurons projecting to the tegmentum are excitatory, but inhibit the DA system via GABAergic neurons residing in the rostromedial tegmental nucleus (RMTg, Jhou et al., 2009; Perrotti et al., 2005; Quina et al., 2015). Despite the recent focus on the Lhb > RMTg > VTA pathway, older studies in the rat have shown a diverse set of Lhb outputs, including efferents to the lateral and posterior hypothalamus (LH, PH), the median raphe (MnR), dorsal raphe (DR), and pontine central gray (Sutherland, 1982). In recent work (Quina et al., 2015), we used anterograde tracing with adeno-associated virus (AAV), combined with serial two-photon tomography, to map the efferents of the LHb on a standard coordinate system for the entire mouse brain, reconstruct the efferent pathways of the LHb in three dimensions, and show quantitatively the PH, LH, MnR and pontine central gray are major recipients of Lhb efferents. Using retrograde tract tracing with cholera toxin subunit B (CTB), we showed that Lhb neurons projecting to these brainstem centers reside in characteristic regions of the Lhb.

**Methods:** In the present study we sought to: 1) Define the neurotransmitter phenotype of neurons receiving direct Lhb synaptic inputs in the mesopontine raphe. 2) Identify transgenic markers that could be used to define functional subsets of Lhb neurons. To determine the phenotype of the synaptic targets of Lhb efferents, we used channelrhodopsin (ChR2)-assisted circuit mapping in transgenic mice that express a fluorescent marker in either GABAergic (Gad67-GFP) or serotoninergic (ePet-GFP) brainstem neurons. AAV encoding ChR2-mCherry was injected in the ventromedial quadrant of the Lhb, and coronal slice preparations were analyzed by intracellular recording of GFP-labeled postsynaptic raphe neurons in voltage clamp mode (“visual patch” recording). To identify an experimentally addressable subset of Lhb neurons that may innervate targets other than the RMTg, we examined the specific properties of Lhb neurons that express the GABA biosynthetic enzyme Gad65, product of the Gad2 gene, using Gad2Cre mice. Anterograde labeling of Gad2Cre Lhb neurons was performed using a Cre-activated AAV-GFP tracer. For retrograde tracing of these projections, Gad2Cre mice were interbred with a reporter line, Ai75, which conditionally expresses a tdTomato nuclear marker. CTB was injected into brainstem nuclei receiving Lhb efferents, and the co-incidence of the induced tdTomato and CTB labels in the Lhb was examined.

**Results:** ChR2-assisted circuit mapping of Lhb efferents to the mesopontine raphe demonstrates that neurons in the ventromedial Lhb form direct synaptic connections to both
GABAergic and serotonergic neurons in this region. As expected, the direct LHb input to both of these cell types is excitatory. Further studies will be required to determine the net effect of LHb input on serotonergic neuron activity in the raphe, which may be distinct for different subsets of 5HT neurons.

Anterograde labeling of Gad2Cre LHb neurons showed expression of GFP mainly in the rostral and ventromedial parts of the LHb, in agreement with the distribution of Gad2 mRNA in this nucleus. These neurons do not project strongly to the RMTg. Instead, both anterograde and retrograde tracing show that Gad2Cre LHb neurons project strongly to the inferior subnucleus of the dorsal raphe, DRI, and to the hypothalamus. In “typical” GABAergic neurons the GABA biosynthetic enzymes Gad65/Gad2 and Gad67/Gad1 are co-expressed, along with the vesicular GABA transporter, Vgat. Remarkably, gene expression profiles in the Allen Brain Atlas suggest that Gad2-expressing LHb neurons do not co-express Gad1 or Vgat. Global analysis of mRNA expression for a Gad2Cre-driven transgenic reporter and Gad1 mRNA using double-label fluorescence in situ hybridization (DFISH) confirms that the LHb neurons identified by Gad2Cre express Gad2, but not Gad1. A very limited number of neurons elsewhere in the brain share this expression pattern. Pharmacological experiments are underway to determine the principal fast neurotransmitter used by these unusual LHb neurons.

Conclusions: We conclude that many brain circuits downstream of the LHb are yet unknown. Functional analysis of the LHb outputs to the hypothalamus and brainstem, including the 5HT system, will require tools with a high degree of anatomical and molecular specificity.

Keywords: Lateral Habenula, Ventral tegmental area (VTA), raphe, optogenetics

Disclosures: Nothing to disclose.

T56. The Complications Associated with Treating Depression and Traumatic Brain Injury: A Case Report of a Suicide Attempt in a 26 Year Old Male

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Background: Traumatic brain injury (TBI) is a debilitating illness that often causes comorbid depression. Treating depression is often difficult with only 30%-40% of patients responding to their first antidepressant. Medical comorbidities exacerbate the struggle of determining optimal pharmacotherapy for a patient. Psychiatrists, in particular, may be exposed to patients with traumatic brain injury (TBI) secondary to the mental illness and substance abuse that may accompany TBI4. While first line pharmacotherapies for depression remain selective serotonin reuptake inhibitors (SSRIs), patients with TBI cannot necessarily be treated with these medications. It is well documented in the literature that both SSRIs and SNRIs may cause hyponatremia. Likewise, traumatic brain injury is often complicated by SIADH or cerebral salt wasting syndrome (CSWS).

Methods: We present a case of a 26 year old male status post suicide attempt via gunshot wound resulting in traumatic brain injury.

Results: CT brain scan was done and significant for a penetrating ballistic injury to the face, brain, and calvarium with a trajectory extending from the left inferior face, through the left maxillary sinus, ethmoids, posterior right orbit, right frontal region, and through the right frontal calvarium. Hospitalization was complicated by diabetes insipidus, CSF leak, increased pneumocephalus, and hyponatremia.

Conclusions: One needs to consider electrolyte abnormalities, seizure propensity, fall risk, potential weight loss, and confusion in TBI patients when choosing psychotherapeutic agents. This case report highlights the difficulties accompanying choosing safe and effective treatment modalities for depression as many psychotherapeutics have side effects that limit their use in patients with traumatic brain injury.

Keywords: Depression, suicide, Brain, Pharmacology, Antidepressants

Disclosures: Nothing to disclose.

T57. The Ankyrin 3 Bipolar Disorder Risk Gene Regulates Neuronal Cytoskeleton Dynamics

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Background: Genome-wide association studies (GWAS) have identified the ankyrin 3 gene (ANK3) among the strongest and most replicated genetic risk factors for bipolar disorder. Ankyrin 3 encodes the ankyrinG protein that tethers integral membrane proteins to the cytoskeleton and has essential functions in neurons. However, there is a fundamental gap in our knowledge of how ankyrin 3 contributes to bipolar disorder. We have previously shown that Ank3+/− mice that have reduced ankyrin 3 expression in brain exhibit features that parallel bipolar disorder symptoms, including increased impulsive behavior and motivation that are reversed by lithium treatment, and heightened response to stress indicating hypothalamic-pituitary adrenal (HPA) axis dysfunction. As the mechanism by which ankyrin 3 regulates these processes is unknown, we performed a transcriptome analysis of Ank3+/− mouse hippocampus to identify novel biological pathways that may underlie the observed behavioral and physiological abnormalities.

Methods: RNA was extracted from hippocampus from adult male Ank3+/− mice and wildtype Ank3+/+ mice exposed to chronic stress (isolation housing) or control conditions (group housing). RNA integrity numbers (RIN) were above 8.4. RNA was sequenced using the Illumina HiSeq platform (>25 million 50 base reads). Sequencing reads were trimmed by 15 bases on the $5'$ end prior to analysis using the Tuxedo package and Ingenuity Pathway Analysis. A subset of differentially expressed genes was validated by
SYBR green quantitative RT-PCR. Cellular alterations were examined by Western blot analysis of protein from Ank3 +/− and Ank3 +/+ mouse hippocampus and a neuronal cell line in which Ank3 expression was reduced by RNA interference.

**Results:** Tuxedo package analysis of RNA sequencing data from hippocampus of Ank3 +/− and Ank3 +/+ mice under control conditions identified 283 differentially expressed genes, while Ank3 +/− mice exposed to stress had 213 differentially expressed genes compared to Ank3 +/− mice under control conditions (≥ 2x read coverage, fold change ≥ 1.2, p < 10−3). Ingenuity Pathway Analysis of the differentially expressed genes converged on pathways involved in cytoskeletal dynamics (e.g., cytoskeleton organization, microtubule dynamics, cell outgrowth; all p < 10−3), suggesting cytoskeletal instability in Ank3 +/− mice that may impact axonal transport and nuclear localization of transcription factors. Exposure to chronic stress appeared to attenuate the cytoskeletal instability in Ank3 +/− mice, in that many of the cytoskeletal genes had comparable expression to wildtype Ank3 +/+ control mice (p > 0.05). Western blot analysis identified changes in microtubule-associated protein levels in Ank3 +/− compared to Ank3 +/+ hippocampus (p < 0.05) that support cytoskeletal instability in Ank3 +/− mice. Knockdown of Ank3 in cultured neurons altered the abundance of microtubule-associated proteins, in line with the hippocampus data. The protein changes in neurons were reversed by treatment with lithium, which inhibits glycogen synthase kinase 3 (GSK3), or a selective GSK3 inhibitor, suggesting that Ank3 regulation of cytoskeleton dynamics involves GSK3 signaling.

**Conclusions:** Our novel data showing transcriptional alterations in the hippocampus of mice with reduced Ank3 expression suggest a role for ankyrin 3 in cytoskeletal dynamics in neurons. Cytoskeletal instability may underlie the behavioral and physiological alterations that we previously reported in Ank3 +/− mice. Ongoing studies are examining GSK3-dependent mechanisms in ankyrin 3 regulation of cytoskeleton dynamics. Our data linking ankyrin 3 cytoskeletal function with behavioral and physiological (stress) regulation opens up a new line of investigation of the disease-relevant functions of psychiatric risk genes.

**Keywords:** genetic mouse model, RNA Sequencing, Hippocampus, cytoskeleton

**Disclosures:** Nothing to disclose.

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**T58. The Stress-Antidepressant-diet (SAD) Paradigm and Weight Gain**

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**Background:** Antidepressants are the most frequently prescribed class of drugs; about 264 million antidepressant prescription were issued in the US in 2011. The use of most antidepressants is associated with weight gain; however, the pathophysiological mechanisms of this association are still unknown. Our lab has developed an animal model that addresses “paradoxical weight loss” by investigating the interactions between short-term exposure to stress, antidepressant administration and exposure, and long-term exposure to an obesogenic high-fat diet.

**Methods:** Male Sprague-Dawley rats are subjected to the following paradigm: Short-term exposure to recurrent restraint stress and antidepressants for 2 weeks, followed by long-term high-fat diet intake, were studied for 295 days. We have classified animals as obesity-prone (upper 50% of body weight) or obesity-resistant (lower 50% of body weight). On study day 295, animals were sacrificed and various organs were collected and weighed. Measurements: Body weight, food intake ratio, behavioural testing, and bone weight.

**Results:** Obesity-prone rats treated with fluoxetine (RFX) had increased body weight, in comparison to the control group treated with saline (RC) and non-restraint control group (NRCF). The RFX and the imipramine treated group (RIM) groups had significantly lower food intake ratio in comparison to the non-restraint control group (NRCF). The obesity-prone RFX rats had significantly longer body length in comparison to the NRCF, RC and RIM groups. The obesity-prone RFX and RIM rats had significantly larger body circumference in comparison to two control groups. The RFX group were significantly less anxious and had heavier bones.

**Conclusions:** Our data suggest that the association between stress, exposure to antidepressant treatment, and the long-term intake of an obesogenic high-fat diet is associated with greater weight gain, bone weight and body length in obese-prone RFX rats. We show that animals with antidepressant exposure had a greater degree of weight gain after long-term exposure to an obesogenic diet than animals on the same diet, but without exposure to antidepressants. We advance here the novel concept that antidepressant exposure represents a long-term risk factor for obesity and present the testable hypothesis that antidepressant exposure might be a major hidden contributor to our current obesity epidemic.

**Keywords:** Antidepressants, Obesity, Major depression

**Disclosures:** Nothing to disclose.

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**T59. Neural Correlates of Attention Bias in Irritability and Anxiety**

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**Background:** There is substantial clinical and pathophysiological overlap between anxiety disorders and severe irritability (Beesdo et al., 2009; Brotman et al., 2010). Clinically, anxious youth have high levels of irritability (Stoddard et al., 2014), and irritable youth have high rates of anxiety disorders (Deveney et al., 2015). Indeed, longitudinal studies suggest that early irritability may be a precursor to later anxiety disorders.
(Stringaris et al., 2009). Moreover, behavioral tasks demonstrate that both youth with anxiety disorders and those with irritability demonstrate an attention bias towards threatening (i.e., angry) faces (Bar-Haim et al., 2007; Hommer et al., 2014).

Whereas functional magnetic resonance imaging (fMRI) studies in anxiety find dysfunction in regions associated with emotional processing (e.g., amygdala) and attention [e.g., ventrolateral prefrontal cortex (vPFC), dorsolateral prefrontal cortex (dPFC)] (Monk et al., 2008), researchers have yet to explore the neural correlates of threat bias in youth with irritability.

The purpose of the present investigation is to examine the neural correlates of attention bias to threat in youth with varying degrees of irritability across a range of diagnoses and healthy comparison (HC) youth. Integrating dimensional and diagnostic categorical approaches, we hypothesized that the pathophysiology of irritability in disruptive mood dysregulation disorder (DMDD), anxiety (ANX) and attention deficit hyperactivity disorder (ADHD) would differ from each other and HC youth. Specifically, we anticipated that regions previously implicated in threat processing and salience detection (i.e., amygdala, vPFC and dPFC) would show between-group differences in activation with increasing levels of irritability.

**Methods:** fMRI data were acquired from 110 participants (9-19 years), including DMDD (N = 28), ANX (N = 30), ADHD (N = 22) and HC (N = 30) youth. Parents and children completed the Affective Reactivity Index (ARI; Stringaris et al., 2012) to assess irritability. We used a dot probe task to ascertain the impact of a threat cue on attention function. Youth viewed two facial expressions simultaneously (angry-neutral or neutral-threat). Following the face-pair, a probe replaced either the angry face (i.e., congruent trials) or neutral face (i.e., incongruent trials for angry-neutral trials). Neutral-neutral face trials served as the control. Participants were instructed to respond to the probe location as quickly and accurately as possible.

To examine the neural correlates of threat bias, we conducted two whole-brain corrected ANOVAs. First, we conducted a Diagnosis (DMDD, ADHD, ANX, HC) x Irritability (mean parent and child ARI score) x Condition (incongruent, congruent, control) ANOVA. Second, we examined diagnostic between-group differences on the incongruent vs. congruent contrast.

**Results:** Groups did not differ on age, IQ, or sex distribution. There were no between-group differences in behavioral threat bias (i.e., mean reaction time on incongruent trials – mean reaction time on congruent trials). Whole-brain analysis revealed a significant Diagnosis x Irritability x Condition interaction in the dPFC (k = 89 voxels, p < 0.005 uncorrected). During neutral-neutral trials, higher levels of irritability were associated with lower dPFC activation in DMDD (p = 0.001), but with greater dPFC activation in HC youth (p = 0.03). Moreover, in ANX youth, higher levels of irritability were associated with greater dPFC activation during angry congruent trials (p = 0.04). Three whole-brain corrected clusters (inferior parietal lobule, k = 131, p < 0.005; middle temporal gyrus, k = 458, p < 0.005; and precentral gyrus, k = 139, p < 0.005) showed significant Diagnosis x Condition interactions. In all three regions, the same pattern emerged; compared to other patient groups, DMDD demonstrated hyperactivation during incongruent relative to congruent trials (all ps < 0.05), whereas ANX showed hypoactivation in this contrast relative to HC youths (all ps < 0.005).

**Conclusions:** Using a dot probe task, we compared the neural correlates of attention bias to threat in DMDD, ANX, ADHD and HC youth with varying levels of irritability. The dPFC has been repeatedly implicated in the pathophysiology of anxiety and threat detection (Monk et al., 2008). Here, we found the neural signature of irritability varied by diagnosis. Whereas higher levels of irritability were marked by decreasing dPFC activation in DMDD, irritability in HC youth was associated with increasing dPFC activation. We also found neural dysfunction in the inferior parietal lobule, which is involved in the perception of face emotions (Sarkheil et al., 2013) and spatial attention (Corbetta et al., 2000). During incongruent compared to congruent trials, DMDD showed greater activation than the other diagnostic groups, whereas ANX and ADHD showed hypoactivation relative to HC youth. Additional work is needed to disaggregate shared and unique neural mechanisms of irritability and anxiety. Moreover, functional connectivity analyses are needed to characterize network interactions among the regions identified in this study.

Finally, future research should explore potential treatment implications [e.g., Attention Bias Modification Training (ABMT)] of these findings for youth with severe irritability.

**Keywords:** attentional bias, irritability, Anxiety, fMRI

**Disclosures:** Nothing to disclose.

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**Abstracts**

**T60. Sex Differences in Nucleus Accumbens Transcriptome Profiles Associated with Susceptibility vs Resilience to Sub-Chronic Variable Stress**

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**Background:** Depression and anxiety disorders are more prevalent in females, but the majority of research in animal models, the first step in finding new treatments, has focused predominantly on males. There is growing concern that off-target effects and insufficient treatment efficacy are due in part to sex differences. The studies presented here examine transcriptional sex differences in reward circuitry that mediate susceptibility and resilience to stress. These data suggest that stress induces different transcriptional profiles in males and females and these sex differences are regulated by epigenetic mechanisms.

**Methods:** Using the subchronic variable stress (SCVS) model, which consists of 6-days of alternating stressors, we explored sex differences in stress responses on a battery of depression- and anxiety-like behaviors and on transcriptional regulation in the nucleus accumbens (NAc) with RNA sequencing (RNA-seq). To determine the mechanism regulating these sex differences in transcriptional regulation we examined a time course of Dna methyltransferase (Dnmt) expression in mice and validated our results in...
post mortem tissue from human patients with a diagnosis of major depressive disorder. To determine the functional relationship between DNA methylation, stress susceptibility and transcriptional regulation we used viral mediated knockout strategies to manipulate DNA methyltransferase (DNMT) 3a levels in NAc and then exposed animals to SCVS. Transcriptional profiles and depression/anxiety-like behavior was measured in males and females.

Results: We show here that exposure to SCVS induces depression-associated behaviors in female mice, whereas males appear resilient in that they do not develop these behavioral abnormalities. In concert with these different behavioral responses, transcriptional analysis in NAc by RNA-seq revealed markedly different patterns of stress regulation of gene expression between the sexes. Among the genes displaying sex differences was DNA methyltransferase 3a (Dnmt3a), which is expressed at higher levels in female NAc at baseline and shows a greater induction in females after SCVS. Interestingly, Dnmt3a expression levels were increased in the NAc of depressed humans, an effect seen in both males and females. NAc specific Dnmt3a knockdown rendered females more resilient, directly implicating this gene in stress responses. Associated with this enhanced resilience of female mice upon NAc knockdown of Dnmt3a was a partial shift of the NAc female transcriptome toward the male pattern after SCVS.

Conclusions: These data indicate that males and females undergo different patterns of transcriptional regulation in response to stress and that a DNA methyltransferase enzyme in NAc contributes to sex differences in stress vulnerability.

Keywords: Major depression, sex differences, Epigenetics, chronic stress

Disclosures: Nothing to disclose.


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Background: Low-affinity, high-capacity transporters for biogenic amines help regulate neurotransmitter homeostasis. They include the plasma membrane monoamine transporter (PMAT) and three organic cation transporter isoforms (OCT1-3). Our published data, using decynium-22 (D22), a non-selective blocker of PMAT and OCTs, suggests OCT3 and/or PMAT limit the therapeutic efficacy of selective serotonin reuptake inhibitors (SSRIs). To aid in discerning the precise transporter(s) involved, we have characterized newly synthesized D22 analogs to identify PMAT and OCT isoform selective compounds.

Methods: Effects of ANSTO analogs on locomotor activity and antidepressant-like behaviors were assessed in adult male, wild type (WT) mice using beam break measurements and the tail suspension test (TST). The activity of 7 analogs (ANSTO 301-307) to inhibit [3H]MPP+ uptake were measured in synaptosomes and HEK cells stably expressing human PMAT, OCT2 or OCT3.

Results: Unlike D22, locomotor activity was not fully suppressed at higher doses up to 1.0 mg/kg, of ANSTO analogs 301, 303-306, and up to 10 mg/kg for ANSTO 307. Similarly to D22, ANSTO compounds (up to 1.0 mg/kg) did not show an antidepressant-like effect in the TST when given alone, however at 3.2 mg/kg, ANSTO 305 and 306 produced antidepressant-like effects. In synaptosomes, all ANSTO compounds, with the exception of analogs 303 and 306, inhibited [3H]MPP+ uptake more potently than D22. In contrast, ANSTO compounds were less potent than D22 and shifted [3H]MPP+ uptake 1- or 2 log-rightward in OCT3-HEK cells. ANSTO analogs displayed similar potencies to corticosterone, which is a blocker of OCT3. ANSTO analogs inhibited [3H]MPP+ uptake through OCT3 more favorably than PMAT (except analog 302) and OCT2 (except analogs 301 & 302).

Conclusions: The significantly enhanced potency for several ANSTO compounds to inhibit [3H]MPP+ uptake in OCT3-HEK cells suggests these compounds may have higher affinity for OCT3 than PMAT or OCT2. Studies measuring behavioral outcomes and competition assays with an SSRI in WT, OCT3 knockout & PMAT knockout mice for physiological comparisons to cell overexpression systems are underway. These studies will reveal more about the pharmacological profile of these novel compounds and their potential therapeutic application to treat disorders, such as depression and drug abuse.

Keywords: organic cation transporters, uptake 2, Antidepressants, inhibition

Disclosures: Nothing to disclose.

T62. Baseline Differences in Two Depressed Populations: Major Depression with Mixed Features vs Bipolar I Depression

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Background: Accumulating evidence indicates that manic symptoms, below the threshold for hypomania (mixed features), are common in individuals with major depressive disorder (MDD). Mixed MDD has been conceptualized as a severe variant of “pure” MMD, however, little is known about the 2 populations with respect to their clinical and demographic features. Studies indicate that MDD with mixed features (compared with pure MDD) is often more severe, and is associated with an increased risk for recurrence, suicide attempts, substance abuse, and functional disability. The aim of this post hoc analysis was to compare baseline demographic and clinical characteristics for two samples of convenience comprising patients entering two short-term clinical trials: an MDD-mixed patient group and a bipolar I depression group.

Methods: In the MDD-mixed study, patients meeting DSM-IV-TR criteria for MDD, with no history of bipolar disorder, who presented with 2 or 3 protocol-specified manic
symptoms, were randomized to 6 weeks of double-blind treatment with either lurasidone 20-60 mg/d (N = 109) or placebo (N = 100). In the second study, patients meeting DSM-IV-TR criteria for bipolar I depression were randomized to 6 weeks of double-blind treatment with either lurasidone 20-60 mg/d (N = 166), lurasidone 80-120 mg/d (N = 109) or placebo (N = 170). The MDD-mixed study required a Montgomery-Asberg Depression Rating Scale (MADRS) total score ≥ 26, and the bipolar depression study required a MADRS ≥ 20, however for the purposes of comparison, the subgroup of patients with a baseline MADRS total score ≥ 26 were analyzed (N = 405/485; 83.5%). In addition, the bipolar depression study limited entry to patients with a Young Mania Rating Scale (YMRS) total score ≤ 12.

Results: At Baseline, the demographic and clinical characteristics of the MDD-mixed (N = 209) vs bipolar depressed (N = 405) populations were as follows: male (30.6% vs 42.2%), mean age (44.9 vs 41.9 years), proportion of patients with ≥ 4 prior psychiatric hospitalizations (12.4% vs 14.1%), mean MADRS total score (33.3 vs 31.8), mean YMRS total score (10.7 vs 4.4), mean HAM-A total score (16.9 vs 16.6) and mean SDS total score (20.2 vs 20.3). At Baseline, the mean MADRS item scores for the MDD-mixed vs bipolar depressed populations were as follows: apparent sadness (4.0 vs 3.8), reported sadness (4.4 vs 4.1), inner tension (3.3 vs 2.9), reduced sleep (4.2 vs 3.7), reduced appetite (3.0 vs 2.7), concentration difficulties (3.9 vs 3.6), lassitude (3.6 vs 3.7), inability to feel (3.8 vs 3.6), pessimistic thoughts (2.5 vs 2.8) and suicidal thoughts (0.5 vs 0.9). At Baseline, the mean YMRS item scores for the MDD-mixed vs bipolar depressed populations were as follows: elevated mood (0.4 vs 0.05), increased activity/energy (1.0 vs 0.11), sexual interest (0.2 vs 0.04), sleep (2.0 vs 1.5), irritability (1.9 vs 1.5), rate/amount of speech (2.1 vs 0.07), language/thought disorder (1.3 vs 0.3), content (0.4 vs 0.07), disruptive-aggressive behavior 0.7 vs 0.4), appearance (0.5 vs 0.4) and insight (0.2 vs 0.5).

Conclusions: In this baseline comparison of two patient groups entering separate clinical trials, demographic and clinical characteristics were similar, with the exception of a higher proportion of females in the MDD-mixed versus the bipolar I depression population. The pattern of symptom severity observed on individual MADRS items was also similar for patients in both populations, suggesting that these two illnesses are closely related.

Clinicaltrials.gov: NCT01421134 and NCT00868699.

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Keywords: Bipolar I Depression, Major Depressive Disorder (MDD), atypical antipsychotic drug

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T63. Inflammasome Signaling Affects Anxiety- and Depressive-Like Behaviors and Gut Microbiome Composition

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Background: The inflammasome is hypothesised to be a key mediator of the response to physiological and psychological stressors, and its dysregulation could be implicated in the development of major depressive disorder. Inflammasome activation causes the maturation of caspase-1 and activation of interleukin (IL)-1β and IL-18, two pro-inflammatory cytokines involved in neuroimmunomodulation, neuroinflammation, and neurodegeneration. In this study, mice with genetic deficiency or pharmacological inhibition of caspase-1 (casp1) were screened for anxiety-like, depressive-like and locomotor activity at baseline and after chronic stress. The microbiota-gut-brain (MGB) axis is a complex multi-organ bidirectional signaling system between the microbiota and the brain that plays a fundamental role in host physiology, homeostasis, development, metabolism and behavior. A growing body of work shows reproducible and consistent effects of microbial states on mice behavior, supporting a role for microbiota in modulating behavior.

Methods: Mice with genetic deficiency or pharmacological inhibition of caspase 1 (casp1) were screened for anxiety-like, depressive-like and locomotor activity at baseline and after chronic stress. Fecal pellets were collected and gut microbiota was evaluated in wild-type mice after stress with pharmacological inhibition of casp1 and compared to controls.

Results: Genetic deficiency of casp1 decreased depressive- and anxiety-like behaviors, and increased locomotor activity and skills. Moreover, casp1 deficiency prevented the exacerbation of anxiety-like behaviors following chronic stress; furthermore, pharmacological casp1 antagonism prevented stress-induced increase in depressive-like behavior. Restraint stress or pharmacological inhibition of casp1 affected fecal microbiome composition and were both associated with a dysbiotic state. Analysis of individual bacterial taxon relative abundance provided evidence of both synergistic and antagonistic effects of chronic restraint and casp1 inhibition.

Conclusions: Casp1 inhibition has a protective effect modulating the relationship between stress and microbiota composition, which support the concept of a microbiota-gut-inflammasome-brain (MGB) axis, in which the gut microbiota via the inflammasome signaling platform modulates inflammatory pathways that will alter brain function and affect anxiety- and depressive-like behaviors. Our data suggest that the MGB
axis represents a potential new target for antidepressant treatment.

**Keywords:** Gut Microbiome, Depression, Anxiety

**Disclosures:** Nothing to disclose.

### T64. Epigenetic SLC1A2 Promoter Hypomethylation in Bipolar Disorder with Comorbid Addiction

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**Background:** Several genes involved in the glutamate-glutamine system regulation, including SLC1A2 encoding excitatory amino acid transporter 2 (EAAT2), the principal transporter normalizing or clearing excessive synaptic glutamate, were identified as candidate genes increasingly implicated in the underlying neurobiology of bipolar disorder (BD), depression, and alcohol addiction. DNA methylation of the SLC1A2 promoter highly correlates with EAAT2 mRNA expression indicating a prominent role for epigenetic regulation.

Here we present the expansion of our initial studies on methylation changes in the 5’ UTR of SLC1A2 promoter between -1759 and -1468 or a group of 150 BD patients and 32 non-psychiatric subjects to include an additional region of the SLC1A2 promoter but also to analyze for interactions with multiple demographic and clinical variables.

**Methods:** In this IRB-approved study, a subset of 150 BD subjects from the Mayo Clinic Bipolar Biobank completed questionnaires for demographic and illness-related variables, provided DNA samples, and were divided into five groups: BD without comorbid alcohol use disorder (AD), nicotine use disorder (ND), or binge eating disorder (BE) (n = 30); BD + AD (n = 30); BD + ND (n = 30); BD + ND + AD (n = 30); and BD + BE (n = 30). Participants from the Mayo Clinic Community Biobank were used as controls (n = 32). DNA was extracted from the peripheral blood. Bisulfite conversion and methylation-sensitive high resolution melt (HRM) PCR (performed in triplicate) were used to compare methylation status of the SLC1A2 promoter. Two regions in the 5’ UTR of the SLC1A2 promoter CpG island were examined [-1759 to -1468 (forward primer: TTTAGTT AGAAGGTGGTGAAGATTTAAGTT; reverse primer: AAAC AAAAAAACCTCACTTTCCTC) for 32 non-psychiatric subjects and 150 BD; and -785 to -654 (forward primer: AAT AGTATTTTTATATTT; reverse primer: ATCCCTCCTC TACCATCCTCC) for a subset of 15 non-psychiatric subjects and 75 BD].

Statistical analyses performed were one-way analysis of variance (ANOVA) to examine an overall difference across the six groups, followed by pairwise post hoc tests adjusted for multiple comparisons using Bonferroni’s method. A general linear regression model was used to examine the specific effects of BD, AD, ND, and BE.

**Results:** CpG island between -1759 and -1468 in the 5’ UTR of the SLC1A2 One-way ANOVA revealed a statistically significant difference in methylation measured by HRM PCR between the groups (p = 0.0003). Comparison of the mean melting temperatures using Bonferroni adjusted t-tests showed lower temperatures (hypomethylation) in BD with comorbidities [AD (p = 0.025), ND (p = 0.001), AD + ND (p = 0.006), and BE (p = 0.001)] than in the BD only group. In the general linear regression model ND (p = 0.014) and BE (p = 0.0006) predicted hypomethylation while AD approached (p = 0.053) significance. After adjusting for age and gender, the impact of ND (p = 0.0009) and BE (p = 0.0002) remained statistically significant. Methylation temperature was significantly higher in females (p = 0.036). There was no significant effect of any illness measure other than mood instability sum (p = 0.03).

CpG island between -785 and -654 in the 5’ UTR of the SLC1A2 gene HRM PCR methylation analysis in a subset of five BD subgroups with and without addiction comorbidities (n = 75) and non-psychiatric subjects (n = 15) tested by one-way ANOVA revealed a statistically significant difference between the groups (p < 0.0001). Comparison of the mean melting temperatures using Bonferroni adjusted t-tests showed decreased temperatures in BD with comorbid AD (p = 0.025), AD + ND (p < 0.001), and BE (p < 0.001) compared with the BD only group. In the general linear regression model, the presence of BD was a predictor of hypermethylation (p = 0.035), whereas AD (p < 0.001), ND (p = 0.011), and BE (p < 0.001) predicted hypomethylation. There was no significant effect of demographic and clinical variables on methylation levels.

**Conclusions:** DNA methylation is altered in two distinct regions of SLC1A2 promoter of BD patients. For both regions, comorbidity with addiction to alcohol, nicotine, and food had a significant impact on the level of promoter hypomethylation, while BD predicted hypermethylation in one of the promoter regions. These molecular findings are in accord with increasingly recognized clinical observations that comorbidities, including AD, ND, and BE, may more narrowly define the phenotype in BD, which in turn may lend more focused study to epigenetic regulation.

Limitations of this study are single technique used to assess the methylation status and paucity of data on epigenetic changes in the SLC1A2 promoter in brains of BD patients. To overcome these shortcomings, we are currently mapping the methylation sites using direct sequencing. Also, post-mortem human brain studies of SLC1A2 promoter are planned for the near future.

Epigenetic rather than genetic features more dynamically reflect disease progression and have the potential for future development of both diagnostic and treatment response biomarkers. Meanwhile, excellence in clinical phenotyping remains essential for identifying comorbidities and individualized treatment selection in persons living with BD.

**Keywords:** glutamate, EAAT2, Bipolar Disorder, Epigenetics

**Disclosures:** Nothing to disclose.

### T65. The Role of Extracellular Signal-Regulated Kinases in the Antidepressant-Like Effects of Scopolamine

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**Background:** Scopolamine, a nonsubtype selective muscarinic receptor antagonist, has been shown to have robust,
rapid acting antidepressant effects in the clinic, however, little is known about its mechanism of action downstream to muscarinic receptor blockade. Recently, several diverse rapid acting antidepressant treatments, including ketamine, repetitive transcranial magnetic stimulation, and electroconvulsive shock have been shown to active extracellular signal-related kinases 42 and 44 (ERK). The activity of ERK is regulated by phosphorylation and de-phosphorylation, with phospho-ERK (pERK) being the activated form. We hypothesized that scopolamine might also work by rapidly increasing pERK, which might be a convergent immediate intracellular action common to rapid acting antidepressants. The purpose of this study was to examine the role of ERK signaling in the pathogenesis of depression and antidepressant action of scopolamine in animal models.

Methods: The effect of scopolamine (1.5 mg/kg i.p.) on rapid accumulation of pERK relative to total ERK in mouse frontal cortex and hippocampus 30 min after injection was determined by Western blot. Levels of pERK relative to total ERK were measured in prefrontal cortex and hippocampus in rats subjected to the learned helplessness paradigm and compared to control rats. A follow up study tested the effects of scopolamine (1.5 mg/kg i.p., 30 min) or vehicle in learned helpless, with frontal cortex and hippocampus collected after the test for analysis of pERK levels for comparison to control rats. M1 receptors are coupled to Gq and have been reported to stimulate pERK. The effects of an M1 positive allosteric modulator (PAM), 10 mg/kg s.c., on pERK was determined by itself and in combination with scopolamine. A subthreshold dose of the M1 PAM (2.5 mg/kg) combined with a subthreshold dose of scopolamine (0.025 mg/kg s.c.) was tested on immobility time in tail suspension.

Results: Rats subjected to the learned helpless paradigm, to induce a depressed-like state, had significantly decreased pERK levels in frontal cortex and hippocampus compared to naïve rats, with no difference in total ERK levels. Scopolamine increased pERK levels in frontal cortex and hippocampus at 30 min without affecting total ERK levels (p<0.04, Duncan’s post-hoc compared to vehicle on treatment x analyte interaction). Scopolamine was able to significantly attenuate helpless-like behavior in the learned helpless test and increase number of escapes. Scopolamine treated animals did not have significantly decreased pERK levels compared to control animals, but did have significantly increased pERK levels, but not total ERK, compared to vehicle-treated helpless animals. The M1 positive allosteric modulator (PAM) had no effect on pERK per se at the dose tested, but significantly potentiated scopolamine-induced pERK in hippocampus, but not frontal cortex. A low dose of the M1 PAM that had no effect on locomotor activity or in tail suspension by itself was able to potentiate the effect of an inactive dose of scopolamine in tail suspension (p<0.03, Duncan’s post-hoc comparing M1 PAM + scopolamine to vehicle + scopolamine and p<0.03, Duncan’s post-hoc comparing M1 PAM + scopolamine to vehicle + scopolamine, on treatment x treatment interaction).

Conclusions: These data suggest that depressed-like states are associated with decreased ERK signaling in hippocampus and frontal cortex. Scopolamine is able to stimulate ERK signaling in hippocampus and frontal cortex while having antidepressant-like effects in tail suspension and learned helplessness. A drug which is able to potentiate scopolamine-induced ERK signaling in hippocampus is sufficient to potentiate an inactive dose of scopolamine in an animal model of depression. Thus, increased ERK signaling may be involved in the rapid acting antidepressant-effect of scopolamine, similar to other rapid acting agents.

Keywords: muscarinic acetylcholine receptor, Fast-acting Antidepressant, Scopolamine, kinases, Animal Models

Disclosures: The authors are employees of Janssen Research and Development.

T66. Effects of Gonadal Steroids on Mood and Emotion Processing in Women with a History of Postpartum Depression

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Background: Neuroendocrine factors are purported to play a role in the etiology of postpartum depression (PPD), but direct evidence for this role is lacking. We are currently investigating the effects of changes in gonadal steroid levels on brain function and mood by simulating two hormonal states related to pregnancy and parturition, respectively, in euthymic women with and without a history of postpartum depression. Here we present preliminary data from this ongoing pharmaco-fmri study.

Methods: Participants included non-pregnant, euthymic women with a history of PPD (hxPPD; n=6) and those without such a history (controls; n=12). The supraphysiological gonadal steroid levels of pregnancy and withdrawal from these high levels to a hypogonadal state were simulated by inducing hypogonadism with the gonadotropin-releasing hormone agonist leuprolide acetate, adding back supraphysiologic doses of estradiol and progesterone for 8 weeks (‘addback’), and then withdrawing both steroids under double-blind conditions (‘withdrawal’). Functional magnetic resonance images (fMRI) were collected at the baseline session, which took place during the early- to mid-follicular phase, and during withdrawal (two weeks after the last hormone dose). Participants performed a monetary incentive delay task ( MID; Knutson et al., 2000), to probe the neural circuits implicated in reward processing, and an emotional face-matching task (EFMT; Hariri et al., 2002) to probe the neural circuits implicated in emotional arousal. Whole brain analyses were conducted to examine group differences in percent signal change in the BOLD response during the presentation of reward versus non-reward during the MID and of the faces versus shapes during the EFMT. Analyses were conducted using FMIRB’s Local Analysis of Mixed Effects (FLAME) within the fMRI Expert Analysis Tool (FEAT). Group-level activation maps were thresholded using a z score of 2.3 (p<.01) to define contiguous clusters of activation. We examined self-reported mood symptoms during all three hormone states (baseline, addback, and withdrawal) using the Inventory of Depression and Anxiety Symptoms (IDAS; Watson et al.,...
2007). A repeated measures ANOVA was used to examine effects of group and hormone states on mood symptoms.

**Results:** During the MID reward (versus non-reward) anticipation, there were significant group x hormone state interaction effects in the left nucleus accumbens, orbitofrontal cortex, and precuneous. During the MID reward (versus non-reward) outcome, there were significant group x hormone state interaction effects in the left caudate and superior frontal gyrus. During the EMFT (faces versus shapes), there were significant group x hormone state interaction effects in the caudate, putamen, accumbens, and frontal pole. In general, hxPPD showed increased activation during hormone withdrawal relative to baseline (both MID and EMFT), whereas controls showed no change. There also were significant group x hormone state interaction effects on self-reported dysphoria ($F = 6.6, p < .02$), ill temper ($F = 9.5, p < .007$), and panic ($F = 8.4, p < .01$). hxPPD reported increased mood symptoms during hormone addback and withdrawal (compared with baseline), whereas controls showed no change in mood symptoms over time.

**Conclusions:** Although preliminary, our data provide direct, experimental evidence for the role (and potential mediating mechanisms) of the gonadal steroids estradiol and progesterone in the development of postpartum depression in a subgroup of women. Reproductive hormone changes were associated with dysregulation of the neural circuits underlying emotional arousal and reward processing and consequent depressive symptoms in hxPPD but not controls. Thus, these neural circuits may underlie both the susceptibility to and mediation of hormone-related affective dysfunction. Understanding these neurobiological mechanisms will subsequently improve our ability to identify those at risk for PPD.

**Keywords:** postpartum depression, estrogen, progesterone, functional magnetic resonance imaging, neuroendocrinology

**Disclosures:** Nothing to disclose.

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**T67. CRH Acts Anxiolytic by Modulating Dopamine Release through a Subset of GABAergic Long-range Projection Neurons**


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**Background:** Dysregulated and/or hyperactive corticotropin-releasing hormone (CRH) circuits were shown to be involved in neuroendocrine and behavioural disturbances of stress-related psychiatric disorders including anxiety and depression. While the role of CRH as an indispensable initiator of the neuroendocrine cascade of the hypothalamic-pituitary-adrenal (HPA) axis is well defined, we are just starting to comprehend the function of extrahypothalamic CRH with regards to emotionality and behavioral responses to stress. In this regard, we were recently able to provide a clearer understanding about the interaction of CRH and other neurotransmitter systems by unravelling that anxiety-related behaviour is modulated by an imbalance between CRH receptor 1 (CRHR1)-controlled anxiogenic glutamatergic and anxiolytic dopaminergic circuits (Refojo et al., Science 2011). However, the identity of CRH-releasing neurons and sites of CRH action that modulate anxiolytic behavioral responses through interactions with the dopaminergic system have not been fully established yet.

**Methods:** Double in situ hybridization against Crh and different neuronal markers (Vglut1, Vglut2, Gad65/67 and Camk2α) was used to characterize the identity of Crh neurons across the brain. Crh-ires-Cre mice were bred to Cre-dependent reporter mice in order to assess the morphology of different CRH subpopulation. In order to determine the origin of CRH-positive VTA inputs, Cre-dependent AAV-mediated anterograde tracers were injected into the most prominent CRH-expressing brain regions (amygdala (CeA), bed nucleus of the stria terminalis (BNST), paraventricular nucleus of the hypothalamus (PVN) and hippocampus) of Crh-ires-Cre mice. Additionally, we conducted imaging with CLARITY to visualize the strongest CRH-VTA projections in the intact mouse brain. Using homologous recombination in embryonic stem cells, we generated conditional Crh knockout mice. These were subsequently bred to different Cre-recombinase lines to target specific CRH populations. All conditional Crh knockout mice underwent a battery of behavioral test assessing anxiety-related behavior as well as cue-dependent and contextual fear memory. Possible alterations in dopamine release were investigated with microdialysis under baseline conditions, and following foot shock stress.

**Results:** We identified that cortical and limbic CRH is primarily expressed in GABAergic neurons, which exhibited distinct morphologies depending on the investigated brain region. CLARITY and viral-mediated anterograde tracing studies revealed that a subset of GABAergic CRH neurons in the BNST and CeA send long-range projecting axons to distant brain regions including the ventral tegmental area (VTA), which harbors the majority of CRHR1-expressing dopaminergic neurons. We found that deletion of Crh from GABAergic long-range projection neurons in the CeA and BNST diminished dopamine release in the prefrontal cortex (PFC), and enhanced anxiety and fear memory expression, implicating that this specific CRH circuit is required under physiological conditions to maintain a positive emotional state. Accordingly, we previously observed that deletion of Crhr1 from dopaminergic neurons produces similar behavioral effects and also diminished dopamine release in the PFC.

**Conclusions:** Our data suggests that a subset of CRH-expressing GABAergic projection neurons in the extended amygdala target CRHR1 on dopaminergic neurons to modulate emotional behavior by regulating dopaminergic neurotransmission. This reveals a previously unidentified anxiety- and fear-suppressing CRH circuit which acts by positively regulating dopamine release.

**Keywords:** CRH, Anxiety, Stress circuits, Dopaminergic system

**Disclosures:** Nothing to disclose.
Disclosures: Nothing to disclose.

Keywords: Conclusions: As compared to the rats treated with the vehicle only, DSAP were more immobile during both D1 and D2 of the FST. In addition, A2 cell group significantly more noradrenergic neurons were activated in the Wistar than in WKY rats at D1. At D2, WKYS also showed attenuated FC between the left ventral striatum and bilateral anterior temporal pole. There were no significant between-group differences for the right ventral and dorsal striatum seeds. Among the MDD group, treatment with escitalopram specifically increased the FC of the left dorsal striatum with the left dorsolateral prefrontal cortex (DLPFC).

Conclusions: These findings suggest that the altered resting state FCs of both dorsal and ventral striatum may be related to the pathophysiology of MDD. Our preliminary data also suggest that SSRI treatment in MDD is associated with changes in a DLPFC-dorsal striatum loop involved in executive function, representing a neural mechanism through which SSRIs may exert their actions. These study provide further evidence for a role of the corticostriatal circuits in the pathophysiology of MDD.

Keywords: Major Depressive Disorder (MDD), SSRI, functional connectivity, striatum

Disclosures: Nothing to disclose.

T68. A2 Noradrenergic Neurons Regulate Forced Swim Test Immobility
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Background: The Wistar Kyoto rat (WKY) is an established animal model of depression- and anxiety-like behavior, characterized by high immobility during the forced swim test (FST) along with a generally inhibited phenotype on related tests of emotional behaviors. Extensive literature indicates that deficits in noradrenergic neurotransmission may contribute to these behavioral traits. Previously, we have reported that the WKY rats are more immobile compared to other rat strains from the beginning of their training phase of the FST, and that they become even more immobile during the testing phase on the next day. We hypothesized that higher immobility during the FST and the greater increase in immobility throughout different phases of the FST are two separate components of rats’ behavior likely mediated by different central mechanisms. We sought to identify the central circuits responsible for these behavioral components by studying activation of neurons within central noradrenergic cell groups during different phases of the FST.

Methods: The WKY rats along with its parent strain, Wistar rats that experienced either the: 1) 5 minutes training phase (D1), or 2) entire FST (D1 and D2) were compared.

Results: Using double immunocytochemistry for tyrosine hydroxylase (TH) and c-Fos, we determined that within the A2 cell group significantly more noradrenergic neurons were activated in the Wistar than in WKY rats at D1. At D2, WKYS increased their activation of the A2 noradrenergic neurons, and c-Fos expression within TH-immunoreactive neurons was equivalent to that of the Wistar group. Based on these results, we further investigated the role of A2 cell group during the FST using anti-dopamine beta hydroxylase-conjugated saporin (DSAP) to selectively destroy noradrenergic neurons within the area. The Wistar rats treated with DSAP were more immobile during both D1 and D2 of the FST as compared to the rats treated with the vehicle only.

Conclusions: Together these data indicate that the A2 noradrenergic cell group regulates FST immobility in rats, and that its activation may contribute to the unique behavioral phenotype of WKY rats. Future experiments aimed at temporally restricted activation and inhibition of A2 noradrenergic neurons will be required to fully elucidate their role in mediating behavioral despair and learned helplessness.

Keywords: rat, Depression, behavioral despair
Disclosures: Nothing to disclose.

T69. Functional Connectivity of Striatum and SSRI Treatment in Major Depressive Disorder
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Background: Altered function of corticostriatal circuits has been implicated in the pathophysiology of Major Depressive Disorder (MDD). Resting-state functional magnetic resonance imaging (fMRI) has been successfully used to map brain corticostriatal circuits, providing clear evidence for the existence of functionally organized dorsal executive and ventral affective circuits. However, the contribution of dorsal versus ventral corticostriatal network alterations to the pathophysiology of MDD remains unclear, and even less is known about the effects of selective serotonin reuptake inhibitor (SSRI) treatment on the resting state functional connectivity (FC) of dorsal versus ventral striatum.

Methods: In this study, resting-state fMRI was performed on 22 patients with MDD at baseline and following 6 weeks treatment with escitalopram, an SSRI. Twenty-two age and sex matched healthy controls (HCs) were also received the resting-state fMRI. We investigated FCs of dorsal and ventral subdivisions of bilateral striatum, and voxelwise statistical maps of each subregion’s FC with other brain areas were compared. The study was conducted under a protocol that was approved by the Ethics Committee of Hiroshima University. All participants gave informed consent prior to participation in the study.

Results: Relative to the HCs, the MDD group exhibited attenuated FC between the left dorsal striatum and the cerebellum and stronger FC between the left dorsal striatum and the anterior-medial prefrontal cortex. Patients with MDD also showed attenuated FC between the left ventral striatum and bilateral anterior temporal pole. There were no significant between-group differences for the right ventral and dorsal striatum seeds. Among the MDD group, treatment with escitalopram specifically increased the FC of the left dorsal striatum with the left dorsolateral prefrontal cortex (DLPFC).

Conclusions: These findings suggest that the altered resting state FCs of both dorsal and ventral striatum may be related to the pathophysiology of MDD. Our preliminary data also suggest that SSRI treatment in MDD is associated with changes in a DLPFC-dorsal striatum loop involved in executive function, representing a neural mechanism through which SSRIs may exert their actions. These study provide further evidence for a role of the corticostriatal circuits in the pathophysiology of MDD.

Keywords: Major Depressive Disorder (MDD), SSRI, resting state fMRI, functional connectivity, striatum

Disclosures: Nothing to disclose.

T70. Antidepressant Onset of Scopolamine in a Mouse Model
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Background: Clinical trials have shown that a single dose of scopolamine, a muscarinic acetylcholine receptor antagonist, has antidepressant effects within 3-5 days. Assay tests in rodent models have corroborated scopolamine’s antidepressant effects, but many of these tests, including the acute forced swim test (aFST) are not sensitive to the length of antidepressant treatment. Therefore, after confirming a dose-response for scopolamine in an acute assay, we sought to examine the onset of scopolamine’s antidepressant effects in a mouse model sensitive to antidepressant onset, the chronic forced swim test (cFST). The cFST requires continuous exposure to the
antidepressant and has been shown to be sensitive to chronic, but not sub-chronic, treatment with classical antidepressants. **Methods:** Nine week old female BALB/cj mice were used for both experiments. In experiment 1, mice were injected intraperitoneally with 0, 0.3, 1, or 3 mg/kg scopolamine (n = 15/group). Mice were tested 30 minutes later in the acute forced swim test. Twenty-four hours later, mice were re-tested in the aFST to determine whether effects were still present a day later. In experiment 2, mice were implanted with subcutaneous mini-pumps attached to a catheter. The catheter was filled with differing volumes of saline followed by 20 mg/kg/d scopolamine to create the following 4 conditions: 3 days of saline, 2 days of saline + 1 day of scopolamine, 1 day of saline + 2 days of scopolamine, or 3 days of scopolamine. The 20 mg/kg/d dose was chosen based on the half-life of scopolamine and the goal of maintaining a steady-state concentration in the range effective in the aFST. On day 3, all mice were tested in the cFST. Behavior was videotaped and the last 4 minutes was scored for time immobile, time swimming, or time climbing. **Results:** In experiment 1, all three doses of scopolamine decreased immobility and increased swimming at the 30 minute time-point, interpreted as an antidepressant effect. Effects on immobility were no longer present when mice were retested 24 hours later. However, there was a decrease in climbing and an increase in swimming at this time-point. In experiment 2, mice receiving 1, 2, or 3 days of continuous scopolamine showed significantly reduced immobility in the cFST. All three treatment durations also increased swimming in the test. **Conclusions:** This study confirms scopolamine’s antidepressant effects in an acute assay and shows that the effects on immobility do not persist 24-hours post-treatment. On the other hand, 24-hours of continuous scopolamine exposure is sufficient to induce antidepressant effects in a model sensitive to antidepressant onset. This study suggests that scopolamine’s onset of antidepressant effect is as early as one day in a mouse model, consistent with anecdotal reports of a therapeutic effect of scopolamine a day after treatment in depressed patients. Importantly, an antidepressant assay and an onset-sensitive test are likely responding to different molecular and neural changes. Knowing scopolamine’s onset is important to ensure that our future mechanistic studies are measured at a time-point to uncover molecular changes underlying an antidepressant effect rather than changes representing an assay-sensitive effect. **Keywords:** Depression, Fast-acting Antidepressant, Animal Models **Disclosures:** Nothing to disclose.

T71. Affective Processing Bias in Unaffected Siblings of Bipolar Disorder Patients

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**Background:** Bipolar disorder (BD) is characterized by emotion processing deficits. In a subset of the present sample, we previously reported that an affective processing bias, measured using an affective Go/No-Go task, is a potential neurocognitive endophenotype in BD. Stable BD patients and their unaffected siblings had a response bias toward negative affective stimuli compared with healthy controls and schizophrenia patients. We aimed to expand our prior finding in a larger sample of BD patients, their unaffected siblings (UAS) and healthy controls (HCs).

**Methods:** An affective Go/No-Go task was used to evaluate inhibitory response to negatively valenced, positively-valenced, and neutral stimuli in 48 BD patients, 29 unaffected siblings and 73 healthy controls. Accuracy (d') and response bias (log beta) served as dependent variables in a series of repeated measures ANCOVAs.

**Results:** We found a significant main effect of subject type on measures of accuracy (d’) when subjects were asked to identify negatively-valenced stimuli (F = 3.44; df = 2; p = 0.035). BD patients were significantly less accurate in the negative condition as compared with HCs (Post Hoc p = 0.01). Premorbid estimates of IQ significantly influenced these results (F = 22, df = 1, p < 0.001; Effect size –partial Eta squared- 0.15); when included as a covariate, the main effect of subject type became non-significant.

When analyzing response bias parameters (logbeta), we found a significant interaction (AGNG condition x subject type) even after covarying for affective symptoms and premorbid IQ (F = 3.3; df = 2; p = 0.038). Specifically, unaffected siblings showed a greater response bias (logbeta) toward negatively-valenced stimuli compared to BD patients and healthy controls.

**Conclusions:** In a larger sample than previously reported, we replicate our previous finding of an affective processing bias toward negatively-valenced stimuli in unaffected relatives of BD patients, which may be a neurocognitive endophenotype. The fact that the BD patients did not differ significantly from HCs on this measure may be due to the fact that they were medicated at the time of testing.

**Keywords:** Bipolar Disorder, emotional response inhibition, emotion processing

**Disclosures:** Nothing to disclose.

T72. Simplifying Acute Tryptophan Depletion (ATD): A New Two Amino Acid Formula Decreases Tryptophan Influx across the Blood-Brain Barrier

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**Background:** Acute Tryptophan Depletion (ATD) is a well-established method in translational brain research used to briefly lower central nervous serotonin (5-HT) synthesis. However, available depletion protocols contain a high number of amino acids and are thus difficult to be composed and monitored. As a consequence, novel and
effective depletion protocols with a simplified amino acid composition are necessary.

Methods: A simplified two amino acid ATD formula consisting of phenylalanine (PHE) and leucine (LEU) was developed to improve tolerance and effectiveness while reducing the overall amount of amino acids (ATD-PHE/LEU). This study investigated tryptophan (TRP) influx rates across the blood-brain barrier (BBB) after ATD-PHE/LEU administration relative to the ATD Moja-De protocol. Seventy-two healthy adults (50% females) were randomized into four groups and administered ATD Moja-De, its TRP-balanced control condition (BAL), ATD-PHE/LEU or its respective control mixture (BAL-PHE/LEU) in a counterbalanced, double blind, between-subjects design. Blood samples were collected at baseline and at hourly intervals for six hours after amino acid intake. Questionnaires about mood, taste and challenge tolerance were completed at fixed time points. The influx of TRP across the BBB was calculated using Michaelis–Menten kinetics with a correction for multiple substrate competition.

Results: Both challenge mixtures significantly reduced central nervous TRP influx relative to baseline and the respective control conditions with only mild and comparable side effects. A greater depletion magnitude after ATD-PHE/LEU administration when compared with ATD Moja-De was detected without group effects for taste, challenge tolerance and mood.

Conclusions: The simplified composition of only two amino acids and the lower overall amino acid amount support the use of the newly developed ATD-PHE/LEU protocol in future research investigating the effects of reduced central nervous 5-HT synthesis. The altered composition of ATD-PHE/LEU, composed of only two LNAAs (PHE and LEU), enabled the reduction in the overall amino acid amount, had acceptable tolerance and ensured simplified blood value monitoring due to the reduced components.

Keywords: Serotonin, Synthesis, humans, Tryptophan depletion, Amino acid mixture

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Together with his former employer (RWTH Aachen University, Germany) FDZ has submitted a patent application which is related to the used new amino acid mixture outlined in this submission. He was the recipient of an unrestricted award donated by the American Psychiatric Association (APA), the American Psychiatric Institute for Research and Education (APIRE) and AstraZeneca (Young Minds in Psychiatry Award). He has also received research support from the German Federal Ministry for Economics and Technology, the European Union (EU), the German Society for Social Pediatrics and Adolescent Medicine, the Paul and Ursula Klein Foundation, the Dr. August Scheidel Foundation, the IZKF fund of the University Hospital of RWTH Aachen University, and a travel stipend donated by the GlaxoSmithKline Foundation. He is the recipient of an unrestricted educational grant, travel support and speaker honoraria by Shire Pharmaceuticals, Germany. In addition, he has received support from the Raine Foundation for Medical Research (Raine Visiting Professorship), and editorial fees from Co-Action Publishing (Sweden). All other authors report no conflicts of interest.

T73. Lateral Habenula and Infralimbic Prefrontal Cortex Differentially Regulate Ventral Tegmental Area Dopamine Neurons: Relevance to Depression

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Background: Dysregulation of the mesolimbic dopamine (DA) system has garnered increasing attention as a key component of major depressive disorder (MDD). It is thought to be particularly relevant to anhedonia, the reduced interest in pleasurable stimuli, which is considered to be a core symptom of MDD. Recent converging studies from our laboratory exposing rodents to either Chronic Mild Stress (CMS) or Learned Helplessness, two stress-induced animal models of depression, resulted in stress-exposed animals showing a reduction in ventral tegmental area (VTA) DA neuron population activity, i.e. the number of DA neurons active and available to respond to environmentally salient rewarding stimuli. This suggests that in MDD, there is a reduced ability of the DA system to respond to rewarding stimuli, which could therefore represent the neural substrate of clinical anhedonia. An important question that remains largely unanswered, however, is what afferent circuitry is driving the altered function of DA neurons in the setting of MDD. An initial study by Chang and Grace (2013) using the CMS rodent model of MDD demonstrated involvement of the basolateral amygdala and ventral pallidum in the decrease in DA neuron population activity that follows CMS. However, the roles of other brain regions established as relevant to MDD in human studies have not yet been examined with regard to their contributions to this CMS-induced DA system hypofunction. Drawing from human neuroimaging research, we identified two candidate regions that were investigated in the present study. The infralimbic prefrontal cortex (ILPFC) is the rodent homologue of human Brodmann Area 25, a region that is established to be key to MDD pathophysiology and is under investigation as a target of deep brain stimulation for treatment resistant depression. Our lab has previously shown that the ILPFC can exert an inhibitory influence over VTA DA neuron population activity, but its role in CMS-induced DA system hypofunction has not yet been assessed. Similarly, the lateral habenula (LHB) more recently has become a focus of intense research efforts in the field due to the region’s strong ability to inhibit the DA system and its recently identified relevance to MDD in human studies. In the present study, we compared the effects of activating the ILPFC or LHB on VTA DA neuron population activity. We then determined whether inactivation of either region in animals exposed to CMS could reverse the induced deficit in VTA DA neuron population activity that occurs following CMS.
Methods: We first performed single-unit extracellular recordings of identified VTA DA neurons from anesthetized rats following LHB or ILPFC pharmacological activation using localized microinfusion of N-methyl-D-aspartate (NMDA), in order to better understand the effect of activating these regions in normal rats. Specifically, we made 9 electrode passes through the VTA in a preset pattern to determine the number of DA neurons firing (population activity), their firing rate and pattern. We then assessed whether inactivation of the ILPFC or LHB using localized tetrodotoxin (TTX) microinfusion in animals that previously underwent 5-7 weeks of CMS (inducing a hypodopaminergic phenotype) would restore DA neuron population activity to normal levels.

Results: Activation of either ILPFC or LHB in normal rats potently suppressed VTA DA neuron population activity (p<0.05), albeit in different patterns. ILPFC activation primarily affected medial VTA DA neurons, whereas LHB activation inhibited more central and lateral VTA DA neurons. In rats that underwent CMS (which impacts primarily medial VTA DA neurons), only ILPFC inactivation restored VTA DA neuron population activity to normal levels, while LHB inactivation had no restorative effect on DA neuron population activity.

Conclusions: These data suggest that the ILPFC and LHB regulate different subpopulations of DA neurons within the mesolimbic system. This appears to have important relevance to understanding the DA system deficits observed in the CMS model of MDD, as this striking pattern of differential regulation appears to explain the unique restorative capacity of ILPFC inactivation in reversing the abnormal DA system hypoactivity observed in this widely used model. Furthermore, these data highlight the importance of the ILPFC as a critical node in depressive circuitry and a potential link between affective and motivational systems in the rodent brain.

Keywords: Dopamine, Depression, Lateral Habenua, infralimbic cortex, chronic mild stress

Disclosures: Dr. Grace receives consulting fees from Johnson & Johnson, Lundbeck, Pfizer, GSK, Merck, Takeda, Dainippon Sumitomo, Otsuka, Lilly, Roche, Asubio, Abbott and receives research funding from Lundbeck, Lilly, Autofon, and Johnson & Johnson. Mr. Moreines and Ms. Owrutsky have nothing to disclose.

T74. Ketamine’s Antidepressant Efficacy is Not Correlated with Baseline Subcortical Volumes in a Major Depressive Disorder Replication Sample

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Background: A single subanesthetic dose ketamine infusion induces a rapid antidepressant response in major depressive disorder (MDD). Several neuroimaging treatment response biomarkers have been identified, but none have been replicated in independent samples. In 13 unmedicated MDD subjects, Abdallah et al. (2015) described a correlation between raw 3-Telsa (T) FreeSurfer-obtained hippocampal volumes and ketamine’s antidepressant efficacy, i.e. smaller hippocampal volumes correlating with better antidepressant efficacy, at 24 hours post-infusion. This association, however, did not survive correction for clinical and demographic variables (total brain volume, handedness, age, gender, height and race). In this study, we attempted to reproduce these findings in a larger MDD sample using several subcortical brain volumes implicated in the pathogenesis and/or antidepressant treatment response. In addition, as the brain-derived neurotrophic factor (BDNF) rs6265 (val6met) single nucleotide polymorphism (SNP) has been associated with hippocampal volume in MDD subjects, we also performed a secondary analysis between this SNP (val/val allele vs. met carriers) and ketamine’s antidepressant response.

Methods: After signing informed consent, 55 unmedicated treatment-resistant major depressive disorder (TRD) subjects underwent a baseline structural 1.5 or 3T magnetic resonance imaging (MRI) session. FSL (FMRIB Software Library)/FIRST (FMRIB’s Integrated Registration and Segmentation Tool) and FreeSurfer software were used to automatically process subcortical volumes, and the following brain regions were selected a priori for analysis: FSL/FIRST – bilateral hippocampus and thalamus; FreeSurfer – bilateral amygdala, hippocampus, and thalamus. These volumes were then corrected for total intracranial volume (TIV; FSL/FIRST) and whole brain volume (FreeSurfer). All subjects then received a single subanesthetic dose (0.5mg/kg) 40-minute ketamine infusion. Bivariate parametric (Pearson) correlations were performed with baseline subcortical volumes and percent change in Montgomery Asberg Depression Rating Scale (MADRS) from the same day baseline to 230 minutes, one day and seven days post-infusion. For the BDNF rs6265 SNP analysis, we assessed the relationship between ketamine’s antidepressant response and subcortical brain volumes in a univariate analysis of covariance (ANCOVA) model.

Results: The a priori-selected subcortical raw and corrected volumes did not correlate with ketamine’s antidepressant response at any time point (all p values > 0.05). In the secondary BDNF rs6265 SNP analysis, FSL-FIRST TIV-corrected thalamic volumes (left: F = 11.51, uncorrected p = 0.002; right: F = 11.53, uncorrected p = 0.002) were associated with ketamine’s antidepressant response at 230 minutes post-infusion. These associations survived Bonferroni correction for multiple comparisons. In the val/val TRD subjects (n = 23), corrected left thalamic volume was positively but non-significantly correlated with ketamine’s antidepressant response (r = 0.34, p = 0.12), while, in the met carriers (n = 14), corrected left thalamic volume was negatively correlated with ketamine’s antidepressant response (r = -0.76, p = 0.004). Similar magnitude and directionality were observed with the corrected right thalamic volumes and ketamine’s antidepressant response at 230 minutes post-infusion. These associations survived Bonferroni correction for multiple comparisons. In the val/val TRD subjects (n = 23), corrected left thalamic volume was positively but non-significantly correlated with ketamine’s antidepressant response (r = 0.34, p = 0.12), while, in the met carriers (n = 14), corrected left thalamic volume was negatively correlated with ketamine’s antidepressant response (r = -0.76, p = 0.004). Similar magnitude and directionality were observed with the corrected right thalamic volumes and ketamine’s antidepressant response at 230 minutes post-infusion. These associations survived Bonferroni correction for multiple comparisons.
Conclusions: Baseline raw and corrected subcortical volumes did not correlate with ketamine's antidepressant response at three post-infusion time points in the largest ketamine unmedicated TRD imaging sample to date (n = 55). In a secondary analysis, BDNF rs6265 (val66met) SNP differentiated correlations between FSL-FIRST corrected thalamic volume and ketamine's antidepressant response at 230 minutes post-infusion. The combination of baseline thalamic volume and BDNF genotype in ketamine-treated TRD subjects may be a rapid antidepressant response biomarker that warrants attempted replication in independent sample(s).

Keywords: Major Depressive Disorder, Ketamine, brain volume, Thalamus, BDNF

Disclosures: A patent for the use of ketamine in depression has been awarded that lists Dr. Zarate among the inventors. He has assigned his rights on the patent to the U.S. government but will share a percentage of any royalties that may be received by the government. All other authors have no relevant financial interests to disclose.

T75. Exploring Intrinsic Topologies of the Human Connectome

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Background: Analogous to the concept of genome for genetic data, a connectome is a whole-brain comprehensive map of neural connections. As neural connections exhibit complex patterns of function and structure, the intrinsic geometry of brain data, that is, the topological space where brain connectivity natively resides (independent of anatomy).

Methods: T1w and DWI images were acquired with a b0 image and 32 gradient directions at b = 700s/mm2. Tractography-based structural connectomes, using FreeSurfer parcellation, were generated from 46 healthy subjects. To reduce dimensionality - while preserving connectivity information - we used Isomap with graph distance matrix as the input. Prototype virtual reality software was developed that could be employed in both large-scale CAVE2 environment and portable Oculus Rift platforms. Connectome visualizations were compared after removing nodes based on clustering coefficient, betweenness centrality, rich club, or random removal.

Results: Virtual lesions created by selected node removal lead to significant conformational changes in the intrinsic topology of connectomes. For example, targeted rich-club node removal reveals a ring-like structure with a "hole" in the middle. Visually, rich-club nodes thus keep the entire network intact by forming the center. When they are removed, remaining brain regions are now topologically dispersed and less coupled. Similar simulations were further conducted by removing an equal number of nodes with respect to the following criteria: a) nodal strength (high to low), b) clustering (low to high), c) nodal path length (low to high), and d) random removal. While random removal (top middle) as expected only induces subtle changes to the intrinsic geometry, interestingly removing nodes based on clustering (lower left) also minimally changes the overall shape, supporting the fundamental differences in what local properties such as clustering capture relative to global properties.

Conclusions: We describe a new paradigm for virtual-reality brain connectomics that provides novel ways of understanding connectome abnormalities in key brain regions-of-interest as seen in neuropsychiatric disorders.

Keywords: neuroimaging, connectome, informatics

Disclosures: Nothing to disclose.

T76. Treatment of Bipolar Depression with Minocycline and/or Aspirin: An Adaptive, Double-Blind, Randomized, Placebo-Controlled Clinical Trial (NCT01429272)

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Background: Conventional antidepressant pharmacotherapy has not been robustly effective for the treatment of bipolar depression (BD). For example, no difference was found between patients with BD depression when randomized to treatment with a mood stabilizer plus either an adjunctive antidepressant or versus placebo. New medication classes are thus needed. Given emerging evidence for immune dysregulation in mood disorders, the aim of the study was to evaluate the antidepressant efficacy in bipolar depression of minocycline, a drug with neuroprotective and immune-modulating properties, and of aspirin, at doses expected to selectively inhibit cyclooxygenase 1 (COX-1).

Methods: One hundred outpatients between 18 and 55 years of age, who met DSM-IV-TR criteria for BD (type I or II or NOS) and for a current major depressive episode were recruited to participate in a randomized, double-blind, placebo-controlled, parallel-group, clinical trial that initially followed a 2x2 design (NCT01429272) involving four treatment arms: placebo-minocycline + placebo-aspirin (PP), active-minocycline + placebo-aspirin (MP), placebo-minocycline + active-aspirin (AP) or active-minocycline + active-aspirin (MA). The dose of minocycline and aspirin was 100 mg twice daily and 81 mg twice daily, respectively. Antidepressant response was evaluated by assessing changes in the Montgomery Asberg Depression Rating Scale (MADRS) scores between baseline and the end of the 6-week trial. Response to medication or placebo was defined as a > 50% reduction in MADRS scores between the baseline and final assessment. Remission was defined as having a MADRS score of <10 on the final visit.
T77. Expanded Safety and Feasibility Data for a New Method of Performing Electroconvulsive Therapy (ECT). Focal Electrically Administered Seizure Therapy (FEAST)

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Background: Electroconvulsive Therapy (ECT) remains our most rapid and effective antidepressant treatment. Refinements in electrode positioning, alterations in electrical stimulation parameters, and individualized dosing strategies have all resulted in an improved side effect profile without compromising the efficacy of ECT. Building on these developments, FEAST was designed to increase stimulus focality and bring stimulus parameters more in line with neurophysiology to further reduce cognitive side effects. We recently reported on the safety and feasibility of this new form of ECT (FEAST) in a small cohort of depressed patients (1). Since that initial report we have made changes in electrode size and the current amplitude used for treatment. We now report on the safety, feasibility, preliminary efficacy, and preliminary cognitive effects of FEAST in an expanded cohort of depressed patients.

Methods: 21 depressed adults (6 men; 3 bipolar disorder; age 49 ± 10) were recruited after being referred for ECT at MUSC. Open-label FEAST was administered with a modified spECTrum 5000Q device and a traditional ECT dosing regimen until patients clinically responded. Clinical and cognitive assessments were obtained at baseline, and end of course. Time to orientation recovery, a predictor of long-term amnestic effects, was assessed at each treatment. Non-responders to FEAST were transitioned to conventional ECT.

Results: Treatment was well tolerated by participants with no dropouts. Four participants transitioned from FEAST to conventional ECT due to non-response. After the course of FEAST (Median 11 sessions), there was a 62.8 ± 32% improvement in Hamilton Rating Scale for Depression (HRS24) scores compared to baseline (p < .0001, paired t-test). 15 of 21 (71%) patients met response criteria (50% decrease in HRS24) and 12/21 (57%) met remission criteria (HRS24 ≤ 10). Patients achieved full re-orientation (4 of 5 items) in 5.1 ± 5.3 mins (median 3 mins), timed from when their eyes first opened after treatment.

Conclusions: This data in an expanded cohort of depressed individuals further supports the safety and feasibility of FEAST. The remission and response rates are in the range of those found in other investigations using conventional ECT and the time to re-orientation may be quicker. Due to the lack of a comparison group it is not possible to make firm conclusions regarding efficacy or time to reorientation as compared to conventional ECT. Further randomized or controlled studies are needed.

Keywords: mood disorders, electroconvulsive therapy, brain stimulation, Depression, bipolar depression

Disclosures: MECTA Corporation has provided both a Custom-modified spECTrum 5000Q device, as well as an unrestricted educational grant in support of this project. Additionally a patent application is pending on the technology (Inventor: Harold A. Sackeim; Patent Publication number: US 2010/0292750 A1, Filing date: May 12, 2009.)

T78. Dysregulated Luteal Phase Startle Response in Premenstrual Dysphoric Disorder is Corrected by Luteal Sertraline Treatment

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Background: Premenstrual dysphoric disorder (PMDD) is characterized by impairing mood and physical symptoms...
present in the premenstrual (luteal; L) phase of the menstrual cycle and absent in the post-menstrual (follicular; F) phase. Psychophysiologic measures, such as the acoustic startle reflex (ASR), are sensitive to hormonal and pharmacological agents, and provide a means of assessing physiologic arousal at different points in the menstrual cycle. Heightened arousal in the premenstrual may be associated with increased reactivity to stress among women with PMDD. We have demonstrated that in PMDD, baseline ASR is heightened in the L phase, but controls do not exhibit this increase. It is unknown whether specific aspects of startle response, anxiety potentiated startle (APS) (mediated by the allopregnanolone (ALLO)-sensitive bed nucleus of the stria terminalis), and fear potentiated startle (FPS) (mediated by the amygdala), differ between PMDD and controls during the L and F phases. We hypothesized that 1) Women with PMDD would demonstrate heightened APS compared to controls only during the L phase; consistent with our hypothesis that the BNST, not the amygdala, contributes to the heightened L phase arousal observed in women with PMDD, we expected no difference in fear-potentiated startle (FPS) magnitude between controls and those with PMDD, and 2) By increasing allopregnanolone (ALLO) biosynthesis during the L phase via administering a selective serotonin reuptake inhibitor (SSRI), we expect decreased luteal ASR in PMDD participants. The significance of this line of investigation is the translational approach it takes to furthering our knowledge of the hormonal milieu and brain structures underlying this prototypical female disorder and to advancing our appreciation of the role of neurosteroids as potential treatment interventions.

Methods: Female control and PMDD participants were recruited; PMDD diagnosis was confirmed via prospective rating with the Daily Record of Severity of Problems (DRSP). Participants underwent a threat of shock task to assess APS and FPS during the L and F phases. ASR was measured via eyeblink reflex and recorded with PsychLab hardware and software (Contact Precision Instruments). Participants in the PMDD group received the SSRI sertraline and controls only during the L phase; was not responsive to sertraline treatment. Controls and those with PMDD, and 2) By increasing allopregnanolone (ALLO) biosynthesis during the L phase via administering a selective serotonin reuptake inhibitor (SSRI), we expect decreased luteal ASR in PMDD participants. The significance of this line of investigation is the translational approach it takes to furthering our knowledge of the hormonal milieu and brain structures underlying this prototypical female disorder and to advancing our appreciation of the role of neurosteroids as potential treatment interventions.

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Results: Participants included n = 6 controls and n = 6 PMDD who completed both L and F visit. APS was heightened (M 156% increase) in the L (M 10.22, SD 16.7 µV) compared to F phase (M 3.99 µV, SD 4.9) in women with PMDD but not in controls; FPS remained relatively constant across the menstrual cycle in both PMDD and controls. Sertraline treatment during the L phase in PMDD participants reduced ASR magnitude by 60%, for the APS condition, but as expected, FPS was not affected by sertraline treatment.

Conclusions: As hypothesized, these findings suggest that BNST-mediated APS is elevated during the L phase in women with PMDD and not in controls; APS was corrected with sertraline treatment during the L phase in PMDD women. Conversely, amygdala-mediated FPS did not show a change from the F to L phase, and was not responsive to sertraline. This is consistent with the hypothesis that activity of the neurosteroid-sensitive BNST is dysregulated in women with PMDD, likely due to suboptimal ALLO function.
Keywords: Atypical antipsychotics, Women’s Mental Health, pregnancy


T80. Analysis of 23andMe Antidepressant and Efficacy Survey Data: Genome Wide Association Analyses and Genetic Heritability and Correlation Estimates

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Background: Despite the Psychiatric Genomic Consortium (PGC)’s collaborative efforts of Major Depressive Disorder (MDD) meta-analysis, variants predictive of disease susceptibility remain elusive, partly due to the fact that MDD is a heterogeneous disorder. Treatment for MDD is equally challenging. There are ~30 antidepressants available for MDD patient care and response to treatment varies in terms of time to onset of benefit, overall efficacy, and duration of effect. Genetic predisposition may contribute to the differences in drug-specific, class-specific, or antidepressant-wide treatment non-response/resistance, but clinical studies with genetic data are often collected from limited sample sizes. Drug response information obtained from self-reported questionnaires may offer an alternative approach to conduct a study with much larger sample size. Disease subtypes may also be defined by treatment response status to antidepressant therapy. There are inherent challenges of interpreting single arm observational studies where self-reported outcome assessment is the only data collection modality. These challenges include lack of diagnostic certainty, recall biases, and qualitative nature of outcome assessment.

Methods: Using phenotype data collected from 23andMe surveys (“Antidepressant Efficacy and Side Effects” survey, “Your Profile and Health History” survey) and genotype data from 23andMe’s research participants, we performed genome-wide association analyses on 4 groups of phenotypes (a) treatment resistant depression (TRD) vs. non-treatment resistant depression, (b) SSRI non-responder vs. SSRI responder, (c) citalopram/escitalopram non-responder vs. citalopram/escitalopram responder, and (d) NDRI (bupropion only) non-responder vs. NDRI (bupropion only) responder. The drug efficacy responses from Antidepressant Efficacy and Side Effects survey were coded as 4 (great deal), 3 (fair amount), 2 (somewhat), 1 (little) and 0 (not at all). The SSRI class of antidepressants includes fluoxetine, paroxetine, and sertraline in addition to citalopram and escitalopram. All subjects included in the analyses self-reported taking antidepressants for depression indication in the last five years and were of European ancestry. TRD (n = 1290) were defined as subjects who reported efficacy ≤ 1 to at least 2 antidepressants and never reported efficacy ≥ 3 to any antidepressant, while non-TRD (n = 7500) were defined as subjects who reported efficacy ≥ 3 to at least one antidepressants and never reported efficacy ≤ 1 for any antidepressant. SSRI non-responders (n = 3280) were defined as subjects who reported efficacy ≤ 1 to at least one SSRI and never reported efficacy ≥ 3 to any SSRI antidepressant, while SSRI-responders (n = 6100) were defined as subjects who reported efficacy ≥ 3 to at least one SSRI and never reported efficacy ≤ 1 to any SSRI antidepressant. Citalopram/escitalopram non-responders (n = 1980) were subjects who reported efficacy ≤ 1 to either citalopram or escitalopram and never reported efficacy ≥ 3 to both citalopram and escitalopram, while citalopram/escitalopram responders (n = 2880) were subjects who reported efficacy ≥ 3 to either citalopram or escitalopram and never reported efficacy ≤ 1 to both citalopram and escitalopram. Likewise, bupropion non-responders (n = 1800) were subjects who reported efficacy ≤ 1 to bupropion and bupropion-responders (n = 2600) were subjects who reported efficacy ≥ 3 to bupropion. For each of the four phenotype groups, the resistant/non-responder group and the non-resistant/responder group were also compared to healthy controls (n ~ 35,000) self-reported to be free of any of the following conditions: ADHD, anxiety, schizophrenia, depression, bipolar, OCD, autism, PTSD, and insomnia. We imputed genotypes based on the reference haplotypes from the 1,000 Genomes project prior to genome-wide association analysis and age, gender, the first 5 principal components representing population substructure, and genotype platform were included as covariates in the logistic regression analysis. Heritability (h2) for each of the four phenotype groups and the genetic correlation between traits were estimated using LD Score regression.

Results: The most significant association in the genome-wide association study (GWAS) was from bupropion responders vs. non-responders analysis. Variant rs1908557 (p = 2.6 x 10^-8) passed the conventional genome-wide significance threshold (p = 5x10^-8) and was located within the intron of human spliced expressed sequence tags (EST) in chromosome 4 overlapping with enhancer/promoter-associated histone mark H3K4Me1. No single nucleotide polymorphism (SNP) passed genome-wide significance threshold in all other GWAS analyses. The heritability estimates for each responder/non-responder
Background: The dorsal hippocampus (dHipp) is essential for spatial learning and memory, while ventral hippocampus (vHipp) appears to regulate emotional and motivated behaviors. vHipp efferents modulate reward circuitry and emotional behavior through projections to nucleus accumbens, prefrontal cortex and amygdala, and stress responses by regulating the hypothalamic–pituitary–adrenal axis. However, it is unknown how chronic exposure to stress and antidepressants alters vHipp gene expression and function, and whether such alterations may underlie susceptibility to chronic stress or responses to antidepressant treatment. Although the transcription factor ΔFosB is induced in the hippocampus by chronic stress and by chronic fluoxetine exposure, and its expression in other brain regions regulates mood, the role of ΔFosB in vHipp function remains unknown. Therefore, we explored the induction and function of ΔFosB in vHipp. Using viral-mediated overexpression or inhibition of ΔFosB, we sought to determine the role of ΔFosB in response to stress or antidepressants.

Methods: Male, C57Bl6/ J mice from Jackson Labs were used in this study and all experiments were performed in accordance with Michigan State University IACUC-approved protocols. Behavioral assays were performed 21-23 d post-surgery in AAV. Chronic Social Defeat Stress (CSDS) and subchronic SDS were performed essentially as previously described (Vialou et al., 2010). Briefly, mice were exposed to aggressors for 5 min sessions once per day for 10 days while being co-housed but physically separated from the aggressor for the remaining time each day (CSDS), or were exposed to aggressors for three 5 min sessions in a single day (subchronic SDS). Social interaction tests were performed one day after the final defeat session. The open field apparatus consisted of a custom-made, square white polyvinylchloride foam box (38 cm x 38 cm x 35 cm) and elevated plus maze was performed using a custom built apparatus based on plans from ANY-maze (www.anymaze.com). Movement was recorded with a digital CCD camera and automated video tracking software package (CleverSys, Inc.). Viral vectors were purchased from UNC Gene Therapy Center Vector Core and injected into vHipp by stereotaxic surgery (5° angle; -3.6 mm anteroposterior (AP), ±3.2 mm mediolateral (ML) and 0.6 ul of purified high-titer virus was separately infused (0.3 ul/infusion) over 3 min periods at 2 sites: -4.85 mm dorsoventral (DV) and at -3.0 mm DV). For immunohistochemistry, animals were transcardially perfused with (and brains were postfixed in) 10% formalin and 35 μm slices were made on a fixed-blade microtome. Slices of vHipp were stained with a polyclonal FosB antibody (SC-48, Santa Cruz Biotechnology), and positive cells were counted by a double-blind experimenter. Western blots were performed on tissue punches from vHipp using a different polyclonal FosB antibody (5G4, Cell Signaling) and quantified using ImageJ (www.NIH.gov).

Results: We find that multiple FosB gene products, including ΔFosB, are induced in dentate gyrus (DG), CA1, and CA3 of vHipp by CSDS and also by chronic fluoxetine administered to both control and defeated animals. Using AAV-mediated expression of the ΔFosB inhibitor ΔJunD, we demonstrate that inhibition of ΔFosB in vHipp (but not dHipp) promotes susceptibility to subchronic (microdefeat) stress. Inhibition of vHipp ΔFosB had no effect on basal locomotion or on anxiety, as measured by elevated plus maze or open field test. Surprisingly, overexpression of ΔFosB in vHipp also promoted susceptibility to stress, indicating that a balance of ΔFosB-mediated gene expression is required for proper stress response.

Conclusions: ΔFosB is a unique candidate factor for mediating long-term responses to stress and antidepressant treatment because, unlike other immediate early genes, it possesses remarkable stability (weeks in a living brain). Here, we demonstrate that ΔFosB is induced in the mouse vHipp by both stress and antidepressant treatment, and that its activity is required for resilience to stress. Because known gene targets of ΔFosB are key players in hippocampal learning and synaptic function (i.e., CaMKII), we hypothesize that ΔFosB regulates local synapses, but it may also regulate vHipp projections to other brain regions, such as nucleus accumbens (NAc). Indeed, recent evidence suggests that vHipp projections to NAc regulate social defeat responses (Bagot et al., 2015). Overall, these studies
suggest that vHipp ΔFosB may be important for resilience to stress and antidepressant function, and thus that vHipp ΔFosB and its downstream targets may represent novel therapeutic inroads for the treatment or prevention of depression.

**Keywords:** ΔFosB, ventral hippocampus, Social defeat stress, Antidepressants

**Disclosures:** Nothing to disclose.

T82. Results of a Double-Blind Placebo-Controlled Study of the Antidepressant Effects of the mGlu2 Negative Allosteric Modulator RG1578

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**Background:** There is increasing evidence implicating abnormalities in glutamate transmission in major depressive disorder (MDD). In particular recent studies demonstrating fast and persistent antidepressant effects of the NMDA antagonist ketamine have supported this assumption. It has been hypothesized that etiologically chronic stress may ultimately lead – by way of a glutamate 'spillover' – to a disruption of normal glutamate transmission via excessive activation of presynaptic autoinhibitory metabotropic glutamate receptors type 2 (mGlu2). mGlu2 antagonists should correct this abnormal state and thus offer a therapeutic approach. We evaluated the antidepressant and pre cognitve effects of the mGlu2 negative allosteric modulator RG1578 in patients with an inadequate response to SSRIs or SNRIs. This compound demonstrated antidepressant and pre cognitive effects in preclinical models of depression and cognitive impairment.

**Methods:** Patients with MDD who continued to have depressive symptoms (inclusion criterion for severity of illness: MADRS ≥ 25, CGI ≥ 4) despite up to two adequate trials with an SSRI or SNRI were recruited for this study. A total of 744 patients were screened. Among the 387 patients who failed screening 15% did so because they had no detectable levels of the SSRI or SNRI they supposedly were taking and 24% did so because the severity of their depression did not reach the inclusionary threshold of depression severity as assessed by blinded centralized raters. A total of 357 patients passed screening and were randomized to 6 weeks double-blind treatment with placebo, 5 mg/d, 15 mg/d and 30 mg/d of RG1578 as an adjunct to ongoing treatment with an SSRI or SNRI. Cognitive impairment was defined as performing 0.5 SD below normal on the mean of an attentional (RVP), memory (PAL) and executive (SOC) test of the CANTAB battery. The primary endpoint (MADRS) was assessed by fully blinded centralized raters. Secondary endpoints included the IDS-SR30, CPFQ, SDS and Q-LES-QSF. Effects on cognition were assessed with the CANTAB battery.

**Results:** A total of 310 patients completed 6 weeks treatment without major protocol violations (per-protocol population; Placebo: N = 86; female 67%; mean age 46.0 ± 11.2; above 45 years 47%); 5 mg group: N = 89; female 70%, mean age = 46.9 ± 10.7; above 45 years 61%; 15 mg group: N = 88; female 72%; mean age = 46.9 ± 10.9; above 45 years 60%; 30 mg group: N = 47; female 64%; mean age 44.5 ± 13.1; above 45 years 53%) of RG1578. At baseline the mean MADRS total score was 31 (± 6 [SD]) and the CGI-S 4.4 (± 0.7). Compared to historical normative CANTAB data the mean cognitive performance of patients was within normal range with overall 24% of all patients demonstrating deficits exceeding half a standard deviation at baseline (Placebo 23%; 5 mg/d 20%; 15 mg/d 30%; 30 mg/d 24%). At the end of treatment the decreases in the MADRS total score did not differ significantly between any active treatment arm and placebo (placebo: -11.8 ± 11.2; 5 mg: -12.8 ± 11.2; 15 mg: -11.8 ± 11.2; 30 mg: -13.2 ± 11.2). Response rates were 35% for placebo, 40% for 5 mg, 43% for 15 mg and 47% for 30 mg and did not differ significantly. Remission rates did not differ significantly between treatment arms (placebo 29%, 5 mg 38%, 15 mg 30% and 30 mg 32%). Additional analyses in subgroups of patients defined by sex, age, history of previous episodes, family history, baseline cognitive impairment, subjective cognitive complaints or geography did not demonstrate any drug effects over placebo. Similar results were observed for all secondary outcome measures. Exploration of RG1578 exposure-response relationships confirmed these results.

The effects of treatment with RG1578 on cognitive functions did not differ at any dose level from those of placebo. Overall treatment with RG1578 was well tolerated and associated with few side effects leading to study withdrawals. The most frequent side effects included headache, nausea and dizziness with dose-dependent increases.

**Conclusions:** Adjunctive treatment with RG1578 was not associated with significant antidepressant effects in patients with MDD and inadequate response to antidepressants. No effect on cognitive functions was observed. However, only about a quarter of patients showed clinically relevant cognitive deficits limiting the power of the study to show such effects.

**Keywords:** Major Depressive Disorder (MDD), metabotropic glutamate receptor, Cognition, adjunctive treatment, treatment refractory

**Disclosures:** I am an employee of F. Hoffmann - La Roche and own stocks of this company. All co-authors with the exception of Maurizio Fava are employees of F. Hoffmann - La Roche. Maurizio Fava has served as consultant to F. Hoffmann - La Roche.

T83. Major Depressive Disorder Increases the Risk for Diabetes by Impairing Insulin Sensitivity

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**Background:** Reports regarding the associations between major depressive disorder (MDD) and diabetes remain heterogeneous in humans. Our aim was to investigate whether glucose homeostasis and insulin sensitivity were impaired in the MDD patients, and to explore the potential mechanisms.

**Methods:** A total of 30 depressive patients and 30 matched controls were recruited. The oral glucose tolerance test and
dual-energy X-ray absorptiometry scan were performed in each participant. Additionally, insulin signaling pathway in postmortem brain tissues from other depressive patients and controls (obtained from Alabama brain bank) was examined.

**Results:** Insulin sensitivity was reduced substantially in the MDD patients; however, the fasting and 2-h glucose concentrations remained within the normal range through exaggerated and compensatory insulin secretion. Despite increased insulin secretion, 1-h glucose concentrations in the MDD patients were significantly elevated compared with controls. MDD patients had greater visceral fat mass but lower adiponectin levels compared with the controls. Furthermore, phosphorylated-AKT levels in insulin signaling were impaired in postmortem brain tissues in the depressive patients. 

**Conclusions:** These results suggest that MDD patients are at a greater risk for diabetes due to decreased insulin sensitivity, reduced disposition index, and impaired glucose tolerance as manifest by elevated 1-h glucose values following an oral glucose challenge. Mechanistic studies reveal that decreased insulin sensitivity is associated with increased visceral fat mass, lower adiponectin levels and impaired insulin action in postmortem brain tissues in the MDD patients. Our findings emphasize the importance of screening depressive patients to identify susceptible individuals for developing diabetes with the hope of improving their health outcomes.

**Keywords:** Major depression, insulin resistance, type 2 diabetes mellitus

**Disclosure:** Dr. Shelton has some disclosures, but none is relevant to this poster.

T84. Cortisol Response to Dex/CRH Test as Predictor of Future Mood/Anxiety Symptoms

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**Background:** A wealth of research has suggested that interplay between genetic pre-disposition, quality of early environment, and active in situ factors, such as physical health and psychosocial stressors, interact dynamically to produce clinically significant symptoms of depression or anxiety. To date, the field of psychiatry lacks meaningful biomarkers for detecting risk for disorders of mental health. Identification of endophenotypes to aid in stratification of an individual’s risk for developing psychiatric disorders or clinically significant symptoms might permit an opportunity for intervention to prevent or reduce the onset of psycho-pathology. The dexamethasone/corticotropin releasing hormone (Dex/CRH) neuroendocrine test allows examination of an individual’s hypothalamic-pituitary-adrenal (HPA) response to a “chemical” stress stimulus (i.e., intravenous CRH bolus) in a psychologically neutral setting. Previous research has identified cortisol response to the Dex/CRH test as a correlate of mood/anxiety disorders, but prospective studies had not been done to evaluate whether Dex/CRH cortisol response could be a useful biomarker for predicting onset of mood and anxiety disorders.

**Methods:** A community sample of healthy medication-free adults aged 18-50 years were recruited. Diagnostic interviews were used to exclude participants meeting DSM-IV criteria for current or lifetime major depressive disorder, bipolar disorder, posttraumatic stress disorder, generalized anxiety disorder, obsessive compulsive disorder, social anxiety disorder, panic disorder, substance use disorders, and all disorders characterized by psychosis. Participants underwent clinical interviews and completed a standard Dex/CRH test at baseline. Family History Research Diagnostic Criteria (FH-RDC) were used to elicit history of mood or anxiety disorder among first-degree relatives. The Childhood Trauma Questionnaire (CTQ) was supplemented with additional interview questions to survey exposure to a broad range stressors during early life. The Inventory of Depressive Symptomatology (self-report version) was used to capture (subthreshold) symptoms present at baseline. The Dex/CRH was administered as an oral dose of 1.5 mg dexamethasone taken at 23:00 hrs, with placement of an indwelling intravenous catheter at 13:00 hrs the following day, and 100 µg intravenous infusion of CRH at 15:00 hrs. Cortisol assays were performed on blood samples drawn every 15 to 30 minutes from 14:00 hrs to 18:00 hrs and Δcortisol was calculated as change from pre-CRH baseline value to peak value. The Longitudinal Interval Follow-Up Evaluation (LIFE) method, using semi-structured interviews and rating systems, was used to assess the onset of selected mental disorders and subthreshold syndromes every 6 months for up to 84 months. Outcome variables included the onset of threshold psychiatric disorders as well as subthreshold symptoms. Logistic regression models controlling for duration of follow-up, gender, age, family history, and early life stress evaluated the utility of Dex/CRH Δcortisol in predicting onset of “any disorder” and “mood or anxiety disorders.” A second set of analyses examined Δcortisol as a predictor of clinically meaningful subthreshold symptom states.

**Results:** Mean ± SD duration of follow-up was 47.3 ± 25.4 months. A total of n = 202 subjects completed a 6-month follow-up assessment, n = 193 completed a 12-month follow-up assessment, n = 174 completed a 24-month assessment, and n = 101 completed a 48-month assessment. A total 40 out of 202 (19.8%) participants reached full threshold diagnostic criteria for one or more of the disorders that were evaluated and of those, 22 participants (10.9%) had onset of a mood or anxiety disorder during the follow-up period. A total of 132 participants (65.4%) experienced subthreshold (or greater) symptoms of “any disorder” and 125 (61.9%) reported subthreshold mood/anxiety symptoms. Dex/CRH Δcortisol prospectively and significantly predicted increased likelihood of developing (at least) subthreshold mood/anxiety symptoms (p = 0.004) and subthreshold symptoms of “any” psychiatric disorders (p = .034). A 100-unit increase in Dex/CRH Δcortisol increased the odds of having “any” subthreshold symptoms by 1.29 and the odds of having mood/anxiety subthreshold symptoms by 1.43. Dex/CRH Δcortisol did not significantly predict onset of threshold disorders.

**Conclusions:** The incidence of psychiatric disorder onset in this study population was consistent with previously published epidemiological studies. The Dex/CRH summary variable we examined (Δcortisol) did not predict onset of...
threshold mood or anxiety disorders in a model that contained other significant risk factors such as female gender, positive family history, and early life stress. We prospectively observed that clinically meaningful subthreshold symptom states were endorsed by more than half of the sample. Restrained cortisol reactivity to Dex/CRH test emerged as an endophenotype protective against mood/anxiety symptom development.

**Keywords:** endophenotype, Cortisol, hypothalamic-pituitary-adrenal axis, mood and anxiety disorders

**Disclosures:** Nothing to disclose.

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**T85. A “Multiple-Hit” Model of Affective Disorders in Rats Selectively Bred for Differences in Emotional Reactivity: A Novel Animal Model of Treatment-Resistant Depression**

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**Background:** Depression is a debilitating disease that renders millions of people in need of life-long treatments. Moreover, approximately two-thirds of patient with depression do not respond to the current pharmacological treatments adequately, and are considered ‘treatment-resistant’. To better understand the neurobiological underpinnings of response versus resistance to antidepressants, we aimed to create an animal model of treatment-resistant depression. In doing so, we developed a multiple-hit model, and used selectively bred rats (bLRs, bHRs) from our colony that display innate differences in stress reactivity. Namely, compared to bLRs, bHRs display higher novelty-induced locomotion, and lower anxiety- and depression-like behaviors, and we have shown that these lines exhibit stable, predictable and profound differences in multiple facets of affective behavior, indicating a pervasive difference in emotionality. Using this animal model and the multiple-hit paradigm, we investigated how environmental interventions and differences in genetic background interact to alter affective behavior, and lead to treatment-resistant depression in a subset of vulnerable individuals.

In the present study, treatment-resistance was identified by lack of response to a classical antidepressant, fluoxetine (FLX) in bLRHRs that display signs of depressed-like behavior following multiple-hits. Subsequently, efficacy of a novel antidepressant drug in reversing signs of depression-like behavior was assessed in this novel rat model of treatment-resistant depression.

**Methods:** Forty-third, 44th, and 45th generation male, bred LR and HR rats were obtained from our breeding colony. The multiple-hit paradigm consisted of chronic variable stress (CVS) exposure during adolescence, followed by additional “hits” in adulthood. Control animals were left untouched during this period. Following the last hit, rats in each group were further divided into vehicle (VEH), FLX, or L-acetylcarnitine (LAC) injection groups. After 2 weeks of daily VEH or drug injections, animals received a 15-min stress challenge and got tested on the social interaction (SI) test for assessment of social withdrawal. Following SI testing, rats were euthanized via rapid decapitation and brains were collected. Ongoing studies are assessing the transcriptome profile via RNA seq. in the hippocampi of treatment-resistant versus --responsive bLRHRs with or without a history of multiple-hits.

**Results:** Our results showed that adolescent CVS exposure affected bLRs and bHRs in opposite ways; inducing resilience in bLRs and vulnerability in bHRs to a stress challenge in adulthood. Moreover, following multiple-hits while bLRs did not behave different than controls, bHRs showed significantly increased social withdrawal compared to their respective controls. This confirmed that environmental challenges encountered in adolescence differentially interact with genetic background to alter affective behavior later in life. Interestingly, our data showed that genetic background determined antidepressant-response as well; while chronic treatment with FLX resulted in increased social interaction time following a stress challenge in bLRs, the identical treatment was ineffective in bHRs. Most importantly, FLX was ineffective in reversing the increased social withdrawal observed in bHRs following multiple hits, indicating treatment resistance in this phenotype. Similar to FLX; a novel antidepressant, LAC, was ineffective in reversing stress-induced social withdrawal in bHRs. Ongoing studies will reveal the transcriptome profile in the hippocampus of treatment-resistant versus --responsive bLRHRs with or without a history of multiple-hits. Additional ongoing experiments are investigating the effectiveness of tricyclic, as well as novel antidepressants in reversing signs of depressed-like behavior in this novel animal model.

**Conclusions:** Overall, the present data demonstrate the effectiveness of the bLRHR phenotype in modeling how early life challenges and genetic background work together to shape an individual’s response to stressful experiences later in life. Moreover, the bLRHR model proves to be a promising animal model of treatment-resistant depression that can help identify the molecular mechanisms responsible for treatment response versus resistance to antidepressants, and aid in development of novel treatment strategies for this disease.

**Keywords:** Treatment Resistant Depression, antidepressant response, animal model

**Disclosures:** Nothing to disclose.

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**T86. Cortisol Administration Inhibits Sadness-Related Subgenual Cingulate Activity in Depression**

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**Background:** Cortisol dysregulation has been observed in a large subgroup of patients with major depression for over 50 years. Subgenual cingulate hyperactivity has also been observed in major depression since the advent of modern neuroimaging techniques over 20 years ago. A great deal of work has been done to demonstrate that 1) both cortisol dysregulation and subgenual cingulate hyperactivity are more pronounced in patients with more severe
symptoms 2) that these findings normalize with when patients are successful treated 3) that these findings reemerge prior to relapse. However, little work has been done to integrate these findings by investigating the interactions between cortisol signaling and subgenual cingulate activity levels. Our group has previously presented evidence that cortisol inhibition of sadness induced subgenual cingulate activity is a normal neural circuit. In this study we present preliminary evidence that this circuit could be breaking down in major depression, leading to hyperactivity of the subgenual cingulate and overexpression of sadness.

Methods: A preliminary analysis included data from 9 participants suffering from major depression. All participants were medication-free for the duration of the study and all female participants were naturally cycling. Participants were scanned using functional magnetic resonance imaging on a 3 Tesla General Electric MRI scanner equipped with a custom 32-channel head-coil executing a 3X multiband acquisition pulse sequence (TR = 2, 87 horizontal slices, isotropic 1.8mm voxels). Participants viewed 3 separate runs of 14 minutes and 44 seconds. Each run was divided into 3 conditions: 1) sadness induction using previously validated stimuli from the International Affective Picture System and still-frames extracted from validated film clips that the participants had previously viewed 2) control stimuli which consisted of pixel scrambled versions of the IAPS and film clip still-frames 3) fixation cross control, which consisted of a black screen with a white fixation cross. Each run contained 9 sadness induction blocks lasting 32 seconds each, 9 control stimuli blocks lasting 32 seconds each, and 19 fixation blocks lasting 16 seconds each. Between run1 and run2 participants were given either 0.65 mg/kg of orally administered cortisol or a matched placebo.

Results: Preliminary data from the first 9 participants with major depression indicate that before cortisol administration the [sadness > control] contrast produced a significant activation of the subgenual cingulate T(8) = 2.615, p = 0.031. After cortisol administration this activity was significantly reduced T(8) = 2.489, p = 0.0371 by an average of 78.3%.

Conclusions: The preliminary analysis indicates that cortisol-induced suppression of the subgenual cingulate activity occurs in participants with major depression. However the magnitude of this suppression is subtle compared to our previously published work with healthy control participants that showed robustly lower sadness-related subgenual cingulate activation in participants taking cortisol. Additional data are being collected from healthy control participants to allow for a direct group comparison with the depressed participants. If the sensitivity of subgenual cingulate activity to cortisol is reduced in major depression it could represent the key neural circuit breakdown that is responsible for hyperactivity of the subgenual cingulate and the overexpression of sadness.

Keywords: Cortisol, subgenual, cingulate, emotion, Depression

Disclosures: Dr. Schatzberg reports the following disclosures: Grant/Research Support- Pritzker Foundation; NIMH Consultant- Pfizer, Takeda, Forum, Neuronetics, X-Hale, and Naurex. Major Stock Shareholder- Corcept. Merck and X-Hale Other-Genetics use patents for mifepristone treatment, pharmacogenetics of depression treatment, and genetic tests for depression risk.
T88. Repeated Brexpiprazole Administration Alters the Activity of Monoamine System: An In Vivo Electrophysiological Characterization

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Background: Brexpiprazole was recently FDA approved as an adjunctive therapy for depression and for treatment of schizophrenia in adults. In a previous study, where the effects of acute brexpiprazole administration were assessed, we demonstrated in vivo agonistic action of brexpiprazole on serotonin 5-HT1A autoreceptors, partial agonism at dopamine D2 autoreceptors, and antagonistic action at 5-HT2A and α2-adrenergic heteroreceptors. Furthermore, brexpiprazole had more potent agonistic action on 5-HT1A autoreceptors and less agonistic action at D2 autoreceptors compared to aripiprazole, an agent in wide clinical use. To extend insight in the effects of brexpiprazole, the present study aimed to assess the impact of its repeated administration on the activities of, and the status of therapeutically relevant pharmacological targets within monoamine systems.

Methods: Brexpiprazole (1 mg/kg, subcutaneous) or vehicle was administered once daily for two or 14 days. Single-unit electrophysiological recordings from norepinephrine (NE) neurons in locus coeruleus (LC), 5-HT neurons in the dorsal raphe nucleus, dopamine (DA) neurons in the ventral tegmental area (VTA), and pyramidal neurons in the CA3 region of the hippocampus were obtained in adult male Sprague-Dawley rats under chloral hydrate anesthesia.

Results: Brexpiprazole did not alter the firing activity of DA neurons. However, the maximal effective dose (ED100) of the D2 agonist apomorphine (40 μg/kg, i.v.) in controls inhibited VTA DA neurons in both two- and 14-day brexpiprazole treated rats only by 30%. Acute, two- and 14-day brexpiprazole increased the firing rate of NE neurons. The ED100 dose of the 5-HT2A agonist DOI inhibited NE neurons only by 50%, thus demonstrating partial blockade of 5-HT2A receptors by repeated brexpiprazole administration. In the hippocampus, repeated brexpiprazole did not alter NE transporter activity or α2-adrenergic receptor sensitivity. However, acute administration of the α2-adrenoceptor antagonist idazoxan – but not the α1-adrenoceptor antagonist prazosin – significantly disinhibited CA3 pyramidal neurons after 14 days of brexpiprazole administration, revealing enhanced NE tone on α2-adrenoceptors. These effects were likely due to increased firing of LC NE neurons, while brexpiprazole simultaneously blocked postsynaptic α1-adrenergic receptors. The firing activity of 5-HT neurons increased after two, but not 14 days of brexpiprazole administration. Despite this latter effect, administration of the 5-HT1A antagonist WAY100,635 had a strong disinhibitory effect on CA3 pyramidal neurons after two and 14 days of brexpiprazole administration. Since brexpiprazole did not alter the status of terminal 5-HT1B receptors or the 5-HT transporter, the full agonistic action of brexpiprazole on postsynaptic 5-HT1A receptors could, in part, explain this enhancement in tonic activation.

Conclusions: Despite appreciable occupation of D2 autoreceptors in the VTA, brexpiprazole did not alter the sensitivity of this receptor population after two and 14 days of drug administration. Since sustained D2 receptor antagonism is known to sensitize these receptors, these data are the first to demonstrate distinguishable effects of repeated partial agonist administration on D2 autoreceptors. Similarly to other typical antipsychotics, repeated brexpiprazole blocked 5-HT2A receptors. Furthermore, it enhanced 5-HT and NE tone in the hippocampus, effects common to antidepressants. Together, these results extend insight in the effects of repeated brexpiprazole administration on monoamine systems, thereby contributing to a better understanding of the neural mechanisms by which it exerts therapeutic efficacy in the treatment of schizophrenia and as adjunctive therapy to antidepressants in the treatment of depression.

Keywords: brexpiprazole, electrophysiology, monoamines

Disclosures: Dr. Blier received grant funding and/or honoraria for lectures and/or participation in advisory boards for Astra Zeneca, Bristol Myers Squibb, Eli Lilly, Actavis, Euthymics, Janssen, Lundbeck, Merck, Otsuka, Pfizer, Pierre Fabre, Servier, Shire, Takeda, and Valeant.

T89. Reinforcement Learning-Based Analysis of a Decision Making Paradigm Reveals Independent Influence of Distress and Trait Sensation-Seeking

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Background: Reward-related paradigms are receiving increasing attention for the development of biomarkers to distinguish dimensions of pathology in individuals with mood disorders, within the framework of Positive Valence system constructs specified within the Research Domain Criteria (RDoC). In particular, we have applied reinforcement learning-based analytical approaches to clarify the relevant underlying behavioral and neural constructs further. In the present study, we applied a reinforcement learning (RL) algorithm to model choices made while performing a decision making task. This task followed an fMRI card guessing paradigm, and employed the same stimuli as those presented in the guessing paradigm. We also examined fMRI-measured anticipatory neural activation in a key region of interest, the left ventrolateral prefrontal cortex, following our previous observation of its engagement during outcome expectancy within a guessing task.

Methods: Twenty-three young adults (mean age: 21.82; 16 female) were recruited on the basis of help-seeking for distress. Thirty healthy controls (mean age 21.57, 17 female) were recruited from the community. All participants were assessed clinically and performed self-report questionnaires assessing symptoms of depression and impulsive sensation-seeking. Participants performed a card-guessing task, which was rewarded with monetary feedback, during an fMRI scan (multiband acquisition sequence; TR/TE = 1500/31ms). After
the scan, participants performed a decision making task in which they could choose between the cards they experienced in the scanner and 'sure-thing' wins and losses. A simple RL algorithm was used to fit the pattern of choices on the task. In a sub-sample of 35 individuals (including both distressed and control individuals) with available fMRI data, relationships between anticipatory activation in the left ventrolateral prefrontal cortex (LVLPFC) and RL-derived parameters were examined. Non-parametric tests (Mann Whitney U; Spearman's Rho) were used to analyze the data.

Results: Distressed individuals and healthy control participants showed similar learning rates for wins and losses (Z < 1), but distressed individuals showed increased exploration (Z = 2.03, p = 0.048) and a poorer fit of their choices by the model (Z = 2.19, p = 0.029). By contrast, individual differences in trait sensation seeking (Zuckerman Scale) were associated with increasing learning from wins (Rho = 0.34, p = 0.014) and also the difference of win and loss learning rate (Rho = 0.35, p = 0.011), but not loss learning rate, exploration or model fit (p's > 0.2). We also observed a positive relationship between individual differences in LVLPFC activation and negative learning rate (Rho = 0.52, p = 0.001) and a trend level association with positive learning rate (Rho = 0.29, p = 0.09).

Conclusions: Distressed individuals showed increased levels of exploratory decision making on the choice paradigm, which is consistent with previous data from other decision making studies, and may be a consequence of a general insensitivity to reinforcement. By contrast, increasing levels of trait sensation seeking influenced the balance of learning from reward versus punishment, which may underlie altered risk preferences and bipolar-related symptoms as impulsiveness. We also implicate LVLPFC activation in risky decision making, in line with our previous studies.

Keywords: mood disorders, Reinforcement learning, Human Neuroimaging, impulsiveness

Disclosures: Mary Phillips is a consultant for Roche.

T90. Basolateral Amygdala Connectivity in Male and Female Veterans with Psychiatric Disorders

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Background: As of 2013, approximately 9% of the 22.2 million veterans in the US were female and estimates suggest that by 2043, females will make up almost 17% of the Veterans in the US (United States Department of Veterans Affairs, 2014). A significant number of veterans experience a host of physical and psychiatric complications, including posttraumatic stress disorder (PTSD) and depression (MDD). Furthermore there is a large body of evidence suggesting that compared to men, women show higher prevalence of depression, PTSD and anxiety disorders than men (Maguen, 2010). Thus far, there is limited data on the underlying neuropathology that may be associated with sex-differences in psychiatric disorders. This study examined basolateral amygdala connectivity in a sample of female and male in veterans matched for psychiatric disorders to test the hypothesis that connectivity from the basolateral amygdala (BLA) will be different in female compared to male veterans.

Methods: Participants in the study consisted of 19 female and 19 male veterans aged 18-55, who met diagnostic criteria for MDD and/or PTSD. BOLD echo planar images were obtained during the 8-minute resting-state using a 3T Siemens Trio scanner (TR = 2s, TE = 28ms, 40 slices, 3mm slice thickness). FMRI images were analyzed using SPM8 and Matlab. The images were corrected for motion, normalized and spatially smoothed using a Gaussian kernel with 4 mm full width at half maximum. Regions of interest for BLA seeds were created using SPM’s Anatomy Toolbox (Eickhoff, 2005) using methods adapted from Roy & colleagues (Roy, 2009). Functional connectivity maps were computed by using a standard seed-based whole brain correlation method. For each seed region, the time series of the voxels within the seed region were averaged to generate the reference time series. For each subject and each seed region, the correlation coefficient was computed between the reference time series and the time course of each voxel of the brain. Correlation coefficients were converted to z-values using Fisher’s r-to-z transform. One-sample t-tests were done to determine brain regions showing significant functional connectivity to the left and right amygdala within each group (p < .05, FDR corrected; cluster size k > 20 voxels). Two sample t-tests were performed for the two groups controlling for both age and gender for the left and right amygdala separately.

Results: There was no significant difference in age between the groups. Additionally, males and females did not differ on race, children, or rank in the Armed Forces. For both left and right BLA, females demonstrated stronger connectivity to the calcarine, cuneus, fusiform, cerebellum, lingual and occipital gyrus (Left BLA: T = 4.60, 1400 voxels; Right BLA: T = 5.22, 2638 voxels) as compared with males. The increased connectivity in males as compared with females was shown in the temporal, frontal and supramarginal areas (Left BLA: T = 3.56, 378 voxels; Right BLA: T = 4.59, 900 voxels).

Conclusions: Results from the current study suggest that BLA functional connectivity differs between male and female veterans matched for PTSD/MDD diagnoses. Connectivity to limbic and paralimbic regions consistent with the amygdala’s role in processing emotions was seen for both male and female veterans. However, relative to females, male veterans showed increased connectivity to the rolandic operculum and cortical motor areas, which may be associated with premotor representations coupled with affective processing (Koelsch, 2006). In contrast, relative to males, females showed increased connectivity to parieto-occipital regions known to process visual information including faces. Additional studies are needed to determine if the observed differences in BLA projections play a role in the increased prevalence of behavioral disorders found in some veteran populations.

Keywords: Amygdala, gender, Connectivity strength

Disclosures: Nothing to disclose.
T91. Pharmacokinetic Profiles of Ketamine Dosing Regimens Used in Preclinical Studies of its Antidepressant-Like Action

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Background: Ketamine is a non-competitive, subtype non-selective, activity-dependent N-methyl-D-aspartate (NMDA) receptor antagonist which also directly interacts with other receptors and ion channels in a close dose range. In addition to its well-known use as an anesthetic and analgesic, sub-anesthetic intravenous doses of ketamine have also been reported to produce rapid (within hours) and sustained (for days) antidepressant effects in patients with depression, bipolar depression and treatment resistant depression (TRD). In preclinical studies, at a wide dosage range (2.5 up to 100 mg/kg), Ketamine produced antidepressant-like behavioral effects in a variety of depression paradigms, including acute severe or chronic stress maneuvers, immune or neuroendocrine challenges, and monoamine depletion. These animal models are also used to explore underlying mechanisms of antidepressant actions of Ketamine. To address the issue of dose selection for preclinical mechanistic studies of antidepressant actions of ketamine, we conducted the current pharmacokinetic (PK) study in rats, compared the PK data to those from depressed patients, and estimated the NMDA receptor occupancies at the doses used in the studies.

Methods: Male Wistar Kyoto rats were used in the PK studies. Ketamine was administered subcutaneously (s.c.) at 1, 5 and 10 mg/kg as well as intravenously (i.v.) at 1 and 5 mg/kg. Blood samples were collected at 5 min through 8 hours or 2 min through 8 hours following single s.c. or i.v. administration, respectively. For detection, plasma samples were mixed with acetonitrile to precipitate proteins, and then subjected to a centrifugation. The supernatants were injected to the LC/MS/MS system to detect plasma levels of Ketamine and Norketamine.

Results: Ketamine displayed a shorter half-life and higher conversion rate to the metabolite, norketamine, in rats compared to published data in humans. Following s.c. administration at 1 mg/kg, the Cmax was 189 ng/mL (0.8 μM), 730 ng/mL (3.1 μM) at 5 mg/kg, and 2399 ng/mL (10.1 μM) at 10 mg/kg. While following i.v. administration the Cmax were377 ng/mL (1.6 μM) at 1 mg/kg, and 2784 ng/mL (11.7 μM) at 5 mg/kg. Comparing the Cmax observed in these studies, only the 1 mg/kg (s.c. group) was near the estimated Cmax of ~183 ng/mL (0.8 μM) reported previously by Zhao et al. (2012) following 40 minute infusion of 0.5 mg/kg ketamine in patients with treatment-resistant bipolar depression. At this dose, the estimated NMDA receptor occupancies are around 45.0 %. In behavioral experiments in rats/mice, administration the 1 mg/kg, s.c. dose of ketamine was not remarkably effective at producing an anti-depressant-like effect; whereas, doses of 2.5 mg/kg or higher (frequently at 10 mg/kg in literature) were generally required.

Conclusions: In this rodent PK study, the 1 mg/kg dose of ketamine resulted plasma Cmax value(s) similar to that observed in depressed patients. A concerning issue emerged from the current data and those previously reported in the literature is that the plasma concentrations achieved in most preclinical studies demonstrating antidepressant-like efficacy with ketamine likely produce substantially higher NMDAR blockage and other activities at non-NMDAR targets. The targets include Dopamine (D2) receptors, Nicotinic Receptors, and Hyperpolarization-activated Cyclic Nucleotide-gated Channels. Some of these receptors and channels might not be markedly affected in humans when treated with ketamine for depression; therefore, caution should be taken when using preclinical data generated with higher doses of Ketamine to interpret the mechanisms underlying antidepressant actions of Ketamine in depressed patients.

Keywords: Ketamine, Esketamine, Depression, Antidepressant, Animal Models

Disclosures: Full time Janssen employee.

T92. Lurasidone for Major Depressive Disorder with Mixed Features: Effect of Baseline Depression Severity on Clinical Outcome

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Background: Accumulating evidence indicates that manic symptoms, below the threshold for hypomania (mixed features), are common in individuals with major depressive disorder. This form of depression is often severe, and is associated with an increased risk for recurrence, suicide attempts, substance abuse, and functional disability. In this post-hoc analysis of an acute treatment study, the impact of baseline depression severity on treatment response to lurasidone was evaluated in patients with a diagnosis of major depressive disorder with mixed features.

Methods: Patients meeting DSM-IV-TR criteria for major depressive disorder, with a MADRS score ≥26, who presented with 2 or 3 protocol-defined manic symptoms, were randomized to 6 weeks of double-blind treatment with either lurasidone 20-60 mg/d (N = 109) or placebo (N = 100). To evaluate the effect of illness severity on treatment response, 3 baseline depression severity groups were defined post-hoc: a moderate severity group (MADRS = 26-30), a marked severity group (MADRS = 31-35), and a high severity group (MADRS ≥36). For each baseline severity group, changes from baseline in MADRS total score (primary) and CGI-S (key secondary) were analyzed using a mixed model for repeated measures analysis. Additional secondary measures of manic symptoms (YMRS), anxiety (HAM-A) and functional disability (SDS), were evaluated using an ANCOVA.

Results: At baseline, there were 59 patients (28.4%) with moderate depression severity (mean MADRS total, 28.6; CGI-S, 4.2), 95 patients (45.7%) with marked depression severity (mean MADRS total, 33.1; CGI-S, 4.6), and 54 patients (26.0%) with high depression severity (mean MADRS total, 38.7; CGI-S, 4.9). At baseline, the high severity group, compared with the moderate severity group,
was more likely to be female, to present with 3 (vs 2) manic symptoms and a higher mean YMRS total score, and was more likely to have higher levels of concomitant anxiety. On the primary endpoint, change from baseline in MADRS total score at week 6, lurasidone treatment effect sizes (drug vs. placebo) increased as baseline severity increased from moderate to marked to high (d = 0.64, P = 0.022; d = 0.72, P = 0.001; d = 1.24, P < 0.0001); a similar effect size trend was observed for improvement at week 6 in the CGI-I score (d = 0.42, P = 0.137; d = 0.57; P = 0.009; d = 0.90; P = 0.002), the YMRS total score (d = 0.39, P = 0.159; d = 0.64, P = 0.003; d = 0.78, P = 0.006), the HAM-A total score (d = 0.70 vs d = 0.76 vs d = 0.87), and the SDS total score (d = 0.43 vs d = 0.70 vs d = 0.89). The mean modal daily dose of lurasidone was similar in the moderate (41.8 mg), marked (39.6 mg) and high (43.6 mg) severity groups. Lurasidone was well-tolerated regardless of baseline depression severity subgroup; in all 3 subgroups discontinuations rates due to adverse events were lower in the lurasidone group compared with the placebo group. There was a modest trend in the lurasidone group for discontinuation rates to be lower as baseline depression severity increased from moderate to marked to high (8.8% vs 6.4% vs 3.6%).

Conclusions: In this post-hoc analysis of a randomized, placebo-controlled, 6-week trial of lurasidone in major depressive disorder with mixed features, patients with higher baseline depression symptom severity were more likely to be female, and to have higher levels of manic and anxious symptoms. Higher baseline depression severity was associated with larger treatment effects for lurasidone across a spectrum of symptom domains, including depression, anxiety and mania, as well as greater improvement in self-reported functioning.

Keywords: lurasidone, Depression, Mixed features

Disclosures: During the past 12 months, I have had the following relationships with the companies listed. Lecture Honaria: Otsuka (Asia), Bristol Myers Squibb (Canada), Genentech. Consultation: Corcept, Janssen, Atossa. Lecture Honaria: Otsuka (Asia), Bristol Myers Squibb (Canada), Genentech. Consultation: Corcept, Janssen, Genentech, Otsuka. Advisory Board: Genentech, Otsuka. Research Support: NIMH, HRSA, Avid. Stock Ownership: Atossa.
T94. Repeated Ketamine Exposure during Adolescence Produces Changes in Reward Sensitivity and Gene Expression in the Nucleus Accumbens in Adulthood

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Background: Major Depressive Disorder (MDD) is a highly debilitating mood disorder with poor treatment options and high economic burden. MDD afflicts up to 10% of adolescents. However, nearly 50% of those afflicted are considered non-responsive to available treatments. Ketamine (KET), a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist has shown potential as a rapid-acting and long-lasting treatment for MDD. Nevertheless, these effects are not permanent and it is conceivable that patients may require subsequent exposure to KET. We have recently shown that in addition to possessing antidepressant effects in adolescent rats, ketamine administered repeatedly during adolescence produces long-lasting (2 months) behavioral adaptations indicative of a stress-resilient phenotype. Given that adolescence is a critical period of brain development and KET’s high abuse liability, the long-term neurobiological consequences of repeated KET treatment must be characterized.

Methods: Adolescent (postnatal day [PD] 35) male rats were treated with KET (0, 20 mg/kg, twice-daily) for 15 days (PD 35-49) and then left undisturbed until adulthood (PD90). At this point, rats were divided into separate groups to assess their behavioral reactivity to the rewarding effects of KET, as measured by the place conditioning (CPP) and the drug self-administration paradigms, or behavioral reactivity to forced swimming stress. Separate groups of rats similarly treated with KET were also used for assessment of drug-induced biochemical changes in the nucleus accumbens (NAc).

Results: Adult rats pretreated with KET during adolescence showed increased sensitivity to the rewarding effects of KET (5, 10 mg/kg) as measured in the CPP and drug self-administration paradigms as compared to controls. Those rats exposed to forced swimming stress showed increased latency to immobility (i.e., antidepressant-like behavior), and showed a robust locomotor sensitization in response to subsequent KET exposure. RT-qPCR analysis revealed that pretreatment with KET modulates the expression of genes highly implicated in depression and drug-addiction (i.e. dopamine signaling).

Conclusions: These findings indicate that repeated KET treatment during adolescence increases behavioral sensitivity to subsequent KET exposure in adulthood, and implicate the intracellular signaling pathways known to mediate responsivity to drug reward and stress.

Keywords: Ketamine, Reward, Nucleus Accumbens, Adolescence
Disclosures: Nothing to disclose.

T95. Developing an Optimized BDNF Measurement for Mood Biomarkers

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Background: Brain-Derived Neurotrophic Factor (BDNF) is a growth factor in brain and many other peripheral compartments that appears to play an important role in cellular regeneration/survival. BDNF is associated with Mood disorders with a trend towards reduced levels in depression. However, effect sizes are modest and the accuracy of the BDNF assays in sera has been problematic. This may be a result of BDNF in sera existing as multiple forms with different biological activities. BDNF (119 amino acids) is produced from a precursor protein (proBDNF = 247 amino acids) by cleavage with the proteases furin (intracellularly), MMP9, or plasmin (extracellularly). BDNF and proBDNF have opposite functions: The former induces survival via binding to TrkB receptor, while the latter induces apoptosis via binding to the p75NTR and sortilin receptors. Despite this known biology, the majority of studies on BDNF in mood disorders do not discriminate between these forms, suggesting that there is an incomplete understanding. Specifically, the utility of BDNF as a mood biomarker may best be reflected in the levels of proBDNF, mature BDNF, or as a ratio of these two measures.

The purpose of the current work is to develop a robust method that can differentiate proBDNF and mature BDNF in an effort...
to improve the utility of BDNF measures as diagnostic and/or treatment response biomarkers for mood disorders.

Methods: Serum, EDTA plasma, and platelet-poor EDTA plasma (PPP) were collected from 2 healthy human donors. PPP was produced via a second high speed spin (10,000xg for 10 minutes) of traditional EDTA plasma to remove any residual cells/platelets. The plasma was then centrifuged at 10,000 x g for 10 minutes to remove any residual cell components before performing enrichment and/or recovery of total BDNF.

Due to the very high isoelectric point (pl) of BDNF and the difference in pl values between mature and proBDNF, cation exchange chromatography could theoretically be used to separate the two forms. A variety of cation exchange matrices were evaluated (carboxymethyl, sulfoethyl, MonoS) on the AKTA chromatography platform, ultimately selecting MonoS 5/50GL (# 17-5168-01, GE- Healthcare) as the best performing. 0.5 mL of serum or plasma was diluted with 4.5 mL of 50mM MOPS/0.5 mM EDTA/pH 6.6. This sample was then injected on a 1 mL MonoS column, followed by gradient elution with 50 mM Tris/1mM EDTA/1M NaCl/pH 8 with a linear flow rate. Fractions were collected every 0.5 mL and dried overnight in an Explorer 220 Savant SpeedVac (Thermo Scientific). Dried samples were stored at room temperature up to one week before analysis.

For final analysis fractions were reconstituted in either 20ul 1x SDS-PAGE loading buffer/DTT or 500 µl of 0.1%TWEEN/0.05%BSA/0.001%phenol red/150mM Tris/pH 7.8 with shaking at room temperature for 5 minutes. Fractions were analyzed by ELISA for total BDNF (MesoScale Discovery, #N451A-1) or proBDNF (R&D Systems, #DY3175), or SDS-PAGE for total protein or total BDNF (anti-BDNF from Santa Cruz #sc-546, recombinant human BDNF from Thermo Scientific #RP-8642).

Results: Immunoassay of crude serum, plasma, and platelet-poor plasma (PPP) showed that proBDNF was barely detectable in serum, but was present in substantial amounts in both plasma preparations (144 ± 14, 3898 ± 3109, 4106 ± 3290 pg/ml respectively in serum, plasma, PPP; avg ± SEM of 2 donors). In contrast, total BDNF was found in all 3 preparations, with the highest levels found in serum (11479 ± 3149, 1950 ± 917, 271 ± 150 pg/ml respectively in serum, plasma, PPP), suggesting a conversion of proBDNF to mature during clotting. Neither form was detectable in crude material by Western Blot (WB).

The cation exchange chromatography technique developed here resulted in >90% of serum proteins flowing through in early fractions (fractions 1-9, as seen by UV absorbance and Coomassie gel), while BDNF was retained until elution, allowing for excellent purification. Further, the proBDNF and mature BDNF forms eluted in discrete peaks (fractions 13-15 vs. 23-28, respectively, as seen by WB and ELISA), resulting in complete separation.

Additionally, the purification technique allowed for detection of BDNF by WB and recovery of total BDNF at levels equal to or greater than that in crude serum (3296 ± 564 vs. 4884 ± 1206 pg respectively in serum vs. MonoS purified serum respectively; avg ± SEM of 2 donors). The data suggests that some BDNF is masked in typical crude serum/plasma measurements and that purification to a higher degree of purity unvails this population.

Conclusions: We have developed a method for chromatographic enrichment of BDNF in serum that enables separate immunoquantification of the two major BDNF forms. This method detects more BDNF than seen in crude serum/plasma, suggesting matrix interference in typical measurements. Future plans include the separate evaluations of proBDNF and mature BDNF in a cohort of patients with major depressive disorder.

Keywords: Biomarker, Major depression, BDNF

Disclosures: The authors are employed by Janssen Research and Development of Janssen Pharmaceuticals.

T96. Individualized Prediction of Vulnerability to Bipolar Disorders Using Structural Neuroimaging Patterns from 307 Individuals and Machine Learning

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Background: Bipolar disorder (BD) is a highly heritable disease with approximately 85% of risk variance attributed to genetic factors (McGuffin et al., 2003). This highlights siblings of BD patients as a distinct population to examine and develop robust antecedent biosignatures of BD – as well as elucidate underlying pathophysiological mechanisms. Previous neuroimaging studies have reported intermediate average group-level neuroanatomical abnormalities in relatives of BD patients as compared to BD probands and healthy controls (Fusar-Poli et al., 2012, Matsuo et al., 2012). Here, we report a novel analytical approach of identifying siblings of BD patients from BD probands and healthy controls using structural neuroimaging patterns at an individual subject level.

Methods: T1-weighted scans from 128 BD patients and 128 demographically matched healthy controls (age range = 18-62) were acquired and gray matter density (GMD) values extracted using the SPM8 toolbox (Ashburner, 2009). A relevance vector machine (RVM) algorithm (Tipping, 2001) was ‘trained’ and brain regions most relevant in differentiating individual BD patients from healthy controls identified. We refer to this step as a brain signature discovery step. An additional sample of T1-weighted scans from 17 BD patients, 17 siblings of BD patients and 17 matched healthy controls were acquired and GMD values extracted. GMD values from brain regions identified in the discovery process above were entered into a RVM algorithm which was ‘trained’ to predict individual siblings of BD patients from BD patients and healthy controls. A standard leave-one-out cross-validation step was used in both steps to predict individual subjects not included in the algorithm ‘training’ process (Mwangi et al., 2014). Consequently, each individual subject was assigned a probability score ranging from zero to unity (0 – healthy, 1 – bipolar disorder, 0.5 – indecisive).

Results: The brain signature discovery step identified individual BD patients from healthy controls with 65% accuracy, and area under receiver operating characteristic curve (AUC) = 0.79 with chi-square p < 0.005. Relevant brain regions included; inferior frontal gyrus, middle temporal gyrus, thalamus and superior frontal gyrus. In the second step, the RVM algorithm correctly identified 82% of healthy controls and 76.5% of BD patients – translating into 79.4% accuracy and AUC = 0.79 with chi-square p < 0.005. Healthy controls and BD patients were assigned average probability scores of 0.28 and 0.62 respectively. In contrast, BD siblings were assigned intermediate probability scores.
T97. Antidepressant Efficacy in a Rodent Model of Hypoxia-Related Depression: Do SSRIs Lose Efficacy at Altitude?

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Background: Selective serotonin reuptake inhibitors (SSRIs) are often a first-line antidepressant treatment. However, high rates of both depression and suicide (the most negative impact of unresolved depression) are seen in people living at altitude or with chronic hypoxic diseases such as COPD, asthma and cigarette smoking. These observations suggest these populations may be at significantly higher risk for treatment-resistant depression. At high altitude, brain serotonin (5HT) levels drop in rats, and SSRIs have been shown to lose antidepressant efficacy in animal models of low brain 5HT, implying that chronic hypoxia may be linked to SSR1 treatment-resistance. We therefore investigated whether housing at altitude can change antidepressant efficacy of SSRIs using an animal model for hypoxia-related depression.

Methods: Female SD 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 antidepressants, and tested for depression-like behavior (DLB) in the forced swim test (FST). Antidepressant treatment consisted of 3 subcutaneous injections of antidepressant in the 24hrs between pretest and test FST sessions. Rats were treated with the SSRIs fluoxetine (FLU, Prozac), 20 or 40mg/ml, paroxetine (PAR, Paxil), 20mg/ml or escitalopram (ESC Lexapro, 20mg/ml), or the tricyclic antidepressant (TCA) desipramine (DES, 10mg/ml). Controls (C) received saline injections.

Antidepressant treatment effects are presented as percent of control within each altitude. DLB in the FST is defined as less activity (swimming, climbing), more immobility and lower latency to immobility (LTI). Effective antidepressant function is defined as a 20% reduction in immobility and increase in LTI, with increased swimming (SSRIs) or climbing (DES). Data was analyzed by two-way ANOVA with altitude and treatment as independent variables, and significance was determined with the post-hoc Bonferroni multiple comparison test. (n = 7-15).

Results: A. SSRIs (1) DLB: The SSRIs FLU, PAR or ESC did not reduce DLB in female rats in the FST vs. controls at any altitude: immobility and LTI were similar or worse than controls, with a significant effect of treatment (p < 0.0001 each) and of altitude (Immobility: p = 0.02; LTI: p = 0.005), but not of their interaction (p > 0.05). (2) ACTIVITY: Only PAR significantly increased swimming in the FST in this study (4.5K, 10K: p < 0.05 each), while all three SSRIs surprisingly reduced climbing in the FST (p < 0.05 each). (4) ESC exhibited the worst antidepressant efficacy, significantly increasing immobility and decreasing LTI. B. TCA: (1) DLB: DES showed strong antidepressant efficacy in this model: DES reduced immobility by 30% and doubled LTI (p < 0.05 each). (2) ACTIVITY: DES doubled time spent climbing at each altitude (p < 0.0001), but did not alter swimming, as expected.

Conclusions: (1) Three SSRIs currently in use in antidepressant therapy, FLU, PAR and ESC, do not exhibit antidepressant efficacy in the FST in female rats in our model for hypobaric hypoxia. Studies in progress imply a similar lack of FLU effect in male rats. These data imply that SSRIs may not work well for treatment of MDD in chronic hypoxia. (2) The noradrenergic antidepressant, DES, exhibits strong antidepressant efficacy in the FST in our model for hypobaric hypoxia, but this TCA is no longer in widespread use due to side effects and the potential for overdose lethality. (3) The findings of this study imply that a non-serotonergic antidepressant may function better in people at altitude and in chronic hypoxic disorders. (4) Future studies using this model for hypoxia-related depression will assess the efficacy of noradrenergic antidepressants including serotonin-norepinephrine reuptake inhibitors (SNRIs) such as venlafaxine (Effexor) or norepinephrine-dopamine reuptake inhibitors (NDRIs) such as bupropion (Wellbutrin), and will also test the impact of novel non-traditional antidepressant therapeutics with a focus on correcting hypoxia-induced brain deficits.

Keywords: Depression, Altitude, SSRI, forced swim test, Treatment Resistant Depression

Disclosures: Nothing to disclose.

T98. Cognitive Predictors of Sexual Dimorphism in Pediatric Depression

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Background: Sexual dimorphism (differences between males and females) in depression is common, is observed across species, and occurs early in development. The etiologic pathways that lead to these sex differences in depression may stem from cognitive factors that vary as a function of developmental stage. Previous studies, however, have been insufficiently powered to model these risk factors system-
attractively within or between males and females, within and across depression and commonly co-occurring anxiety, or across developmental stages. The present study leverages the large multimodal dataset of the Philadelphia Neurodevelopmental Cohort (PNC) to address the pressing questions of which cognitive factors predict depressive symptoms conditioned on sex (i.e., within males and within females), and which cognitive factors explain sexual dimorphism in childhood-onset symptoms of depression and anxiety.

**Methods:** We used state-of-the-art multivariate model selection methods to develop a parsimonious risk model to investigate 85 cognitive predictors of sexual dimorphism in childhood-onset depressive and anxiety symptoms in 8009 PNC youth (4147 girls and 3862 boys) ages 8-21 years. These PNC youth underwent semi-structured clinical interviews to assess for the presence of psychiatric symptoms and the Penn computerized neurocognitive battery to assess cognitive function.

**Results:** Approximately 21.4% of all youth endorsed depressive symptoms, and 49.7% of all youth endorsed anxiety symptoms. Girls were more likely than boys to endorse depressive symptoms starting at the age of 11. Cognitive predictors of depressive symptoms across all youth included reading ability, abstraction, and age differentiation. Reading ability and working memory were stronger predictors of depressive symptoms in girls than in boys. Cognitive predictors of shared depressive and anxiety symptoms in girls included face memory, sustained attention, and emotion differentiation, whereas in boys, working memory, sustained attention, and emotion differentiation predicted the presence of depressive and anxiety symptoms. Cognitive factors that differentiated girls from boys in predicting depressive symptoms were the capacity to differentiate sad from happy, angry, fearful, and neutral faces, and abstraction.

**Conclusions:** Key cognitive factors predict sexual dimorphism in psychopathology. Identifying and targeting these cognitive predictors prior to the onset of symptoms has the potential to prevent depression and to provide more precise neuroscience-informed treatment targets for childhood-onset symptoms of depression and anxiety.

**Keywords:** Sexual Dimorphism, Cognition, Major depression, Children and Adolescents

**Disclosures:** Nothing to disclose.

**T99. Reduced Global Functional Connectivity of the Medial Prefrontal Cortex in Major Depression Disorder**

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**Background:** Major depressive disorder is a disabling neuropsychiatric condition that is associated with disrupted functional connectivity across brain networks. The precise nature of altered connectivity, however, remains incompletely understood. The current study was designed to examine the coherence of large-scale connectivity in depression using a recently developed technique termed global brain connectivity.

**Methods:** A total of 82 subjects, including medication-free patients with major depression (n = 57) and healthy volunteers (n = 25) underwent functional magnetic resonance imaging with resting data acquisition for functional connectivity analysis. Global brain connectivity was computed as the mean of each voxel’s time series correlation with every other voxel and compared between study groups. Relationships between global connectivity and depressive symptom severity measured using the Montgomery-Asberg Depression Rating Scale were examined by means of linear correlation.

**Results:** Relative to the healthy group, patients with depression evidenced reduced global connectivity bilaterally within multiple regions of medial and lateral prefrontal cortex. The largest between-group difference was observed within the right subgenual anterior cingulate cortex, extending into ventromedial prefrontal cortex bilaterally (Hedges’ g = -1.48, p < 0.000001). Within the depressed group, patients with the lowest connectivity evidenced the highest symptom severity within ventromedial prefrontal cortex (r = -0.47, p = 0.0005).

**Conclusions:** Patients with major depressive evidenced abnormal large-scale functional coherence in the brain that was centered within the subgenual cingulate cortex, and medial prefrontal cortex more broadly. These data extend prior studies of connectivity in depression and that functional disconnection of the medial prefrontal cortex is a key pathological feature of the disorder.

**Keywords:** Depression, Resting State Functional Connectivity, graph theory, subgenual, Medial Prefrontal Cortex

**Disclosures:** Nothing to disclose.

**T100. Conceptual Convergence: Increased Inflammation is Associated with Increased Basal Ganglia Glutamate in Patients with Major Depression**

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**Background:** Inflammation and alterations in glutamate metabolism are two important and potentially complementary pathways in the causal chain of pathophysiology leading to mood disorders including major depression. Interestingly, consistent with this notion, our preliminary studies have demonstrated that activation of inflammatory responses resulting from administration of the inflammatory cytokine interferon (IFN)-alpha led to increased glutamate (normalized to creatine or Glu/Cr) in the left basal ganglia and dorsal anterior cingulate cortex (dACC) as measured by proton magnetic resonance spectroscopy (MRS). Increased glutamate in these brain regions was in turn associated with symptoms of depression including anhedonia and psychomotor slowing. Nevertheless, it remains unclear whether inflammatory activation is associated with increased central nervous system (CNS) glutamate in subjects with major depression. Therefore, we used a sample of patients with major depression to examine if peripheral and CNS markers of inflammation
predicted increased glutamate concentrations in the left basal ganglia and dACC and to examine if changes in glutamate were associated with the severity of depressive symptoms including anhedonia and psychomotor slowing. Furthermore, given the reported association between astrocytic dysfunction and glutamate pathology among major depression patients, we also explored if increased inflammatory activation in the periphery and CNS was associated with increases in concentrations of the astroglial marker myo-inositol (mI).

**Methods:** Fifty medically stable, medication-free outpatients with major depression were recruited. Standardized measurements of depressive symptom severity and neurocognitive function were obtained along with acquisition of plasma and cerebrospinal fluid (CSF) samples for measurement of inflammatory markers including C-reactive protein (CRP) and a series of inflammatory cytokines and their receptors as well as chemokines. All assessments were conducted under identical conditions within 1-2 days following MRS scans. Two independent but complementary MRS techniques were used to address two distinct goals of the study. First, a focused, hypothesis-driven analysis (one metabolite, one inflammatory marker) was conducted to examine the relationship between CRP and absolute tissue-corrected concentrations of glutamate in the left basal ganglia and dACC obtained using single voxel MRS. In addition, we examined if regional absolute glutamate concentrations predicted behavioral and neurocognitive measures. Scans were obtained on a Siemens Trim Trio 3T scanner with settings of TR/TE/averages/data points = 3000/30/128/1024. Second, an exploratory, non-selective analysis was conducted to examine the relationship between CRP and other inflammatory markers and creatine-normalized concentrations (metabolite/Cr) of a broad array of neurometabolite (including astroglial) biomarkers in bilateral basal ganglia regions using chemical shift imaging (CSI) with settings of TR/TE/averages/data points = 1590/30/7/1024, voxel size of 10.3x10.3x15mm3 and acquisition matrix of16x16. 

**Results:** Increased log plasma CRP was associated with increased log left basal absolute glutamate in both a linear and stepwise fashion from low (<1mg/L) to high (>3mg/L) inflammation. Results remained significant after controlling for covariates including age, sex, race, BMI and depression severity. In turn, left basal ganglia glutamate positively predicted symptoms of anhedonia and measures of simple reaction time and negatively predicted performance on the Digit Symbol Substitution Task and the finger-tapping task after controlling for covariates. In addition to CRP, the plasma chemokine monocyte chemoattractant protein (MCP)-1 was also associated with absolute glutamate concentrations in the basal ganglia. Neith plasma CRP nor any of the inflammatory markers were associated with dACC glutamate. A broader omnibus multivariate analysis of variance using all metabolites from CSI demonstrated a significant overall main effect of CRP on the metabolite profile in the basal ganglia. Significant individual associations between CRP status and Glu/Cr and ml/Cr ratios in the left basal ganglia and ml/Cr in the right basal ganglia were revealed following correction for multiple comparisons. CSF concentrations of interleukin (IL)-1beta and plasma concentrations of its receptor antibody (IL-1ra) predicted Glu/Cr in the left basal ganglia and ml/Cr in both left and right basal ganglia. Finally, ml/Cr in the right basal ganglia was strongly predicted by CSF CRP concentrations (p<0.001).

**Conclusions:** These data provide the first evidence that increased inflammation in major depression as measured by CRP and other inflammatory markers is associated with increased absolute glutamate in the left basal ganglia, which in turn was associated with alterations in hedonic tone, psychomotor activity and processing speed. In addition, relationships between proinflammatory markers and myo-inositol point to the possibility of astrocytic dysfunction as a mediator of glutamate changes in activated inflammatory states among depressed patients. These data also suggest that increased peripheral inflammatory markers may identify depressed patients with increased basal ganglia glutamate and therapeutic strategies targeting glutamate may be preferentially effective in depressed patients with increased inflammation.

**Keywords:** MR spectroscopy, Psychoneuroimmunology, Major Depressive Disorder (MDD), cytokines, CRP

**Disclosures:** Nothing to disclose.
remitter versus non-remitter status at 8 weeks, and interacted with type of treatment. Whole brain voxel wise analyses were conducted using an uncorrected threshold of p < 0.001 to test for generalized treatment effects. Further, complementary ROI based analyses utilizing generalized linear models (GLMs), implemented in R, elucidated the moderating impact of antidepressant treatment. Remission status was determined by a Hamilton Depression Rating Score of < 7 at week 8. Age and depression severity at the baseline session were used as covariates in all statistical models.

**Results:** Whole brain voxel wise analyses revealed that remitters relative to non-remitters showed greater resting state connectivity between the PCC and ACC. By contrast, non-remitters showed greater resting state connectivity between PCC and inferior parietal cortex than remitters. Further, linear regression analyses revealed that there was a significant antidepressant by remission status interaction in the ACC. Specifically, remitters who received sertraline and venlafaxine-XR showed greater pre-treatment PCC-ACC connectivity relative to their non-remitter counterparts (t = 3.33, p = 0.002 and t = 2.62, p = 0.01 respectively). However, those who received escitalopram treatment and remitted were not differentiated from non-remitters by PCC-ACC connectivity (t = -0.42, p = 0.68).

**Conclusions:** The general and differential capacity to mount a response to antidepressants was predicted by pre-treatment connectivity within the default mode network. Such findings suggest that neuroimaging derived measures of resting state connectivity may be used for tailoring treatment choices in MDD.

**Keywords:** Depression, Resting State Functional Connectivity, default mode network, Antidepressants, anterior cingulate cortex

**Disclosures:** Nothing to disclose.

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**T102. The Antidepressant-Like Utility of Rho-Kinase Inhibition in Adolescents is TrkB-Dependent**

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**Background:** Adolescence represents a critical period of neurodevelopment, defined by structural and synaptic maturation and reorganization within the prefrontal cortex. Although these processes are critical for the transition to adulthood, structural instability may open a window of vulnerability to neuropsychiatric disorders including depression. A drug that facilitates the synaptic remodeling that occurs during adolescence may be therapeutic. To test this hypothesis, we evaluated the therapeutic-like potential of the brain-penetrant Rho-kinase (ROCKII) inhibitor, fasudil.

**Methods:** Adolescent female mice expressing thy1-derived Yellow Fluorescent Protein were administered fasudil, fluoxetine, ketamine or vehicle at postnatal (P) day 42 prior to the forced swim test and euthanasia. Brains were frozen for immunoblotting experiments or fixed for confocal fluorescence microscopy. Lastly, a truncated (inactive) TrkB receptor was expressed in the ventromedial prefrontal cortex (vmPFC) to determine whether the antidepressant-like effects of fasudil were TrkB-dependent.

**Results:** Fasudil has antidepressant-like properties, and these effects are indistinguishable from those of fluoxetine and ketamine. Fasudil increases expression of TrkB, p110beta (the catalytic subunit of PI3K), AKT, mTOR, and PSD-95 in the adolescent vmPFC. The increase in PSD-95 is accompanied by enhanced dendritic spine pruning in deep-layer vmPFC, resulting in adult-like spine densities. Over-expression of truncated TrkB in the vmPFC occludes the antidepressant-like effects of fasudil in the forced swim test. At the same dose, fasudil has no effects on these signaling factors, dendritic spine density, or forced swim performance in adult mice.

**Conclusions:** Together these findings suggest that ROCKII inhibition may be uniquely therapeutic in the treatment of adolescent-onset depression.

**Keywords:** Adolescent Depression, Antidepressant, Dendritic Spine, TrkB, Medial Prefrontal Cortex

**Disclosures:** Nothing to disclose.

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**T103. Late Pregnancy Thyroid-Binding Globulin Predicts Perinatal Depression**

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**Background:** DSM-IV major depression, minor depression and anxiety disorders are common during pregnancy and the postpartum period (O’Hara and Wisner, 2014). Large endocrine changes that occur during pregnancy and postpartum have been examined as correlates of perinatal depression (Schiller et al., 2015). Previously we found significant correlations between lower total and free thyroxine (TT4, FT4) concentrations at 3 time points between pregnancy weeks (PW) 32 and 37 and greater pre and/or postpartum depressive symptoms (Pedersen et al., 2007; N = 31). The current study was conducted in a much larger cohort (N = 199) that enabled examination of pregnancy thyroid indices, trauma history and major depression history as predictors of perinatal syndromal depression (DSM-IV major and RDC minor) as well as perinatal depression and anxiety ratings. Findings by Bloch et al. (2000) indicated for the first time that sensitivity to postpartum major depression is related to variance in sensitivity to sex hormone elevations during pregnancy rather than differences in hormone levels. Elevated estrogen during pregnancy causes an approximately 150% rise in thyroid-binding globulin (TBG) concentrations suggesting that TBG may be an index of estrogen sensitivity.

**Methods:** Our cohort was 199 low SES, euthyroid women recruited from a public health obstetrics clinic. After screening and blood draws for hormone measures at PW 31-33, subjects were evaluated during home visits at PW 35-36 as well as postpartum weeks (PPW) 6 and 12. Evaluations included psychiatric interviews for current and lifetime DSM-IV psychiatric history, subject self-ratings...
and interviewer ratings for depression and anxiety (Edinburgh Postnatal Depression Scale, Montgomery-Asberg Depression Rating Scale; state part of Spielberger State-Trait Anxiety Inventory, Hamilton Anxiety Inventory), as well as a standardized interview to obtain lifetime trauma history.

**Results:** Our cohort was 63.8% Hispanic, 21.6% Black. 52.8% had lifetime major depression and a mean of 4.5 categories of lifetime trauma. During late pregnancy, 22 (11.1%) subjects met criteria for syndromal depression, 24 (12.1%) met criteria postpartum, 9 met criteria pre and postpartum. Thus 37 (18.6%) met criteria for perinatal syndromal depression. Mean depression and anxiety ratings were significant individual predictors of syndromal depression. (p = .016). Third trimester progesterone (P) levels were a significant individual predictor of depression and anxiety ratings when these variables were analyzed individually or in a combined model including FT4 or TBG (p < .001). FT4, TBG and trauma history, but not major depression history, were significant individual predictors of syndromal depression during the study period (p < .035). In combined models with trauma and major depression history, FT4 and TBG generally were not significantly predictive of mood ratings and FT4 was also not a significant predictor of syndromal depression. However, TBG was a particularly strong predictor of perinatal syndromal depression (p = .005) as was trauma history (p = .016). Third trimester progesterone (P) levels were a significant individual predictor of depression ratings (p < .035) and, in a combined model with trauma, FT4 and lifetime major depression, a predictor of syndromal depression. (p = .02)

**Conclusions:** We validated our previous report that 3rd trimester FT4 predicts perinatal depression ratings and also found that FT4 is a significant individual predictor of anxiety ratings and syndromal depression. Of greatest interest, though, is that TBG, in a combined model with trauma and major depression history, is the strongest predictor of syndromal depression. In other analyses, P had a relationship to TBG that differs significantly when adjusted for interactions with trauma history and perinatal syndromal depression. These findings, in addition to the strong relationship we now report between lower TBG and perinatal depression after adjustment for trauma and major depression history, suggest that further investigation of interactions among TBG, sex hormones and risk factors may provide new insights into the basis of variance among women in vulnerability to mood destabilization by the hormone conditions of pregnancy.

Supported by R01 MH077838-01A2 awarded to CP.

**Keywords:** thyroid, perinatal depression, perinatal anxiety, trauma history, major depression history

**Disclosures:** Nothing to disclose.

Supported by R01 MH077838-01A2 awarded to CP.

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**T104. Abnormal Neurochemistry in the Left Ventrolateral Prefrontal Cortex of Young Offspring of Bipolar Disorder Parents**

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**Background:** Bipolar disorder symptoms may arise from dysfunction within the anterior limbic network. Adolescence is a common period of onset of bipolar disorder, and offspring of bipolar parents have an increased risk of developing mood disorders compared with the general population. Thus, the study of young bipolar offspring prior to development of a mood episode may help identify potential neurobiological predictors of disease. We used proton magnetic resonance spectroscopy (1H MRS) to investigate possible neurochemical abnormalities in the left and right ventrolateral prefrontal cortices (VLPFC), and the anterior cingulate cortex (ACC) of youth offspring of bipolar parents as potential vulnerability markers of mood disorders.

**Methods:** 3D T1-weighted magnetic resonance images at 4 T were obtained from 117 offspring of DSM-IV bipolar disorder type I patients (bipolar offspring; mean age ± SD: 13.6 ± 2.7 years girls: 54%) and 62 group-matched healthy offspring of healthy parents (HC offspring; mean age ± SD: 14.2 ± 3.0 years, girls: 55%). Bipolar offspring were free of major mood or psychotic disorders. Metabolite concentrations, including N-acetyl aspartate (NAA), phosphocreatine plus creatine (PCr + Cr), choline-containing compounds (Cho), myo-inositol (mI), glutamate plus glutamine (Glx), and glutamate, were determined using 1H MRS, and corrected for gray/white matter ratio. We compared metabolite concentrations between the bipolar and HC offspring; as a secondary analysis we compared metabolite concentrations between subgroups of bipolar offspring with and without psychopathology, and compared both groups with HC offspring. We also investigated possible correlations between metabolite concentrations and mood rating scale scores in the bipolar offspring.

**Results:** Bipolar offspring presented lower NAA, PCr + Cr, glutamate, and Glx concentrations compared with HC offspring in the left VLPFC, but these differences were not statistically significant. There were no differences in any of the metabolite levels in the right VLPFC and ACC between bipolar and HC offspring. A three group comparison of affected bipolar offspring, unaffected bipolar offspring, and HC offspring showed statistically significant differences among the three groups in concentrations of NAA (p = .009), PCr + Cr (p = .009), mI (p = .05), glutamate (p = .03), and Glx (p = .05), in the left VLPFC. Specifically, affected bipolar offspring presented lower metabolite concentrations than either healthy bipolar or HC offspring. Pairwise comparisons showed that affected bipolar offspring had significantly lower NAA (p = .0008) and PCr + Cr (p = .007) concentrations than healthy bipolar offspring, and significantly lower glutamate (p = .04) and Glx (p = .04) concentrations than HC offspring. There were no differences among the 3 groups in the right VLPFC and the ACC. NAA (r = -.29, p = .002), Cho (r = -.21, p = .03), PCr + Cr (r = -.27, p = .004), glutamate (r = -.19, p = .04), and Glx concentrations (r = -.21, p = .02) negatively correlated with depression ratings in the left...
VLPFC of bipolar offspring. There were no correlations between neurochemical concentrations and depression ratings in the right VLPFC or ACC; there were no correlations between neurochemical concentrations and mania ratings in any of the regions measured.

Conclusions: Abnormalities in the glutamatergic system were observed in the left VLPFC of youth at risk for bipolar disorder. These findings are consistent with previous observations of glutamatergic abnormalities in early-course bipolar disorder patients. These findings may be driven by the lower concentrations of glutamate and Glx observed in the bipolar offspring showing some early evidence of psychopathology, suggesting that it might represent an early marker of the illness. Glutamate and Glx concentrations negatively correlated with depression ratings, reinforcing this suggestion. In addition, the negative correlation between possible markers of neuronal metabolism and depression ratings suggest that the early onset of symptoms might be related to changes in the left VLPFC activity. These results are consistent with prior findings of decreased cortical activity in the left VLPFC of youth bipolar offspring, as well as in adults with bipolar disorder. Early interventions targeting changes in the glutamate system and prefrontal neuronal metabolism might yield therapeutic benefits for youth at risk for bipolar disorder. In addition, these changes might prove to be a marker for youth who might most benefit from early, intensive intervention.

Keywords: Bipolar Disorder, child offspring, ventrolateral prefrontal cortex, glutamate, MR spectroscopy

Disclosures: This study was partly supported by a NIH grant # 5 P50 MH077138. Dr. Fabiano G. Nery held a position of Associate Medical Advisor in Eli Lilly & Co. from 2012 to 2013. His wife is currently an employee of Eli Lilly & Co. Dr. Stephen Strakowski chairs DSMBs for Sunovion and is a consultant to Procter & Gamble. Dr. Caleb Adler has received research support from AstraZeneca, Amylin, Eli Lilly, GlaxoSmithKline, Lundbeck, Martek, Merck, Novartis, Otsuka, Pfizer, Takeda, and Shire. He has been on the lecture bureau for Merck and Sunovion, for which he has received honoraria. Dr. Melissa P. DelBello has received research support from AstraZeneca, Amylin, Eli Lilly, Pfizer, Otsuka, GlaxoSmithKline, Merck, Martek, Novartis, Lundbeck and Shire, and is also on the lecture bureau for Otsuka, Merck and Bristol-Meyers Squibb. She has received Consulting/Advisory Board/Honoraria/Travel support from Merck, Pfizer, Dey, Lundbeck, Sunovion and Otsuka. The remaining authors reported no conflicts of interests.

T105. Inflammatory Cytokines Are Associated with Decreased Psychomotor Speed in Female, but Not Male, Patients with Major Depression

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Background: There has been increasing recognition that inflammation may play a role in the pathogenesis of major depression (MD). Inflammatory cytokines are increased in the peripheral blood and cerebrospinal fluid of MD patients, and administration of inflammatory cytokines or cytokine inducers result in depressive symptoms, especially anhedonia and psychomotor slowing. One mechanism that may contribute to cytokine effects on the brain and behavior is inhibition of dopamine release in the basal ganglia. We therefore hypothesized that inflammatory cytokines would be associated with poor performance on neurocognitive tests of psychomotor speed in patients with MD.

Methods: Patients diagnosed with MD (DSM-IV TR) were recruited at Emory University. Subjects were required to have a 17-item Hamilton Depression Rating Scale score (HAM-D) ≥ 20 for inclusion in the study. Blood sampling for plasma inflammatory markers and neuropsychological testing were completed on the same day at standardized times to control for circadian variations. A range of neuropsychological tests were chosen to probe basal ganglia function, from purely motor tasks (e.g. finger tapping) to those that involved motor activity with increasing cognitive demand and cortical participation (e.g. Trails A and Digit Symbol Substitution Task). The following neuropsychological tests were included: Finger Tapping Task, Reaction Time Task (CANTAB), Trail Making Test Part A (Trails A), and the Digit Symbol Substitution Task (DSST). Of note, the CANTAB Reaction Time Task distinguishes both simple and choice reaction times and movement latencies. Plasma inflammatory variables, including C-reactive protein (CRP), interleukin (IL) 1beta, IL-6, tumor necrosis factor (TNF) and their soluble receptors IL-1 receptor antagonist (IL-1ra), soluble IL-6 receptor (sIL-6R) and soluble TNF receptor 2 (sTNFR2), were measured by Luminex-based multianalyte profiling kits (R and D Systems). Linear regression analyses were performed to determine which inflammatory measures predicted psychomotor task performance, controlling for age, race, education, and body mass index (BMI). Based on previous studies indicating differential responsiveness of males and females to inflammatory challenge, the sexes were analyzed independently.

Results: One hundred seven unmedicated subjects were included in the analysis (71 females). Male and female subjects were significantly different on BMI (p = 0.028) and finger tapping frequency (p = 0.036), but did not significantly differ on any other demographic variables or performance on neuropsychological measures. Linear regression analyses showed significant relationships between all motor tasks and various inflammatory markers in women but not in men. In women, slower frequency of finger tapping was associated with higher levels of sTNF-R2 (B = 0.453, p = 0.001). Slower times to complete Trails A and slower simple movement time was associated with higher concentrations of IL-6 (B = 0.248, p = 0.049 and B = 0.284, p = 0.007, respectively). Slower choice movement time was also associated with higher IL-6 (B = 0.277, p = 0.024) as well as higher IL-1beta (B = 0.334, p = 0.007) and lower IL-10 (B = -0.253, p = 0.040). In addition, lower number of correct responses in ninety seconds on the DSST was associated with higher plasma concentrations of IL-6 (B = -0.240, p = 0.047) and lower concentrations of IL-10 (B = -0.235, p = 0.005). Interestingly, performance on simple and choice reaction time tasks was not significantly affected by inflammatory cytokines in either women or men.

Conclusions: Females, but not males, with MD exhibited a significant association between slowing on neurocognitive tasks with a strong motor component and plasma
concentrations of IL-6 as well as other inflammatory cytokines and cytokine receptors. Tasks with more of an attentional component (reaction time) were unrelated to inflammation. These data are consistent with findings that women may be more sensitive to inflammatory effects on behavior. Moreover, the data suggest that inflammation may be more associated with neurocognitive tasks related to basal ganglia (dorsal striatal) function as opposed to tasks that invoke attentional networks.

**Keywords:** inflammation, Cognition, Major depression, cytokines, Basal Ganglia

**Disclosures:** There are no conflicts of interest specific to the data presented in this poster. I have received a Janssen Academic Research Mentoring Award as well as an American Psychiatric Institute for Research and Education/Janssen Resident Psychiatric Research Scholar Award during residency.

**T106. Intranasal Esketamine in Treatment-resistant Depression, a Dose Response Study – Double Blind and Open Label Extension Data**

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**Background:** Intravenous administration of racemic ketamine, which is composed of both (s)-ketamine (esketamine [ESK]) and (r)-ketamine, has been shown to produce rapid antidepressant effects in some patients with treatment-resistant depression (TRD). The dose response relationship and the capability of maintaining the antidepressant effect over the long term have not been studied systematically. We assessed the dose-response relationship for intranasal ESK in patients with TRD, and its ability to sustain the antidepressant response with a gradual tapering of treatment frequency.

**Methods:** This was a 2-panel, double-blind (DB), placebo-controlled, multicenter study. Panel A was conducted in the United States and Belgium; Panel B is ongoing in Japan (hence not discussed here). Panel A involved a doubly randomized, DB treatment phase using intranasal ESK that included two 1-week periods (Periods 1 and 2), an up to 9-week, optional open-label (OL) treatment phase with tapering of dose frequency, and an 8-week follow-up phase occurring after the last dose. The 1-week periods each included 2 treatment sessions. In Panel A, a total of 67 patients with TRD were randomly assigned (3:1:1:1) to 1 of 4 groups: placebo (n = 33), ESK 28 mg (n = 11), 56 mg (n = 11), or 84 mg (n = 12) in Period 1. In Period 2, 28 placebo patients with continued moderate to severe symptoms as assessed using the 16-item, self-reported Quick Inventory of Depressive Symptomatology (QIDS-SR16: moderate, 11 to 16; severe, >16), were re-randomized (1:1:1:1) to placebo (n = 6), ESK 28 mg (n = 8), 56 mg (n = 9), or 84 mg (n = 9). Patients who received ESK in Period 1 remained on ESK in Period 2. During the OL phase, treatment session frequency was reduced from: twice weekly - first 2 weeks; to once weekly - 3 weeks; and then to every other week - for the subsequent 4 weeks. Patients receiving oral antidepressants prior to study entry continued their regimen throughout the study, while patients who were not currently receiving antidepressants at study entry did not receive concomitant oral antidepressants during the study. The primary efficacy endpoint was the change from baseline to day 8 (each period) in the Montgomery-Asberg Depression Rating Scale (MADRS) total score. Other endpoints included the percentage of patients who responded and remitted during ESK administration. Safety endpoints included treatment-emergent adverse events (TEAEs) and Clinician Administered Dissociative States Scale (CADSS) total score.

**Results:** The mean change in MADRS total score on day 8 (Periods 1 and 2 combined; weighted combination test) in all 3 ESK groups was statistically superior to placebo (28 mg: p = 0.021, 56 mg: p = 0.001, and 84 mg: p < 0.001; 1-sided significance levels). Mean differences (standard error) from placebo (after 1 week treatment; i.e. day 8) were -4.2 (2.09) for ESK 28 mg, -6.3 (2.07) for ESK 56 mg, and -9.0 (2.13) for ESK 84 mg. Of those who continued the same treatment in both Periods 1 and 2, the response rates (day 15, unadjusted) were: placebo: 10%; ESK 28 mg: 37.5%; ESK 56 mg: 36.4%; and ESK 84 mg: 50%. Remission rates (day 15, unadjusted) were: placebo: 10%; ESK 28 mg: 12.5%; ESK 56 mg: 27.3%; and ESK 84 mg: 40%. A total of 57 patients entered the OL phase on day 15 with a starting dose of ESK 56 mg; the dose was then adjusted based on the investigator’s clinical judgment of efficacy and tolerability. The majority of patients received the highest dose, 84 mg, during the OL phase. In a subset of patients (n = 10), the OL treatment phase only lasted 2 weeks (2 sessions/week) before the protocol was amended to extend this phase to 9 weeks (day 74). Thirty-four patients had data available at day 74; the majority were responders (22/34; 64.7%), and 11/34 of these patients (32.4%) also met remission criteria. Post-treatment follow-up data (up to 8 weeks) was available for 41 patients (including those who remained in the OL phase for 2 weeks or withdrew early); the majority sustained their improvement, with 23/41 patients (56.1%) considered responders and 17/41 (41.5%) considered remitters at the end of the 2-month follow-up period. Intranasal ESK was well-tolerated with most TEAEs being similar to those reported with intravenous ketamine and ESK. The most common TEAEs during the DB phase (≥10% of patients in any group) were: dizziness, dissociation, headache, dysgeusia, nasal discomfort, nausea, hypoaesthesia oral, dissociative symptoms, tunnel vision, oropharyngeal pain, throat irritation, blurred vision, hyperhidrosis, feeling abnormal, insomnia, hypertension, vertigo, paresthesia and sedation. Transient elevation in blood pressure (maximum mean change: systolic, 19 mmHg; diastolic, 10.3 mmHg) and heart rate was observed in a majority of patients on ESK on dosing days. The perceptual changes, measured by the CADSS, suggest onset of these symptoms occurred shortly after the start of intranasal dosing and resolved by 2 hours post-dose; the mean CADSS scores diminished significantly with repeated dosing.

**Conclusions:** Intranasal ESK was efficacious in the treatment of TRD and well-tolerated at all doses tested. Efficacy appeared more robust at higher doses and response to 56 mg and 84 mg doses appeared to be more sustained across
the DB treatment period. There was also evidence that efficacy was sustained over the OL phase (with a reduced frequency of treatment sessions) and also for up to 8 weeks after cessation of intranasal dosing. Further studies are needed to provide data on efficacy and safety during long term ESK administration.

**Keywords:** Esketamine, Treatment Resistant Depression, Intranasal formulation

**Disclosures:** Drs. Daly, Singh, Fedgchin, Cooper, Lim, Melman, Manji, Van Nueten and Drevets are employees of Janssen Research & Development and hold company stocks, and will not receive any direct financial benefit from any patent directed to this technology on which he/she may be an inventor. Dr. De Bruin has received research funding from Janssen Research & Development. Drs. Shelton, Thase and Ahmad have nothing to disclose. All authors meet ICMJE criteria and all those who fulfilled those criteria are listed as authors. All authors had access to the study data and made the final decision about where to present these data.

**T107. Neural Functional Changes Resulting from SSRI, ECT, and rTMS Treatments for Major Depressive Disorder: Meta-Analytic Synthesis and Comparison**

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**Background:** Conventional brain-based therapies for major depressive disorder (MDD) have developed mostly serendipitously, leaving open critical questions concerning the routes by which these therapies both ameliorate MDD as well as cause unwanted side effects. Addressing these questions could have important implications both for the development and for the individualization of neural therapies for depression. In this context, we conducted meta-analytic synthesis of studies imaging regional cerebral blood flow (rCBF) to ascertain the neural-systems-level ramifications of applying selective serotonin reuptake inhibitors (SSRIs), electro-convulsive therapy (ECT), or repetitive transcranial magnetic stimulation (rTMS) to the treatment of MDD.

**Methods:** Using PubMed and Web of Science databases as well as examining works cited in and cited by several key articles, we identified for SSRI, ECT, and rTMS treatment modalities studies reporting changes in rCBF as measured with positron emission tomography or single photon emission computed tomography in MDD from before to after administration of antidepressant therapy. We extracted from these studies reported coordinates of changes in rCBF resulting from antidepressant therapy and applied to these data a multi-level kernel density (MLKD) analysis approach to meta-analysis of functional neuroimaging data. After using MLKD analysis to identify regions showing meta-analytically reliable changes in rCBF from SSRI, ECT, or rTMS treatment, we applied intersection analysis to identify: 1) overlap across treatment modalities in neural-functional changes resulting from treatment; and 2) overlap between treatment associated neural changes and regions shown from prior meta-analysis to be abnormally over- or under-active in depressed relative to healthy control samples.

**Results:** SSRI, ECT, and rTMS treatments all resulted in meta-analytically reliable decreases in neural activity as indexed by rCBF, albeit in distinct neural regions. SSRI treatment resulted in reliable decreases in activity in the middle and posterior insula. ECT brought about reliably decreased activation in two primary nodes of the default-mode network (DMN): ventromedial prefrontal cortex and posterior cingulate cortex. Finally, treatment with rTMS resulted in reliable decreases in activity in subgenual cingulate cortex. None of the regions showing reliable change with treatment overlapped with regions in which meta-analytically reliable abnormal activity in MDD has been observed.

**Conclusions:** SSRI, ECT, and rTMS treatments for MDD result in decreased activity in non-overlapping neural regions—regions that have not shown reliably abnormal (relative to healthy control samples) levels of activity in MDD. SSRI-induced reduction of middle and posterior insula activity in MDD provides an intuitive account of both reduced negative affect—an SSRI treatment effect—and affective blunting, a side effect of SSRI treatment for depression. Similarly, ECT effects on the DMN in MDD could account for the rapid reduction in negative, self-relational thought associated with ECT treatment as well as impairment in autobiographical memory, a frequent side effect of ECT.

**Keywords:** Major depression, Positron emission tomography, serotonin reuptake inhibitors, electroconvulsive therapy, transcranial magnetic stimulation

**Disclosures:** Nothing to disclose.

**T108. Neurocognitive Performance as an Endophenotype for Mood Disorder Subgroups**

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**Background:** There is growing evidence that neurocognitive function may comprise an endophenotype for mood disorders (Glahn et al., 2012). The most compelling evidence supports aspects of executive function (e.g., task flexibility, planning and execution, problem solving), verbal memory, working memory, and attention as neurocognitive endophenotypes for bipolar disorder (Bora et al., 2009), whereas memory deficits may be a state marker for MDD (Lee et al., 2012). The goal of this study is to examine the specificity and heritability of cognitive functioning for the full range of mood disorder subtypes including Bipolar I disorder (BP-I), Bipolar II disorder (BP-II), Major Depressive Disorder (MDD), and controls in a community based family study of Mood Spectrum Disorders in order to determine whether these domains may comprise endophenotypes for mood disorders.

**Methods:** The sample includes 298 individuals who participated in a community-based family study in the...
greater Washington, DC metropolitan area. Mood disorder subtypes (i.e., BP-I, BP-II, and MDD) and other disorders were defined by the DSM-IV criteria using information from a semi-structured diagnostic interview and family history information, using Best Estimate Diagnostic procedures. Participants completed the University of Pennsylvania’s Computerized Neurocognitive Battery (CNB), which provides measures of accuracy and speed of performance across multiple neurocognitive domains. Mixed effect regression models in SAS version 9.3 were used to compare diagnostic subgroups, and the fixed effect for the within family component was used to estimate familial inter-correlations. All models controlled for participant age and sex.

**Results:** BP-I participants were more accurate on spatial ability than those with other mood disorder subtypes (p < .03). No other statistically significant associations between mood disorder subtype and accuracy were found. BP-I participants had slower working memory response times than other mood disorder subtypes and controls (p < .05), while BP-II participants performed faster on sensorimotor processing (p < .05). The BP-II group had marginally slower response time on the word memory (p < .05) and delayed word memory (p < 0.05) tasks. There were no speed or accuracy differences in any of the cognitive measures among participants with MDD compared to other mood subtypes or controls. There were significant familial associations for accuracy and speed on more than half of the tasks administered in this study. For accuracy, there were significant coefficients for multiple forms of memory (facial, immediate and delayed word, working), spatial ability, and motor speed. For speed, familial associations emerged for delayed facial memory and working memory, sensorimotor ability, spatial ability, emotion recognition, and motor speed.

**Conclusions:** This work adds to the growing evidence that specific cognitive domains may underlie the risk of bipolar disorder. To our knowledge, this is the first study of potential cognitive endophenotypes that includes the full range of mood disorder subtypes. In addition, the community-based sampling enhances the generalizability of the findings, and enables our ability to examine traits rather than acute states of disorders. Measures that may comprise endophenotypes, because of their specificity for mood disorder subtype and familial correlations, include working memory and spatial ability for BP-I, and sensorimotor ability for BP-II. The differential findings and lack of evidence for any cognitive endophenotypes for MDD should be evaluated in future studies. Future analyses of the associations between these cognitive domains and other study measures (i.e., neuroimaging, psychophysiological reactivity, personality/temperament, sensory thresholds, and objective measures of sleep and activity) may provide insight into the specific biologic processes underlying these mood disorder subtypes.

**Keywords:** Bipolar Disorder, endophenotypes, neurocognition, community based family study, mood disorders

**Disclosures:** Nothing to disclose.

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**T109. Luzindole Hippocampal Neurogenesis and Modulates Depressive-Like Behaviors via Distinct Actions at the MT1 and MT2 Melatonin Receptors in C3H/HeN Mice**

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**Background:** Major depressive disorder is characterized by a constellation of symptoms that affect mood, neurochemical balance, sleep patterns, circadian and/or seasonal rhythm entrainment, as well as promoting anxiety and neuronal atrophy (Srinivasan et al., Psych. Res. 165:201, 2009). Current pharmacological interventions for depression, including selective serotonin and/or norepinephrine re-uptake inhibitors and tricyclic antidepressant, alleviate most symptoms of depression but have little effect on sleep and circadian disturbances. MT1 and MT2 melatonin receptors are targets for the modulation of sleep and circadian rhythms. Activation of melatonin receptors in rodents entrain circadian rhythms and exert antidepressant-like effects in models of behavioral despair. In humans melatonin ligands ameliorate several symptoms of depression. In the C3H/HeN mice acute treatment with luzindole, a competitive antagonist at MT1/MT2 receptors and inverse agonist at constitutively active MT1 receptors, induced an antidepressant-like effect in the forced swim test via the MT2 receptor (Sumaya et al. J. Pineal Res. 39:170, 2005). Furthermore, chronic luzindole treatment increased hippocampal cell proliferation and survival (unpublished). Together these data suggest luzindole may act as an antidepressant-like compound. The current study was aimed to determine the MT1 and/or MT2 melatonin receptor type mediating the effects of chronic luzindole treatment on antidepressant-like activity and hippocampal neurogenesis in the C3H/HeN mice.

**Methods:** Wild type C3H/HeN and C3H/HeN mice with genetic deletion of MT1 and/or MT2 melatonin receptors [wild type, MT1KO, MT2KO, MT1/MT2KO] were given 21 days vehicle (0.4% ethanol) or luzindole (0.02 mg/ml) treatment via drinking water. All mice were subjected to a forced swim test 1 day after completion of treatment. To study hippocampal cell proliferation and neurogenesis, mice were injected with BrdU (4 x 75 mg/kg, ip) every 2 hours on the last day of treatment and sacrificed 24 hours after to assess cell proliferation, or 4 weeks after to assess cell survival and fate. Hippocampal coronal sections (30 um) were collected and used for BrdU immunohistochemistry. Neurogenesis was assessed by quantifying cells with BrdU and NeuN immunofluorescence co-labeling using confocal microscopy. Hippocampal cell proliferation and neurogenesis were separately analyzed in the dorsal and ventral hippocampus. All animal procedures were approved by the Institution 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:** Luzindole decreased immobility time compared to vehicle in the forced swim test in both WT (VEH:171.1 ± 7.0 s, n = 28 vs. LUZ:135.0 ± 9.4 s, n = 28; p <0.05) and MT1KO mice (VEH:162 ± 9.4 s, n = 10 vs. LUZ:119.6 ± 9, n = 10; p <0.05) but not in the MT2KO mice (VEH: 132.4 ± 14.7 s, n = 9 vs. LUZ:162.6 ± 16.9 s, n = 8) suggesting that the...
antidepressant-like effect of luzindole is mediated by the MT2 receptor. Luzindole significantly increased hippocampal cell proliferation (VEH:51 ± 5, n = 8 vs. LUZ:87 ± 9, n = 9; p < 0.05) and neurogenesis (VEH:11 ± 2, n = 8 vs. LUZ:18 ± 2, n = 8) compared to vehicle in wild type mice. The effect of luzindole was specific to neurogenesis in the dorsal hippocampus. Furthermore, luzindole increased hippocampal cell proliferation in MT2KO mice (VEH:46 ± 6, n = 8 vs. LUZ:90 ± 9, n = 8; p < 0.05) but not in the MT1KO (VEH:73 ± 11, n = 7 vs. LUZ:96 ± 11, n = 7) or MT1/MT2KO mice (VEH:94 ± 7, n = 7 vs. LUZ:88 ± 10, n = 5). In addition, MT1KO and MT1/MT2KO mice had higher basal cell proliferation levels compared to wild type mice and MT2KO mice, suggesting that the MT1 receptor negatively regulates basal hippocampal cell proliferation. Together, these observations indicate that the effect of luzindole on hippocampal cell proliferation is mediated by the MT1 receptor.

Conclusions: Chronic luzindole treatment increases hippocampal cell proliferation and exerts antidepressant-like effects through distinct actions at the MT1 and MT2 melatonin receptors. Melatonin receptor antagonists may be candidates for antidepressants discovery. The antidepressant-like effect of luzindole appears to be independent of hippocampal neurogenesis since increasing hippocampal neurogenesis in the MT2KO mice did not lead to an antidepressant-like activity in the forced swim test and vice versa the antidepressant-like effect observed in MT1KO mice did not require a significant increase in hippocampal neurogenesis. Together these results suggest increasing hippocampal neurogenesis is a secondary effect of antidepressant-like compounds rather than a necessary mechanism of antidepressant-like activity. Supported by UB Funds to MLD.

Keywords: Melatonin, Antidepressant, Neurogenesis, Hippocampus, Luzindole

Disclosures: Nothing to disclose.

T110. Early Life Stress Modulates the Developmental Trajectory of the Endocannabinoid System

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Background: Early life stress modulates the development of cortico-limbic circuits regulating stress and emotionality and can increase vulnerability to psychiatric illnesses later in life. Given the important stress-buffering role endocannabinoid (eCB) signaling exerts in the brain, we performed a comprehensive investigation of the developmental trajectory of the eCB system and how it is impacted by exposure to early life stress.

Methods: Early life stress was modelled in the form of repeated maternal separation (MS; 3 hours / day) from postnatal day 2 (PND2) to PND12. Tissue levels of the eCB molecules anandamide (AEA) and 2-arachidonoylglycerol (2-AG) were measured after the first and last MS exposures on PND2 and PND12, respectively, as well as under basal conditions at a juvenile (PND14), adolescent (PND40) and adult (PND70) timepoints in the prefrontal cortex (PFC), amygdala and hippocampus to assess the residual effects of MS. In addition, we also examined the effects of MS on CB1 receptor binding in these three brain regions at PND40 and PND70.

Results: Data demonstrated that exposure to MS resulted in immediate bidirectional changes in AEA and 2-AG tissue levels, in a region specific manner, which persisted into PND14, but only in the amygdala. In adolescence and into adulthood, there were no sustained effects of MS on AEA or 2-AG levels in either the PFC or the amygdala, although both regions exhibited a reduction in CB1 receptor binding at PND40. The hippocampus, on the other hand, exhibited a robust downregulation of both AEA and 2-AG at both PND40 and PND70 and a downregulation of CB1 receptors that was only apparent at PND70.

Conclusions: Collectively, these data demonstrate that early life stress can alter the normative ontogeny of the eCB system, resulting in a sustained deficit in hippocampal eCB function in adulthood. Given the importance of eCB signaling in buffering and constraining the effects of stress, these data support the hypothesis that impaired eCB signaling in adults exposed to early life stress could be a contributing factor to increased vulnerability to stress-related psychiatric illnesses, such as depression and PTSD.

Keywords: endocannabinoid, Hippocampus, Early life stress, stress

Disclosures: MNH is a scientific consultant for Pfizer.

T111. Treatment of Cognitive Deficits in Bipolar Disorder with Galantamine-ER

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Background: Subjects with bipolar disorder experience significant cognitive dysfunction, even when euthymic, but few studies have evaluated potential treatments for such deficits. We completed a two-site, 16-week, randomized, placebo-controlled study to evaluate the efficacy of adjuvant galantamine ER, an acetylcholinesterase inhibitor, in addition to mood stabilizers, for the treatment of cognitive deficits in euthymic subjects with bipolar disorder.

Methods: We randomized N = 73 euthymic subjects who met SCID criteria for bipolar disorder (BP). 52% were female, mean age was 46.5 years (SD = 12.6). All subjects were euthymic (had minimal symptoms of depression or mania for the previous three months) and reported subjective cognitive deficits; 80% of subjects were BPI and 20% BPII. Subjects were randomized 1:1 to adjuvant galantamine ER or placebo (in addition to their existing mood stabilizing treatment) for a 16-week treatment study with flexible doses (8-24 mg/day). At every monthly visit subjects were administered neuropsychological tests of attention (Conners CPT) and self report scales for functional impairment (The Range of Impaired Functioning Tool, LIFE-RIFT). Tests for episodic memory (CVLT) were administered only at baseline and endpoint to reduce learning effects. We used mixed-effects linear regression to compare treatment groups on the repeated assessments of CPT and LIFE-RIFT; changes in CVLT were assessed with ANOVA.

Results: There was no significant difference between the treatment groups on the three co-primary measures:
T112. Can Medication Free, Treatment-Resistant, Depressed Patients who Initially Respond to TMS Be Maintained off Medications? A Prospective, 12-month Multisite Randomized Pilot Study

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**Background:** Repetitive transcranial magnetic stimulation (TMS) is an efficacious intervention for acute treatment of resistant major depressive disorder (MDD), but there is little information on the maintenance use of TMS after acute response. This pilot feasibility study investigated 12-month outcomes comparing two TMS maintenance approaches—a scheduled, single TMS session delivered monthly (SCH) vs. observation only (OBS).

**Methods:** Antidepressant-free patients with unipolar, non-psychotic, treatment-resistant MDD participated in a randomized, open-label, multisite trial. Patients meeting protocol-defined criteria for improvement after six weeks of acute TMS were randomized to SCH or OBS regimens. TMS reintroduction was available for symptomatic worsening in both groups, and all patients remained antidepressant-free during acute and maintenance phases.

**Results:** Sixty-seven patients enrolled in the acute phase and 49 (73%) met randomization criteria. Groups were matched, although more patients in the SCH group had failed ≥2 antidepressants \( p = .035 \). There were no significant group differences on any outcome measure. Participants who got SCH had numerically longer time to first TMS reintroduction than OBS \( (91 \pm 66 \text{ versus } 77 \pm 52 \text{ days}) \), but this difference was not statistically significant. Reintroduction of TMS in the context of symptom re-emergence was given for 14.3 ± 17.8 days (SCH) and 16.9 ± 18.9 days (OBS). 14/18 (78%) SCH patients and 17/27 (63%) OBS patients responded to TMS reintroduction. Sixteen patients (32.7%) completed all 53 weeks of the study.

**Conclusions:** Maintaining treatment-resistant depressed patients off medications with periodic TMS appears feasible in some cases, but we found no statistically significant advantage of SCH vs. OBS regimens with regard to time to relapse. Responders to an acute course of TMS were highly likely to respond to TMS again if needed for symptom re-emergence.

**Keywords:** repetitive transcranial magnetic stimulation, Antidepressant, maintenance treatment

**Disclosures:** This study was sponsored by Neuronetics, Inc. Linda L. Carpenter: C-Path Institute, AbbVie, Taisho, Naurex, Magstim (Consultant); NIMH, (Grant support); Neosync and Cervel (Clinical trials contracts with Butler Hospital). Neuronetics (Sponsored present study, Grant support). Noah S. Philip: Cervel, NeoSync (Clinical trial contracts through Butler Hospital); U.S. Department of Veterans Affairs (grant support); Neuronetics (Sponsored present study, grant support) David L. Dunner: Neuronetics (Sponsored present study, Speaker). Naurex (Consultant). Sheila M. Dowd: Neuronetics (Sponsored present study); NIMH, Pfizer, Edgemont, Janssen (Grant support); Clintara, LLC and the Chicago WIHS Consortium (Consultant). Scott Aaronson: Neuronetics (Grant support, Sponsored present study, Speaker); Genomind (Board member); Sunovion, Takeda, Lundbeck, Otsuka (Speaker). David G. Brock: Neuronetics (Employee, Stock options).

T113. Longitudinal Cognitive Outcomes in Late-life Depression: Effects of Achieving Antidepressant Remission

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**Background:** Cognitive impairment is common in late-life depression and a critical outcome of the illness. Cognitive deficits are well-established predictors of poor response to antidepressants and such deficits often persist even with successful treatment. Depression is further associated with increased risk of dementia, but it is unclear whether this risk differs based on the clinical response to antidepressant treatment. In other words, do antidepressant remitters represent a phenotype that is at lower risk for cognitive decline? In this longitudinal study, we hypothesized that depressed elders achieving remission at key clinical milestones would have less change in neuropsychological measures over time than those with poorer clinical responses.

**Methods:** The sample consisted of 437 nondemented individuals, including 237 depressed and 164 never-depressed adults age 60 years or older, with an average study participation of 68 months. While receiving algorithm-guided antidepressant treatment, participants completed neuropsychological testing annually. Following our previous strategy, we created z-scores for each neuropsychological measure and combined them into three cognitive domain constructs: episodic memory, working memory, and executive speed. In statistical analyses we tested for differences in domain scores between three groups: nondepressed, depressed / remitted and depressed / non-remitted. Remission was defined at 3-months (acute remission).
and 12-months (chronic remission), using a remission definition of a MADRS score of 6 or less. Longitudinal analyses examined each cognitive domain Z-score as a repeated measure, while controlling for independent variables of age, sex, education, race, study time and baseline neuropsychological domain Z-score. Depression severity by CES-D was a repeated independent variable assessed annually. The primary independent variable of interest was an interaction term between time and study group, allowing us to determine whether there was a different effect of time on longitudinal cognitive domain performance across the three study groups.

**Results:** After controlling for covariates, initial analyses demonstrated that compared with the nonremitted group, the depressed group exhibited significantly greater decline in performance over time in all cognitive domains, including episodic memory ($F = 20.00$, $p < 0.0001$), working memory ($F = 20.27$, $p < 0.0001$), and executive speed ($F = 12.02$, $p = 0.0005$). Longitudinal analyses of 3-month status included 77 remitted and 193 nonremitted depressed elders, demonstrating significant differences between diagnostic groups in cognitive domain performance over time (episodic memory: $F = 10.84$, $p < 0.0001$; working memory: $F = 10.90$, $p < 0.0001$; executive speed: $F = 5.95$, $p = 0.0027$). For all cognitive domains there were no significant differences in rate of change between the 3-month remitted and nonremitted groups, and both exhibited greater decline in performance than the nondepressed group. Finally, analyses of 12-month status included 128 remitted and 145 nonremitted depressed elders. We continued to observe significant group differences in domain performance over time (episodic memory: $F = 10.05$, $p < 0.0001$; working memory: $F = 10.22$, $p < 0.0001$; executive speed: $F = 9.04$, $p = 0.0001$). For episodic and working memory, there were no significant differences between 12-month remitters and nonremitters, and both depressed groups exhibited greater decline than did the nondepressed group. However, for executive speed, the nonremitted group exhibited a greater decline in performance than the remitted or nondepressed groups, between which there was no significant difference in change over time.

**Conclusions:** These data demonstrate that, when compared with never-depressed subjects, older adults with depression exhibit greater cognitive decline over time. Contrary to our initial hypothesis, this decline is unrelated to clinical antidepressant response, except for executive speed where individuals who remit to chronic antidepressant administration exhibit rates of change comparable to never-depressed subjects. In other words, individuals who achieve clinical remission continue to exhibit an increased rate of cognitive decline. These findings are concordant with past work associating depression with increased risk of dementia, however they also underscore the lack of understanding of the neurobiological factors underlying this relationship. More work is needed to elucidate this relationship and, as antidepressant treatment does not appear to modify the risk of cognitive decline, identify treatment strategies to preserve cognitive function in this at-risk population.

**Keywords:** Late-life Depression, Cognitive Decline, aging

**Disclosures:** Nothing to disclose.
Consistent with this finding, sexual dysfunction was not reported as an adverse event in the lurasidone group.

Conclusions: Treatment with lurasidone significantly improved depressive symptoms in patients with a diagnosis of major depressive disorder with subthreshold hypomania. Modest but significant improvement in sexual function was also observed. Lurasidone treatment did not appear to be associated with any adverse effects on sexual function.

Clinicaltrials.gov: NCT01421134

Sponsored by Sunovion Pharmaceuticals Inc.

Keywords: Major Depressive Disorder (MDD), Atypical antipsychotics, sexual dysfunction


T115. Effects of Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) Signaling on Social Interaction

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Background: Severe or prolonged stress can trigger psychiatric illnesses including mood and anxiety disorders. The mechanisms by which stress induces these illnesses are not fully understood. Accumulating evidence indicates that pituitary adenylate cyclase-activating polypeptide (PACAP) plays an important role in regulating stress effects. In rodents, exogenous administration of PACAP can produce persistent effects on metrics (e.g., acoustic startle) that may reflect anxiety-like signs including hypervigilance. More recent work shows that PACAP treatment also disrupts other core domains often dysregulated in mood disorders, including motivation and attention. The present studies were designed to investigate how PACAP affects social withdrawal (diminished interest or participation in social behaviors), another core feature of mood and anxiety disorders. In parallel, we are examining the role of the transcription factor cAMP response element binding protein (CREB) in mediating the effects of PACAP within the nucleus accumbens shell (NAS), a brain area involved in encoding reward and aversion, and the bed nucleus of the stria terminalis (BNST) and central amygdala (CeA), brain areas previously implicated in PACAP stress-related signaling.

Methods: Social behavior was measured using the social interaction (SI) test. Rats were habituated for 10 min to the interaction chamber (60 X 60 X 35 cm, Plexiglas) one day prior to testing. On test day, rats were infused with VEH or PACAP (0.25, 0.5, or 1.0 µg, ICV) and placed in the interaction chamber 1 hr later with an untreated weight-matched partner rat. Social behavior was videotaped for 5 min in red light conditions and scored by an observer blind to the treatment conditions. Rats were re-tested 1 week later with a novel partner rat. A separate cohort of rats was tested only at the 1-week post-treatment time point to control for repeated presentation effects. For region-specific infusions, separate cohorts of rats were implanted with bilateral cannula aimed at the NAS, BNST, or CeA. Testing was identical to ICV cohorts, except that rats were tested 30 min post-infusion (1.0 µg/side, based on previous studies). For phospho-CREB (pCREB) immunoblots, tissue was collected 10 min after SI (80 min post-infusion of VEH or 0.5 µg PACAP) and NAS, BNST, and CeA dissections were analyzed by western blot to quantify pCREB expression (normalized to GAPDH). Data were analyzed with ANOVAs followed by post-hoc tests.

Results: ICV PACAP (at 0.5 and 1.0 µg, but not 0.25 µg) significantly decreased active social behaviors (e.g. grooming, sniffing, following partner rat), but had no effect on social avoidance (e.g. fleeing from the partner rat). The highest dose of PACAP (1.0 µg) also produced anxiety-like behaviors (decreased time spent in the center of the arena) during the social interaction test. These effects were not due to differential behavior from the partner rat; approach behavior of the partner rat was unaltered by the dose of PACAP administered to the treatment rat. Rats that had received the 1.0 µg dose PACAP exhibited increased active SI behavior when re-tested with a new partner rat 7 days later, without any additional treatment; this type of effect was not seen at lower doses. PACAP treatment also affected anxiety-like behavior one week later, with nominal increases in anxiety-like behavior in rats that had received the 0.25 µg dose PACAP. PACAP decreased active social interaction when infused directly into the BNST or CeA, but had no effect when infused into the NAS. PACAP treatment (0.5 µg) significantly reduced pCREB protein levels in the NAS compared to VEH-treated rats, with no differences found in the BNST or CeA.

Conclusions: We show that ICV PACAP produces dose-dependent disruptions in social behaviors. At the highest dose (1.0 µg PACAP), these effects may be confounded by increases in anxiety-like behavior. PACAP treatment dysregulated SI behavior up to 1 week later, producing a phenotype opposite to that seen with acute treatment, suggesting “over-shoot” of recovery. The mechanism of this effect is unclear, although biphasic effects of PACAP on fear expression have been previously described. We also show that PACAP dysregulates social behaviors when directly infused into the BNST or CeA, regions previously found to be implicated in stress-related PACAP signaling, but not the NAS. Based on previous work showing that both stress and PACAP can activate CREB, we hypothesized that PACAP treatment would increase pCREB protein expression in regions implicated in stress or social behaviors.
However, we found that PACAP treatment significantly decreased pCREB expression only in the NAS, with no changes in the BNST or CeA. A more comprehensive understanding of the impact and persistence of PACAP on symptoms associated with mood disorders will elucidate how stress contributes to psychiatric illness, and facilitate the development of new medications for stress-related disorders.

**Keywords:** PACAP, social interaction, CREB

**Disclosures:** Dr. Carlezon discloses that within the last 2 years he has received compensation from the ACNP for serving as an editor and Cerecor for serving as a consultant.

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**T116. Effect of a Dietary Supplement on Predisposition to Depressed Mood in Postpartum**

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**Background:** Postpartum depression is the most common complication of childbearing with a 13% prevalence rate. In addition, postpartum depression can adversely affect child development and raise the risk for future major depressive episodes. However, there are currently no widespread prevention strategies for postpartum depression. Greater severity of postpartum blues is associated with increased risk of developing postpartum depression; hence, one approach to prevent postpartum depression is to reduce the intensity of postpartum blues, such as through a dietary supplement. Monoamine oxidase-A levels are elevated by over 40%, particularly in the prefrontal cortex and anterior cingulate cortex, during days 4 to 6 postpartum, when postpartum blues occur. A supplement kit consisting of monoamine precursor amino acids tryptophan and tyrosine, and dietary level antioxidants was designed to counter the elevated monoamine oxidase-A levels that occur during postpartum blues. In previous studies, it has been shown that oral tryptophan and oral tyrosine supplements do not increase their total concentrations in breast milk; despite the significant increase in maternal plasma. The specific aim of this open-label study was to assess whether this dietary supplement can reduce the intensity of postpartum blues at day-5 postpartum, the typical peak of postpartum blues.

**Methods:** Thirty three healthy day-5 postpartum women were recruited and assigned into 2 groups: The control group (n = 16) was not receiving any supplements, and the supplemented group (n = 17), was receiving the dietary supplement consisting of 2 grams of tryptophan, 10 grams of tyrosine and blueberry juice + blueberry extract. Severity of postpartum blues was quantitated by the change in the visual analogue scale mood scores and the profile of mood state depression scores from the sad mood induction procedure.

**Results:** Univariate analysis of variance demonstrated a significantly greater elevation in depressed mood on the visual analog scale mood scores after the sad mood induction procedure in controls versus the supplemented group (F (1,31) = 110.93, p < 0.001). There was a large effect size (approximately 3.6) observed in the ability of the dietary supplement to attenuate the intensity of depressed mood on day-5 postpartum. A similar effect of group on change in profile of mood state depression scores was observed after the sad mood induction procedure (F (1,31) = 17.83, p < 0.001).

**Conclusions:** This is the first study to investigate the effect of a combination of monoamine precursors, tryptophan and tyrosine, and blueberry extract/juice on the intensity of postpartum blues. At the level of open trial, administration of the dietary supplement virtually eliminated the intensity of postpartum blues. This supports further investigation of this dietary supplement in a double-blind, randomized, placebo controlled trial, to reduce the intensity of postpartum blues, as the next phase in developing a dietary strategy to reduce the risk of postpartum depression.

**Keywords:** Postpartum depression, Dietary supplement, Monoamine oxidase A, Prevention

**Disclosures:** Nothing to disclose.

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**T117. Patterns of Caudate Connectivity during Cognitive Control during Late Life Depression and Healthy Aging**

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**Background:** Executive dysfunction is a common feature of depression during late life (LLD), and a robust predictor of treatment response (Sneed et al., 2007) and course of illness (Alexopoulos, 2000). Our previous work demonstrated greater activation in fronto-striatal regions among patients with LLD, relative to never-depressed controls (NDC) during a task of cognitive control, despite equivalent task performance. In fact, NDC deactivated the left caudate head, while LLD demonstrated a pattern of hyperactivity (Rao et al., 2015). The aim of the current study was to extend our previous work by investigating patterns of functional connectivity during a task of cognitive control among LLD relative to NDC using the caudate head as a seed region.

**Methods:** Eighteen adults with LLD (M age = 66.17, SD = 7.16) and 20 NDC (M age = 67.30, SD = 8.47), ages 55-81 completed Level 1 of the Parametric Go No-Go Task (Langenecker, 2003), requiring sustained attention and attentional switching, while undergoing fMRI. Psychophysiological interaction (PPI) analysis was performed with the left caudate head as the seed region (-14 20 -8). Data for each subject were modeled with regressors for one condition (activation during correct hits, the psychological regressor), the time course in the functionally defined left caudate head (the physiological regressor), and the interaction of the timecourse in the caudate head and the correct hits condition (the psychophysiological regressor). Between-group analysis was then performed using a two-sample t test. Thresholds were set using AlphaSim correction (1000 iterations), balancing height (p < .003)
and extent (264 mm3) thresholds to achieve a whole brain correction of p < .05.

**Results:** Both groups demonstrated connectivity of the left caudate head with regions in the frontal cortex relevant to cognitive control, including the middle and inferior frontal gyri, as well as with parietal regions important for maintaining attention. The LLD group demonstrated connectivity with fewer regions, overall, than did the NDC group, and uniquely revealed connectivity with the left claustrum, medial frontal gyrus, and insula. There was no region for which the LLD group demonstrated greater connectivity relative to the NDC group. Relative to the LLD group, the NDC group had greater functional connectivity of the caudate with the left subcallosal (BA34) and inferior temporal (BA37) gyri, in addition to posterior regions relevant for higher-order processing of visual information.

**Conclusions:** Findings suggest that older adults with depression demonstrate less assimilation of cortical regions with the left caudate head during cognitive control than do normally aging individuals. Older depressed patients especially show less integration of higher-order visual processing areas relative to normally aging individuals. They also demonstrate less connectivity with the left subcallosal gyrus, which has been demonstrated to be relevant to detection of irrelevant stimuli (Crottaz-Herbette et al., 2005), a necessary sub-component of the task used in the current study. These findings are in the context of greater activation of fronto-striatal regions during cognitive control, as demonstrated in our previous work (Rao et al., 2015). Taken together, findings imply compensatory processes, in addition to de-differentiation of networks during cognitive control in older adults with depression. While the current study was not longitudinal, variation in patterns of activation and connectivity among older depressed persons might be explored as predictors of illness course and future cognitive decline, given that functional brain changes often precede behavioral changes.

**Keywords:** Late-life Depression, fMRI, cognitive control, connectivity

**Disclosures:** Nothing to disclose.

### T118. Increased Susceptibility of Hypogonadal Male Mice to Social Stress is Mediated by Estradiol

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**Background:** Major depressive disorder is a public health concern and afflicts approximately 10-12% of the male population at some point in their lives. Hypogonadism has been shown to correlate with increased propensity of developing depression in men. Although testosterone administration exerts antidepressant effects on its own, it is not clear whether these effects are mediated by the direct actions of testosterone on the androgen receptor or by its aromatase-dependent conversion to 17β-estradiol (E2), and activation of estrogen receptors. In fact, while E2 is primarily considered a female steroid hormone, it is also found at physiologically relevant concentrations in the male brain. In the present study, we sought to investigate the specific role of gonadal hormones in the propensity to develop depressive-like behaviors following social stress in male mice.

**Methods:** Male C57BL/6J mice underwent sham or orchectomy surgery to mimic the human condition of hypogonadism and were behaviorally characterized in the forced-swim test, sucrose preference and female urine sniffing test (FUST; measure of anhedonia). Other cohorts of male mice underwent sham or orchectomy surgery and they received vehicle, testosterone, 17β-estradiol, or 10β,17β-dihydroxyoestra-1,4-dien-3-one (DHED; a brain-selective prodrug of E2) implants. Following 10 days of treatment, half of the mice were subjected to a subthreshold social defeat to assess the susceptibility to develop social avoidance and anhedonia induced by social stress. Mice subjected to social defeat stress had three cycles of 2-min physical and 15-min psychological/sensory contact with an aggressive CD-1 mouse for one day. Mice were then tested in the social interaction/avoidance and FUST tests 24 and 48 hours following the defeats respectively. For the social interaction test, the experimental mouse simultaneously explored an apparatus containing two identical small cages, one with a non-aggressive CD-1 mouse and the other empty. Time spent by the test mouse in close proximity to the cage containing the CD-1 mouse compared to the empty cage was measured. For the FUST two cotton-tipped applicators were placed in the cage; one applicator with fresh estrus female mouse urine and the other with fresh male mouse urine. Time spent sniffing the female urine compared to the male urine was scored.

**Results:** No effect of orchectomy was observed on the forced-swim test, sucrose preference or FUST under baseline conditions. However, combined hypogonadism and social stress resulted in a susceptible phenotype as indicated by social avoidance in the social interaction test, and lack of preference for the female urine vs male urine in the FUST. While testosterone replacement reversed these depressive-like effects, blockade of the androgen receptor with flutamide had no effect on the social interaction and hedonic behaviors following social defeat, suggesting that the actions of testosterone might be mediated by its conversion to E2. Aromatase inhibition with letrozole induced a susceptible phenotype similar to that observed following orchectomy, indicating that these pro-depressive symptoms of social stress require conversion of testosterone to E2 and not derived by testosterone per se. In support of this, both E2 and DHED treatment reversed the depressive-like effects induced by a combined hypogonadism and social stress. Finally, to assess the involvement of estrogen receptor beta (ERβ) in the mediation of social stress-induced pro-depressive phenotype of hypogonadal male mice, we characterized the effects of subthreshold social defeats on mice lacking the ERβ gene. We found that ERβ knockout mice manifested increased susceptibility to develop both social avoidance and anhedonia following social defeat.

**Conclusions:** Our findings demonstrate that hypogonadism increases the risk to develop mood-related disorders following a social stress and these effects are caused by the low levels of E2, rather than testosterone. The present...
findings highlight a novel role for E2 in males to modulate depressive-like behaviors via an ERβ-dependent mechanism. Although peripheral side-effects of E2 limit its clinical use, DHED administration, converted to E2 selectively in the brain of males, fully reversed the hypogonadism/social stress-induced pro-depressive behaviors suggesting an E2 brain-selective pharmacotherapy as a potential option for the treatment of mood-related conditions in men caused by low testosterone.

**Keywords:** estradiol, Major depression, Social defeat stress, Gonadal Hormones

**Disclosures:** Nothing to disclose.

**Conclusions:** Finally, it is important to determine if our results are restricted to neurons. Co-culture of astrocytes and neurons may identify important differences in their behavior, or it is possible that both cell types are involved in BP. 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:** Induced pluripotent stem cells (iPSCs), CACNA1C, Bipolar Disorder, calcium

**Disclosures:** Nothing to disclose.

**T119. Stem Cell Models of Bipolar Disorder**

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**Background:** A major challenge in understanding human neuropsychiatric disorders has been the lack of viable cells and tissues for analysis. Patient-derived induced pluripotent stem cells (iPSC) offer the opportunity to examine the full complement of neural tissues and the prospect of identifying stem cells (iPSC) offer the opportunity to examine the full complement of neural tissues and the prospect of identifying underlying disease mechanisms.

**Methods:** To study Bipolar Disorder (BP), we have derived and characterized iPSC from fibroblasts obtained from control (C) and BP patients, and have differentiated them into neurons and glia.

**Results:** RNA from undifferentiated and differentiated iPSC were analyzed using microarray. Compared with the Allen BrainSpan database, BD neurons have gene expression profiles most similar to 20-week post-conception brain whereas C neurons were most similar to 16-week post-conception brain. Comparing microRNA expression in neurons between groups, 82 differentially expressed micro-RNAs were identified—including miRs previously identified as altered in neuropsychiatric diseases, one of which targets the BP risk gene CACNA1C. We have also identified differences in neuronal lineage allocation between groups, with BP neurons favoring differentiation into ventral forebrain neurons (as measured by Nkx2.1 expression) and C neurons forming dorsal cortical precursors (as indicated by Emx2/Pax6 expression).

We have also identified differences in calcium signaling in BP neurons, which are consistently more active than C neurons. Calcium transients and wave amplitude are greater in BP neurons compared to C, and lithium pre-treatment reduces these significantly, providing a tractable model system to examine the response of iPSC-derived neurons to pathway perturbagens and potential therapeutics. The AA genotype of the SNP rs1006737 in the voltage-gated calcium channel gene, CACNA1C, has been associated with BP, and may be important for expression, localization, or function of the CaV1.2 calcium channel. We are investigating the function of this SNP in BP neurons using CRISPR/Cas9 to edit BP fibroblasts with the AA genotype to the non-risk GG genotype, and are using these cells to assess differentiation potential and CACNA1C expression patterns.

**Conclusions:** These results suggest that stress-related psychopathologies may be due to PACAP induced increases in BNST cell activity resulting from maladaptations in BNST PACAP systems. Therefore, new classes of drugs that target PACAP may have the potential to alleviate the various behavioral and physiological consequences of stressor exposure.
T121. Interoceptive Insula Activity to Food Cues is Negatively Correlated with Behavioral Ratings of Expected Food Pleasantness in Depressed Patients with Increased Appetite

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Background: Changes in appetite and weight are variable diagnostic markers of major depressive disorder (MDD), with some patients manifesting increased appetite when they become depressed, while others manifest decreased appetite. Previously, we have demonstrated that unmedicated currently-depressed adults with increased versus decreased appetites exhibit dissociable patterns of activity to the sight of food cues (Simmons et al., under review). Depression with increased appetite was associated with potentiated activity in mesocorticolimbic reward circuitry, including the orbitofrontal cortex, ventral striatum, and ventral pallidum, whereas depression with appetite loss was associated with attenuated activity in interoceptive regions of the insula. It remains unclear, however, how the neural activity that distinguishes between these groups during perception of food cues relates to individuals’ moment-to-moment hedonic inferences about foods, which presumably play an important role in real-world food decision-making. To address this gap, the present study examined the relationship between behavioral ratings of expected food pleasantness and brain activity to food pictures measured separately while subjects underwent functional Magnetic Resonance Imaging (fMRI).

Methods: Forty-five participants performed a Food Pleasantness Rating Task in which they saw photographs of 144 different foods and made behavioral ratings of how pleasant it would be to eat each food item at that particular moment, thereby requiring subjects to make hedonic inferences about the foods. The stimuli depicted four broad classes of food items, including high-fat high-sweet foods (e.g., primarily “dessert” foods such as cake and ice cream), high-fat low-sweet foods (e.g., savory foods such as pizza), low-fat high-sweet foods (e.g., fruits), and low-fat low-sweet foods (e.g., vegetables). On the same day as the Food Pleasantness Rating Task, the same subjects also underwent fMRI while performing a task in which they viewed a different set of food photographs as well as photographs of non-food objects. The participants were drawn from three populations: 16 unmedicated subjects with MDD and increased appetite during the current depressive episode, 15 unmedicated subjects with MDD and decreased appetite during the current depressive episode, and 14 healthy control subjects with no history of psychiatric diagnosis. All three groups were matched for age and body mass index (BMI). The two depressed samples were group-matched for mean ratings of depression severity, anhedonia, and anxiety. Using regression analyses, we assessed the relationship between the subjects’ behavioral ratings in the Food Pleasantness Rating Task and their fMRI hemodynamic activity to food (versus non-food) cues.

Results: Compared to the other two groups, depressed subjects with increased appetite inferred that visually perceived foods would be more pleasant to eat (p < .02 in each group comparison). The ratings provided by healthy control subjects and depressed subjects with appetite loss, however, did not differ from each other (p > .27). Compared to both healthy control subjects and depressed subjects with appetite loss, depressed subjects with increased appetite rated high-fat high-sweet foods (p < .03 in each group comparison), and high-fat low-sweet foods (p < .005 in each group comparison), as being more hedonically pleasing. Significant negative correlations were observed between the food pleasantness ratings of the depressed subjects with increased appetites and hemodynamic activity to food images in the left and right dorsal mid-insula, a region previously implicated in interoception and monitoring of the body’s homeostatic needs.

Conclusions: Depressed subjects with increased appetite rated food stimuli as being more pleasant to eat, and the hemodynamic activity in these subjects’ left and right dorsal mid-insula to food images was negatively correlated with their food pleasantness ratings. In other words, the depressed individuals with increased appetite who exhibited the weakest hemodynamic response to food cues in interoceptive regions of the insula were the ones who subsequently anticipated that visually-perceived foods would be the most pleasant to eat. This negative association suggests that interoceptive signals about the state of the body represented by increased activity of the mid-insula may act as a brake on food anticipation in those with over-active food reward signals (i.e., the increased appetite depressed group in the present study). Depression-related appetite increases may result from a dysregulation of the balance between increased reward circuit activity (reported previously in depressed adults with increased appetite) and interoceptive signals in the insula about the homeostatic consequences of perceived foods, which alter hedonic inferences about those foods.

Keywords: Major depression, Appetite, Interoception, fMRI

Disclosures: WCD is an employee of Johnson & Johnson, Inc. WKS has served as a consultant with Zafgen, Inc. All other authors have no financial interests to disclose.
two main disadvantages. First, these cut-off values are ultimately arbitrarily determined and categorical, and thus do not necessarily reflect the complexity and the continuous nature of brain connectivity. Second, despite the hierarchical nature of the human connectome, connectomic metrics (such as z-score and participation coefficient) are still defined after restricting to a specific modular hierarchy, and thus they do not properly capture the potential scale-dependent nature of a node’s role in the network as a whole. In this abstract, we thus seek to address these issues by proposing a new approach to collectively probe the scale-dependence of information transfer across all levels of modular hierarchy, without resorting to arbitrary thresholding, binning, or binarization of scalar-valued data points.

Methods: Image Acquisition and Processing.

DTI images were acquired with a single shot EPI sequence with 67 slices, b = 700/s/mm2, and a B0 image. Images were preprocessed using DTIstudio and tractography was performed using the FACT algorithm. T1-weighted images were used to generate label maps using the Freesurfer software, defined by 82 cortical and subcortical regions. Each of these 82 Freesurfer labels was then further subdivided until we attained cuboid brain regions equivalent to about 4, 2, and 1 cm3 respectively. Community structure was defined using the path length associated community estimation (PLACE) framework that extracts the connectome’s hierarchical modular structure by finding groups of nodes that are highly efficiently integrated amongst themselves while separated from others.

Measuring Modular Scale-Dependence Information Transfer.

To probe the proposed scale-dependent information transfer across the entire brain connectome’s modular hierarchy, we first define the following variable $\tau$ for any node i at any hierarchical level $L$: $\tau_{i}^{L} = (\sum_{i = 1,2,...n}^{i} (j \neq i) (1/2)^{min(L,Level(i,j))} PL(i,j)^{-1}/\sum_{i = 1,2,...n}^{i} (j \neq i) (1/2)^{min(L,Level(i,j))})$. Here $n$ is the total number of ROIs, and Level*(i, j) indicates the most local hierarchy at which nodes i and j are still assigned to the same module (in PLACE global scales are at low levels). Note that the above equation collapses to the standard nodal efficiency when computed at the root ($L = 0$). Furthermore, the weighting term $(1/2)^{min(L,Level*(i,j))}$ gives more weights to the information exchange efficiency between nodes that remain in the same module at higher levels of hierarchy, and as a result $\tau$ can be interpreted as a hierarchically weighted nodal efficiency.

Next, plotting $\tau_{i}^{L}/\tau_{i}^{0}$ (y axis) against level of bifurcation (x axis) is fitted with an exponential function $\tau_{i}^{L}/\tau_{i}^{0} = \exp(\mu_{i} L)$ where the rate constant $\mu_{i}$ is unique to each region. Intuitively, the rate constant $\mu_{i}$ thus represents the rate of decay in a given node’s information exchange efficiency with other regions, as we move from fine- to coarse or local-to-global along the modular hierarchy.

Results: We find that nodal efficiency decreases as the resolution or granularity of parcellation increases from the base Freesurfer labels to 1 cm3 size brain regions, while the novel variable $\mu_{i}$ is relatively insensitive to parcellation resolution. Interestingly, we note that in general, those with low nodal efficiency tend to also have higher rates of decay; by contrast, nodes that have lower decay rate can have either low, medium, or high nodal efficiency. In fact, the correlation between $\mu_{i}$ and $\tau_{i}^{L}/\tau_{i}^{0}$ becomes statistically nonsignificant after controlling for multiple comparisons.

These results support that the decay rate constant $\mu_{i}$ captures scale-dependent properties of the connectome that are not measured by single-scale graph metrics such as standard nodal efficiency.

We also propose to form the ratio $\tau_{i}^{L}/\tau_{i}^{0}/\mu_{i}$, which can be thought of as a measure of hierarchical “embeddedness” of any brain region. These regions that are highly embedded not only communicate overall more efficiently with other brain regions, they do so across all levels of hierarchy (i.e., insensitive to scale changes). Highly embedded brain regions consist of primarily the bilateral subcortical structures including the thalamus and basal ganglia, the regions forming the limbic system, the precuneus, superior parietal regions, and the medial orbitofrontal cortex.

Conclusions: This work presents a novel connectome approach to understand the property of embeddedness, i.e., the degree of scale-dependence of information exchange efficiency across levels of hierarchical modularity. Our results support that the structural human connectome exhibits overall near-decomposability and selective embeddedness in brain regions within the “limbic network” (including the limbic system, subcortical structures, and regions known to be part of the default mode network). Results may have clinical implication, in that such topological differences may provide structural evidence of the prioritization of limbic network-mediated information, possibly in the context of its enhanced evolutionary value.

Keywords: hub analysis, community structure, connectomics

Disclosures: Nothing to disclose.

T123. WITHDRAWN

T124. Genomic and Brain Transcriptomic Variation of Mouse microRNAs

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Background: MicroRNAs (miRNAs) are small non-coding RNAs that function in the post-transcriptional regulation of gene expression. A single miRNA can target hundreds of genes, often within the same biological pathway. miRNAs have been suggested as putative drug targets as by manipulating the levels of a single miRNA it may be possible to affect the expression levels of hundreds of target genes within the same biological pathway. The role of individual miRNAs in various neurobiological functions, such as development of the nervous system, synaptic plasticity, and neurodegeneration has recently been revealed. However, it is not understood how genetic variation within miRNA loci influence the function of miRNAs. Genetic variation within miRNA genes and their putative regulatory regions may affect either the expression level of miRNAs or target gene recognition, thus resulting in phenotypic differences. Inbred mouse strains offer an excellent model system to study genetic variation and its effect on miRNA expression and behavioral phenotypes as
the whole genome sequence of a large number of the strains is publicly available and their behavioral phenotypes are well characterized.

**Methods:** We systematically investigated genetic variation within the miRNA genes in the mouse genome. We used publicly available whole genome sequence dataset of 36 inbred mouse strains, including the most common laboratory and wild-derived strains (http://www.sanger.ac.uk/resources/mouse/genomes/) that are used as models of a number of human diseases. We performed a genome-wide analysis of single nucleotide polymorphisms (SNPs) and structural variation (SV) within miRNA genes. We also carried out miRNA and mRNA sequencing (miRNAseq and RNAseq) from frontal part of the cortex and hippocampus of 6 laboratory strains (A/J, 129S1/SvImJ, C57BL/6J, C3H/HeJ, DBA/2J, and FVB/NJ) to investigate the brain miRNA transcriptome. We conducted extensive bioinformatics analyses of the DNA sequence, RNAseq and miRNAseq data to investigate the basic characteristics of mouse miRNAs, to study the distribution of SNPs and SV within the mouse miRNA genes, and to assess the effect of genetic variation on brain miRNA expression levels.

**Results:** We observed, as expected, genomic variation occurring less frequently within the miRNA loci compared to the rest of the genome. Furthermore, the seed region of the miRNAs, important in the target recognition, harbored significantly less polymorphisms than the rest of the mature miRNA sequence, the pre-miRNA sequence, and the putative promoter region. miRNAs located in clusters had significantly fewer variable sites (SNP %) and lower nucleotide divergence (pi) rate compared to unclustered miRNAs in the pre-miR, mature, and seed regions. miRNAs located within genes (mirtrons) did not differ significantly in this respect from other miRNAs. According to the miRNAseq data, 719 miRNAs were expressed in the frontal part of the cortex and 779 in the hippocampus. By comparing the DNA variation data with the miRNAseq data we found consistent RNA editing of the seed region in three miRNAs: miR-411-5p, miR-376b-3p, and 467d-5p (all of which were A->I transitions). To investigate how RNA editing or SNPs in the miRNA seed region (N=29 seed region SNPs among the six inbred strains) affect their putative mRNA targets, we carried out bioinformatic target predictions using several algorithms with both alternative alleles. As expected, the target sets of the two alternative alleles had minimal overlap. Consequently, the biological processes and pathways potentially regulated by the two miRNA alleles were different as revealed by the Ingenuity Pathways Analysis or DAVID. Using two de novo miRNA predictions tools, miRDeep2 and sRNAbench, with our miRNAseq data, we identified 105 putative novel miRNAs supported by both software.

**Conclusions:** We have performed a comprehensive characterization of mouse miRNA genes and their expression patterns in two brain regions. Although mouse miRNA genes are highly conserved, we found a number of seed region SNPs among inbred mouse strains that putatively affect which miRNAs the polymorphic miRNAs target. This variation likely affects expression levels of numerous genes contributing to the phenotypic differences between the mouse strains. Therefore, inbred mouse strains provide a genetic model system for investigating the effect of genomic variation within the miRNA genes on various behavioral phenotypes.

**Keywords:** inbred mouse strains, MicroRNA, polymorphism

**Disclosures:** Nothing to disclose.

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**T125. Mapping Functional Connectivity Networks in the Individual**

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**Background:** The capacity to identify the unique functional architecture of an individual subject’s brain is a critical step towards personalized medicine and understanding the neural basis of variations in human cognition and behavior. Clinical and imaging studies have demonstrated marked inter-individual variability in the organization of different functional systems of the brain, particularly in higher order association areas. Localizing specific functional circuitries in a particular subject is therefore a fundamental requirement in clinical procedures such as surgical planning or non-invasive brain stimulation therapies. However, functional imaging techniques are generally limited in accuracy and reliability at the single-subject level.

**Methods:** Here we developed a novel brain parcellation approach to accurately map functional organization at the individual level using resting-state fMRI. A population-based functional atlas and a map of inter-individual variability were employed to guide the iterative search for functional networks in individual subjects. This strategy allows the idiosyncratic functional organization of the individual to drive the network solution. Reliability and accuracy of the resulting functional maps were tested in several independent datasets.

**Results:** Functional networks mapped by this approach were highly reproducible within subjects and effectively captured the variability across subjects, including individual differences in brain lateralization. The algorithm performed well across different subject populations and data types including task fMRI data. The resulting parcellation networks were significantly more reliable than networks localized by traditional task-evoked response. This novel technology can also reliably identify the ventrolateral prefrontal (vPFC)-striatal circuitry at the single subject level, a potential neurostimulation treatment target for OCD.

**Conclusions:** Functional connectivity variability has a specific topographic distribution with heteromodal association cortex being most variable therefore within-subject functional mapping is particularly important in psychiatric research. The novel functional mapping technique developed in this study can provide an individual-level functional atlas which may help the identification of personalized therapeutic targets for various diseases including OCD.

**Keywords:** Resting State Functional Connectivity, obsessive-compulsive disorder, fMRI, individual differences
Disclosures: The authors are listed as inventors on submitted patents on mapping functional brain organization using fMRI.

T126. Raman Spectroscopy for Reagentless Workflow Enhancement to Monitor Human iPSC Research

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Background: Induced pluripotent stem cell (iPSC) technologies enable us to generate, culture and manipulate limitless numbers of live, ‘iPSC-neurons’ from specific individuals, but progress in iPSC research is hampered by the high cost and complexity of differentiation workflows. The current ‘gold standard’ assays to monitor differentiation, such as immunocytochemistry or gene expression analyses entail time consuming, reagent-based, labor intensive analyses. We investigated the utility of Raman Molecular Imaging (RMI), a reagentless molecular analysis technique that combines digital imaging with Raman Spectroscopy (RS). RS relies on the ‘Raman effect’, a characteristic shift in the wavelength of incident laser light scattered by individual molecules. Thus, solids and liquids produce characteristic spectral patterns with unique signatures that can be analyzed rapidly using advanced statistical techniques. While RMI is used routinely in industry, microbiology and in biotechnological manufacturing processes, it is also being used increasingly in cell biological research, including cancer diagnosis and staging, analysis of atherosclerotic vascular disease, and recently in cellular differentiation. RMI can also be used for single cell microscopy and to monitor cell density and viability in cell cultures. The feasibility and reliability of RMI for monitoring iPSC differentiation is unknown.

Methods: iPSCs from 5 individuals were differentiated progressively into neural progenitor cells (NPCs) and iPSC-neurons (D’Aiuto et al, 2015, Organogenesis, in press). The cell types were identified using cell specific fluorescent antibodies: iPSCs - OCT4, TRA-1-60; NPCs - Nestin, PAX6; iPSC-neurons - Tuj1 and MAP2. Three biological replicate culture samples from each cell type were lyzed and 25 spatially resolved RS analyses were obtained from each sample, following a Raman sampling protocol developed originally for pathogen detection (Stewart et al, 2014, J Raman Spectroscopy: 45, 274-280). We used partial least squares discriminant analysis (PLS-DA), a supervised classification model, to seek group-wise discrimination.

Results: Accuracy matrices based on probability distributions of PLS-DA scores indicated 95% overall accuracy for distinguishing among all iPSCs, NPCs and neurons. The identification accuracies for individual cell types against the other cell types were: iPSCs - 100%, NPCs - 100%, neurons - 85%. The intra-individual coefficients of variation (CV) were: iPSCs – 0.7%, NPCs - 16.9%, neurons - 16.3%. The within class CVs were: iPSCs – 2.6%, NPCs – 28.8%, neurons – 17.8%.

Conclusions: RMI can distinguish cell lysates from iPSCs, NPCs and neurons with a high degree of reliability. The variation in CV between the three cell groups may be related to cellular heterogeneity in each group. RMI shows promise for non-invasive, real time analysis of iPSC differentiation workflows. The potential of RMI for high throughput, semi-automated analysis of iPSC differentiation should be considered. Comparison of RS between iPSCs from individuals with and without specific disorders should also be investigated.

Keywords: iPSC, Raman spectroscopy, neurons
Disclosures: Nothing to disclose.

T127. Missense Mutation in FOLH1 is Associated with Differences in Gene Expression and Neuroimaging Phenotypes

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Background: N-acetyl aspartyl glutamate (NAAG) is a selective agonist for the metabotropic glutamate receptor 3 (mGlur3, gene GRM3), which is associated with schizophrenia at the whole-genome level. Subjects with a GRM3 risk-associated variant have decreased NAAG in the prefrontal cortex, as measured by spectroscopy (H1MRSI), as well as diminished frontal cognition. The NAAG catalyzing enzyme in the brain is folate hydrolase 1 (FOLH1), also known as glutamate carboxypeptidase II (GCPII). GCPII inhibitors have shown to increase brain NAAG, as well as reduce schizophrenia-like symptoms and learning and memory impairments in preclinical models. We hypothesize that increasing NAAG levels in patients with cognitive impairments, such as schizophrenia, will lead to cognitive improvement.

Methods: We aimed to test the effects of functional variation in FOLH1 on gene expression in post-mortem human brain, and on the neural activation patterns during cognitive and emotional tasks in living human brain. We chose two known functional variants in FOLH1, missense mutations rs202676 and rs595139. Gene expression was measured using RNAseq in the dorsolateral prefrontal cortex (dPFC) of 237 normal adult brain specimens. Functional MRI data was collected in healthy subjects during a working memory task (N-back, n = 478), an emotional scenes encoding task (n = 258 for rs202676, n = 259 for rs595139), an emotional control task (flanker, n = 232 for rs202676, n = 233 for rs595139). Genotype groups were balanced for age, sex, IQ, years of education, and task performance.

Results: Both missense mutations in FOLH1 were significantly associated with expression of FOLH1 (total transcripts) in the dorsolateral prefrontal cortex. Each copy of the G allele of rs202676 was associated with a 10% increase in the expression of FOLH1 (p = 7.99e-11), and each copy of the A allele of rs595139 was associated with an 11% increase in the expression of FOLH1 (p = 1.63e-08). Healthy subjects who carry the G allele of rs202676 have decreased activity in the expression of FOLH1 (n = 316 for rs202676, n = 319 for rs595139). Functional MRI data in healthy subjects was collected during an emotional scenes encoding task (n = 316 for rs202676, n = 319 for rs595139), an emotional control task (flanker, n = 232 for rs202676, n = 233 for rs595139). Genotype groups were balanced for age, sex, IQ, years of education, and task performance.

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the superior frontal gyrus (xyz = 24, 6, 63) during the no-go stimuli of the flanker task (whole brain p-unc < 0.001, p-FWE-corr = 0.040, T = 4.39, k = 37). Healthy subjects who carry the A allele of rs595139 have increased activation of the dorsolateral prefrontal cortex (xyz = 54, 15, 36) during the N-back working memory task (whole brain p-unc < 0.001, p-FWE-corr = 0.015, T = 4.39, k = 48). Carriers of the A allele of rs595139 also have decreased activity in the inferior frontal gyrus (xyz = 51, 18, 6) during the contrast between incongruent > congruent stimuli of the flanker task (whole brain p-unc < 0.001, p-FWE-corr = 0.072, T = 4.23, k = 41). Neither SNP is associated with differences in neural activity during the emotional tasks, nor is SNP rs202676 associated with neural activity during the N-back task.

Conclusions: The missense mutation, rs202676, is associated with increased expression of FOLH1 and therefore potentially lower NAAG levels in the dlPFC, and is associated with decreased activity in the superior frontal gyrus during cognitive control. The missense mutation, rs595139, is also associated with increased expression of FOLH1, and is associated with decreased efficiency of the prefrontal cortex during working memory, a neural pattern found in patients with schizophrenia, and decreased activity in the inferior frontal gyrus during distraction. These neuroimaging phenotypes could be used as biomarkers for the development of GCPII (FOLH1) inhibitors, and these genotypes may predict whether someone would respond to treatment. Future studies of this novel drug target should take into account the genetic variability of FOLH1.

Keywords: fMRI, RNA Sequencing, genetics, glutamate, pharmacogenetics

Disclosures: Nothing to disclose.

T128. Multimodal Neuroimaging of Visual Plasticity in Healthy Humans
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Background: Learning and memory deficits are linked to poorer functional outcomes and quality of life in several psychiatric and neurological diseases. These deficits may reflect alterations in long-term potentiation (LTP), a form of synaptic plasticity underlying learning. This study combined fMRI to assess visual plasticity using a paradigm known to induce LTP in rodents, 1H-MRS to assess occipital glutamate, actigraphy to assess sleep efficiency, and blood measures of kynurenic acid (KYNA), an endogenous alpha7 nicotinic acetylcholine and NMDA receptor antagonist known to modulate learning in rodents. The specific aims of the study were to determine if 1) the fMRI paradigm induces visual plasticity, and 2) whether occipital glutamate, sleep efficiency, and KYNA predicted visual plasticity in healthy humans.

Methods: Seventeen healthy participants completed this study. MR scanning was conducted on a 3T Siemens Tim Trio with a 32-channel head coil. Spectra were acquired from the visual cortex using a STEAM sequence optimized for detection of glutamate. The fMRI plasticity paradigm consisted of a low-frequency visual stimulation to assess occipital visual processing, followed by a high-frequency stimulation to induce visual plasticity, and another low-frequency stimulation. fMRI analyses were conducted in SPM, and 1H-MRS data were quantified using LCModel. Occipital ROIs were selected from a 2x2 ANOVA using the main effect of time that fell within the spectroscopic voxel. Sleep quality during the night prior to scanning was objectively assessed using actigraphy, and a fasting blood draw was conducted to measure KYNA in the plasma. Participants also completed tests of explicit memory function.

Results: Occipital fMRI signal increased following high frequency stimulation, indicating that visual plasticity was induced. Glutamate occipital levels were related to occipital visual plasticity (r = -0.573, p = 0.016). Sleep efficiency was also significantly related to occipital visual plasticity (r = -0.665, p = 0.007). However, there was no significant correlation between KYNA and visual plasticity, but KYNA was related to verbal (r = -0.453, p = 0.021) and spatial memory (r = -0.588, p = 0.021).

Conclusions: These results support previous work showing that the paradigm is effective in inducing visual plasticity in healthy humans. A significant novel finding is that higher glutamate concentrations are predictive of greater visual plasticity response in healthy participants. Given the significant role of glutamate in LTP, this relationship was expected but shown for the first time. Greater sleep efficiency was related to greater visual plasticity response, which was also expected given the role of sleep in brain and cognitive function, especially learning and memory. Blood KYNA levels were not related to visual plasticity but were related to explicit memory function. Future work will determine if visual plasticity is impaired in schizophrenia and whether glutamate levels, sleep efficiency, and peripheral KYNA levels relate to visual plasticity in patients with schizophrenia.

Keywords: 1H MRS, Kynurenic acid, fMRI, sleep, learning and memory

Disclosures: Nothing to disclose.

T129. Relationship Between the Effects of Nicotine on Subjective State and Vigilance Task Performance in Healthy Non-smokers
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Background: There is extensive evidence that nicotine has beneficial effects on cognitive task performance, especially in attention tasks requiring vigilance and simple stimulus detection. However, it is unclear how this ties in with the subjective effects of nicotine. The subjective effects profile associated with objectively measured performance benefits
may provide a new level of insight into the nature of these potentially therapeutic effects.

**Methods:** Healthy non-smokers (N = 19) participated in a double-blind, placebo-controlled study. Participants came into the research facility on two separate test days, on which they received a transdermal patch upon arrival. On one of the days, the patch contained 7mg/24 hrs of nicotine and on the other day the patch was a placebo. After patch application subjects completed the Profile of Moods States (POMS) questionnaire every hour. Participants rated how well each of 65 adjectives related to how they were feeling at that moment. The POMS provides subscale scores for six factors; vigor, fatigue, depression, tension, confusion, and total mood disturbance. The factor vigor was composed of only positively valenced items, while the remaining factors were composed of only negatively valenced items.

After wearing the patch for 4.5 hours, a 30-minute computerized vigilance task was performed in which eleven dynamic stimuli were visible at any one time at random locations. The stimuli gave the appearance of raindrops growing and fading on a windshield, with target stimuli differing subtly from the standard stimuli. On average, 6 target stimuli were presented per minute. The percentage of correct detections and the reaction time to target stimuli was recorded. Six hours after the initial patch application, participants underwent two hours of intensive cognitive testing while undergoing an MRI scan (results not reported here). The POMS was completed once more after this scan, eight hours after patch application. A blood draw was then collected and analyzed for concentrations of nicotine.

Ratings on the six POMS factors were averaged for hours 4, 5 and 6 post patch application, which were close in time to performance of the vigilance task. These ratings were compared to the 8-hour measurement after the lengthy and cognitively intensive MRI scan, to test for potential state dependency of the subjective effects of nicotine. Data was analyzed by a two-factor ANOVA for repeated measures (drug x time). The effects of nicotine on performance of the vigilance task were correlated with the effects on subscale scores of the POMS averaged over hours 4, 5, and 6.

**Results:** Nicotine significantly increased subjective self-reports of vigor on the POMS (main effect of drug p = .039). There were also trend effects reflecting reduced fatigue (p = .130), confusion (p = .079), and total mood disturbance (p = .058) with nicotine. A significant main effect of time on fatigue (p = .035) reflected greater fatigue after the cognitive MRI scan. However, there was no drug x time interaction on any of the POMS subscales (p > .4 in each case), suggesting that the subjective effects of nicotine were not state-dependent. RT on the vigilance task was significantly faster in the presence of transdermal nicotine relative to placebo; however there was no effect on stimulus detection. Nicotine-induced reductions in RT correlated significantly with nicotine-induced reductions in the POMS factor of confusion (r = .517, p = .034). There were also trend correlations between drug effects on RT and on self-reports of fatigue (r = .403, p = .108) and total mood disturbance (r = .412, p = .100). Surprisingly, even though the effect of nicotine on the factor vigor was the most robust, there was a complete absence of an association with the effects on vigilance task performance (r = -.173, p > .5). Controlling for blood levels of nicotine did not change this pattern of results.

**Conclusions:** Although one would expect the subjective state most related to attention and vigilance to be vigor, this factor was not associated with the performance-enhancing effects of nicotine, despite a significant increase in self-reported vigor with nicotine. Instead, RT benefits of nicotine were associated with greater reductions in confusion, with trends also for fatigue and total mood disturbance, all negatively valenced factors. This suggests that the performance-enhancing effects of nicotine may have resulted from a reduction in the distracting influence of negative subjective mood states. Because all participants were non-smokers, this did not reflect alleviation of nicotine withdrawal.

**Keywords:** nicotine, subjective effects, Attention

**Disclosures:** Nothing to disclose.

T130. Circuit Components and Dynamics of a Putative Top-Down Attentional Filter
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**Background:** Appropriately filtering salient cues from the environment is critical for the allocation of scarce attentional resources, but how neural circuits support this critical function is poorly understood. Salience is encoded by multiple brain regions, including the claustrum and the anterior cingulate cortex (ACC), with the latter implicated in executive attentional processing. By virtue of the claustrum’s functional quiescence and wide connectivity with cortex, this structure is situated to relay top-down allocation of attentional command from the ACC to sensory cortices. How the claustrum is built to possibly mediate such top-down attentional gain control of ACC information is not known.

**Methods:** Here, we use a combination of neuronal tract tracing, whole-cell patch clamp electrophysiology and optogenetics to elucidate circuit components and performance of claustral processing of ACC input.

**Results:** Our findings of preferential claustral connectivity with the ACC, claustral functional quiescence conferred by powerful inhibitory microcircuitry, claustral glutamatergic intracortical connectivity and connectivity with widespread cortical areas.

**Conclusions:** suggest a role for the claustrum in top-down attentional filtering and allocation.

**Keywords:** claustrum, anterior cingulate cortex, Attention, electrophysiology, Brain Anatomy

**Disclosures:** Nothing to disclose.

T131. Prenatal LPS Exposure Preferentially Increases Kynurenine Pathway Metabolism in the Fetal Brain
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**Abstracts**
Abstracts

Background: Maternal infection during pregnancy increases the risk for the offspring to develop a broad spectrum of psychiatric disorders, including schizophrenia (Brown and Patterson, 2011). Prenatal exposure of mice to lipopolysaccharide (LPS) leads to brain and behavioral abnormalities in the offspring, but the underlying mechanisms are still unknown. The kynurenine pathway (KP) of tryptophan degradation is strongly regulated by the immune system (Saito et al., 1992) and may constitute a molecular link between immune activation and psychiatric diseases. Notably, the KP contains several neuroactive metabolites, including kynurenic acid (KYNA), an antagonist of the \( \alpha_7 \) nicotinic acetylcholine receptor and the N-methyl-D-aspartate (NMDA) receptor, and, in a competing branch of the pathway, the free radical generator 3-hydroxykynurenine (3-HK) and quinolinic acid (QUIN), an agonist of the NMDA receptor.

Methods: In the present study, we evaluated the effect of an intraperitoneal injection of a low dose of LPS (100 \( \mu \)g/kg) on gestational day 15 on KP metabolism in CD1 mice. To this end, kynurenine, KYNA and 3-HK levels were determined in maternal plasma and brain, as well as in placenta and fetal brain, 4 and 24 h after the immune challenge.

Results: No differences in maternal body weight or number of embryos were observed between controls and LPS-treated mice. Moreover, KP metabolite levels were slightly elevated in placenta 4 and 24 h after LPS injection, but the increases did not reach statistical significance. In contrast, kynurenine levels were significantly increased in the fetal brain 4 h after exposure to LPS \( (p<0.01, n=3) \), compared to the control group, and were still elevated after 24 h \( (p<0.05, n=4) \). A similar trend was observed for KYNA and 3-HK levels, with increases seen 4 h after the LPS injection \( (p<0.01, n=3) \). Interestingly, this significant increase in KP metabolism was not observed in the maternal brain at this low dose of LPS, indicating a higher susceptibility of the developing brain to infection.

Conclusions: These results suggest that even relatively modest prenatal immune activation, by increasing KP metabolism specifically in the fetal brain, may have detrimental long-term consequences during postnatal development. In particular, the observed elevation in KYNA levels in the fetal brain may increase the risk of developing psychiatric disorders later in life (Pocivavsek et al., 2014).

Keywords: kynurenic acid, schizophrenia, development

Disclosures: Nothing to disclose.

T132. Estrogens Suppress a Behavioral Phenotype in Zebrafish Mutants of the Autism Risk Gene, CNTNAP2

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Background: Autism spectrum disorders (ASD) are a group of devastating neurodevelopmental syndromes that affect up to 1 in 68 children. Contactin Associated Protein-like 2 (CNTNAP2) is one of the first genes strongly linked to autism and epilepsy in consanguineous families. At present, our ability to advance rapidly from the identification of risk genes in ASD to the discovery of potential pharmacotherapies remains limited. The zebrafish is a model vertebrate system well-suited for conducting small molecule screens to uncover modulators of signaling pathways. Here we capitalize on this system to investigate the consequences of the loss of Cntnap2 in zebrafish and to conduct rational pharmacological screens to identify phenotypic suppressors and novel pathways with relevance to autism.

Methods: To investigate the function of Cntnap2, we generated double mutants of cntnap2 in zebrafish using zinc finger nucleases. We analyzed inhibitory and excitatory neuronal populations in cntnap2 mutants during early brain development using transgenic lines that co-label these cell populations. To predict neural pathways that are disrupted in mutants, we conducted quantitative behavioral profiling of cntnap2 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: Zebrafish cntnap2 mutants display GABAergic deficits particularly in the forebrain and increased sensitivity to drug-induced seizures. High-throughput behavioral profiling identified a phenotype of nighttime hyperactivity in cntnap2 mutants, while pharmacological testing revealed dysregulation of GABAergic and glutamatergic systems. Specifically, GABAergic agonists elicited the most differential responses in mutant versus wild-type fish, while mutants displayed increased sensitivity to behavioral activation by NMDA antagonists. In addition, we found that both GABA- and NMDA- antagonists are significantly enriched among compounds that correlate with the mutant behavioral profile. Finally, we found that estrogen receptor agonists elicit a behavioral fingerprint anti-correlative to that of cntnap2 mutants. Further, we show that the phytoestrogen biochanin A specifically reverses the mutant behavioral phenotype.

Conclusions: These results identify estrogenic compounds as phenotypic suppressors and illuminate novel pharmacological pathways with relevance to autism.

Keywords: Autism, genetics, zebrafish, Pharmacology

Disclosures: Nothing to disclose.

T133. The National Neuroscience Curriculum Initiative (NNCI): Progress on Preparing for the Future of Psychiatry

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Background: Neuroscience research is dramatically expanding our understanding of the biological basis of psychiatric illness. And yet, for a host of reasons, most psychiatry training programs do not have rigorous neuroscience curricula. Of those that do, fewer still have moved past
neuroscience” is not currently included in the core teaching neuroscience in psychiatry during a time when drawing attention at a national level to the importance of the APA assembly at its May meeting. This support NNCI in this effort. The position statement was endorsed by comprehensive understanding of neuroscience and its application to teaching and learning neuroscience include articles published about neuroscience in psychiatry residency training programs are using our materials and/or increasing neuroscience teaching. In the year 2014. The NNCI is a collaborative effort of educators and neuroscientists around the country. The initiative is supported by the American Association Directors of Psychiatry Residency Training (AADPRT) and the American Psychiatric Association (APA) Council on Medical Education and Lifelong Learning, with funding from the NIMH. Here we present data on the impact and uptake of the NNCI since its inception.

Methods: To date, we have designed six different modules, each of which reflects a different experiential approach to teaching and learning neuroscience. Each of the modules has been published online (freely available for registered users) along with a detailed facilitator’s guide and supplementary resources (including videos, worksheets, answer keys, and additional readings). We have also begun a database of resources for self-study, including a collection of short and engaging expert videos in which leading neuroscientists summarize and translate essential elements of their research to a clinically focused audience.

To enhance dissemination and to engage stakeholders, we have conducted a large number of faculty development workshops at the annual meetings of major national organizations and at universities in the U.S. and Brazil. We have also used these opportunities to solicit content submissions from neuroscientists and clinician educators.

Results: Our primary outcome measure is whether psychiatry residency training programs are using our materials and/or increasing neuroscience teaching. In the year following our first major outreach event, 73 out of 96 individuals surveyed reported incorporating NNCI teaching approaches within their residency curriculum. In the first three months after launching our website in March, 2015 the site hosted 4,161 users from 50 countries and had more than 21,551 page views.

Additional outcome measures for the initiative include the reception of the NNCI mission (to integrate a modern neuroscience perspective into the clinical practice of psychiatry) by the field at large. Evidence of our success in stimulating dialogue about the importance of neuroscience in psychiatry include articles published about the NNCI in JAMA Psychiatry, the Journal of the American Academy of Child and Adolescent Psychiatry, the NIMH Director’s blog, the New York Times, and the Huffington Post. Additionally, an APA workgroup recently created a report entitled “Training the Psychiatrist of the Future”. The report includes a position statement that “A comprehensive understanding of neuroscience and its application to psychiatric treatment should be one of the core requirements of training” and highlights the role of the NNCI in this effort. The position statement was endorsed by the APA assembly at its May meeting. This support demonstrates the significant impact of our efforts in drawing attention at a national level to the importance of teaching neuroscience in psychiatry during a time when “neuroscience” is not currently included in the core Accreditation Council for Graduate Medical Education training requirements for psychiatry.

As a final measure of success, we hope to engage stakeholders to contribute back to the mission of the NNCI. In addition to the number of individuals and programs who are using NNCI materials, we are also tracking submissions of new content to the NNCI. To date, we have had more than 30 submissions of new session material.

Conclusions: During the initial phase of the NNCI we have designed a set of unique, experiential approaches to teaching and learning neuroscience that have been widely adopted by residency programs. We have a large - and ever growing – collection of resources that are freely available through our website. We have engaged in extensive outreach to disseminate the core message of the NNCI with evidence of widespread impact. Significantly, the initiative appears to be building on its momentum, with an increasing number of individuals contributing new content – a crucial step for the long-term success of the program.

Keywords: education, Training, Translational Neuroscience, User-centered design

Disclosures: Nothing to disclose.

T134. The Cooperation of Hemispheres and Consciousness
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Background: The aim of this poster is to elucidate the origin of consciousness. Corpus-callosum enables the contact of the hemispheres of the brain. The stimulus from the left (dominant) hemisphere meets with those from the right hemisphere in corpus-callosum. The stimulus with the origin in the right hemisphere is three times faster than the stimulus from the left hemisphere. What occurs in the time passing during the different velocities? The right hemisphere takes stimuli from external and internal world. The left hemisphere is slower in the reading and interpreting of the meaning of the information which has come, whereas the right hemisphere continues to accept new stimuli. We assume that there is no thought without words. The time necessary for the left hemisphere to read the information coming from the right hemisphere is too short to be able to interpret this information.

Methods: Because the results are known earlier than the explanation of the method, the method is just reviewing and deducing the literature.

Results: Results can be understood thanks to a model of the following event. In the night, a driver goes through a forest and a deer jumps suddenly into the road. The driver breaks, steps on the clutch and turns the steering wheel to avoid the crash. Only afterwards, he realizes what has happened. The time, he needs to realize what has happened, is used by the left hemisphere to translate the event into words. Because the left hemisphere works without being able to translate all stimuli from the right hemisphere, it tries to find meaning of the information gotten and tries hard to catch up with the right hemisphere. Here is the source of energy, which pushes the mental activity forward. The mental activity is not a product of nervous activity. The mental (conscious)
activity is approximately 3-4% of the activity of the brain. All which is conscious must be translated into words. Words are not the grains of sand chaotically accumulated. Words are organized by the grammar. Grammar is the structure of the language. Noam Chomsky postulates that the structure of the language is inherited and is identical in all languages. The majority of the linguists does not agree with Chomsky and considers the ability to learn to speak to be inherited.

**Conclusions:** Cooperation between the hemispheres of the human brain is the source of consciousness.

**Keywords:** consciousness, brain hemispheres, advantages of the combination of therapies, neurophysiological aspects of the origin of consciousness, all is in the timing

**Disclosures:** Nothing to disclose.

**T135. Multi-Tracer PET Characterization of Pre- and Post-Synaptic Dopaminergic Systems**

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**Background:** Important insights into central dopaminergic function, essential for understanding neuropsychiatric illnesses such as schizophrenia, have been generated by single-tracer PET studies using regional analyses of pre- or post-synaptic dopamine (DA) systems, but how these systems interact on a network level remains unclear. DA-relevant mesostriatocortical circuitry has a complex organizational structure with overlapping cortical projection fields throughout the basal ganglia, resulting in functional subregion boundaries that may not follow intuitive neuroanatomical-based landmarks. Thus, conventional region-of-interest or even voxelwise univariate model approaches may not fully account for the spatial interdependencies inherent in maps of DAergic function. Here we apply a data-driven approach, joint independent components analysis (jICA), to a unique series of three complementary DA PET datasets in the same healthy individuals, in order to delineate distinct multivariate DAergic network features (voxelwise joint components) that might enhance our understanding of integrated DAergic systems function.

**Methods:** Seventy-five participants (ages 19-55y, mean 35y; 35 females) were screened for confounding medical or neuropsychiatric problems by clinician-administered semi-structured interview (SCID), history, physical examination, clinical lab work, and structural MRI. All provided consent per the NIH CNS IRB. Each underwent three separate-session PET studies with different DAergic targets: [18F]FDOPA, permitting quantification of the last committed step in DA synthesis in the presynaptic neuron; [18F]fallypride, an assay of in vivo pre- and post-synaptic D2/3 receptor availability; and [11C]NNC112, a measurement of post-synaptic D1 receptor availability. Caffeine and nicotine were restricted for 4 hours prior to tracer administration. For FDOPA only, a fasting state was required and an oral dose of carbidopa was given one hour before injection. Each individual’s separately-collected, T1-weighted anatomical MRI image was segmented for a cerebellar reference region, which excluded both medial regions surrounding the vermis and lateral regions abutting the transverse sinuses, and coregistered to native-space, attenuation-corrected, realigned PET data. After extraction of the reference region’s time activity curve, PET data were normalized to MNIST-space using ANTS software. Voxelwise modeling using PMOD software yielded maps of the specific uptake constant, Ki (FDOPA, Gjedde-Patlak model) and binding potential, BPND (fallypride and NNC112, SRTM). Within each modality, these modeled maps were global variance-normalized and mean-centered, then entered into a joint independent components analysis (jICA) as implemented in the Fusion ICA Toolbox. Resultant joint component sets were evaluated for reliability with ICASSO. Mixing coefficients, representing an individual’s loading parameters for each joint component set, were tested for sex and age effects using a general linear model in SPSS.

**Results:** Five reliable (ICASSO derived Iq ≥0.95) joint components were estimated, including three components featuring strong striatal signal from each of the three tracers, respectively, and two more complex components: one demonstrating robust, opposing dorsal-ventral striatal signal across all tracers, and one revealing opposing FDPFA-fallypride striatal signal. Two of these five joint components showed significant association with age (p <0.005, corrected), and a nominal association (p = 0.014, uncorrected) with sex was identified for one component.

**Conclusions:** Spatially independent pre- and post-synaptic DAergic networks can be delineated via multisession PET imaging and jICA. The modality-specific partitioning of ICs characterized by prominent loadings in high signal-to-noise ratio, DA-rich regions (i.e. mesostriatal circuitry) suggests that regulation of D1, D2, and DA synaptic processes are dissociable at the systems level, in accord with the interacting but divergent cellular populations these aggregate measures represent. Also, we show that some of these networks demonstrate substantial age-dependence, even within this relatively restricted age range. Further work is needed to determine whether these same networks may be specifically altered in DAergic neuropsychiatric illness.

**Keywords:** Dopamine, striatum, PET

**Disclosures:** Nothing to disclose.

**References:**

T136. Presynaptic Midbrain Dopamine Modulates Adaptive Prediction Error Signals in Humans

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**Background:** The dopaminergic system plays a critical role in the processing of prediction errors (PE) associated with reward (Schultz 2007) and punishment (Frank et al. 2004) through its influence on the cortico-striatal system (Wise 2004). Pharmacological manipulation (Pessiglione et al. 2006) and assessments of neuropsychiatric status (Frank et al. 2004; Murray et al. 2008) have defined a role for this neurotransmitter in PE signaling during learning. However, the extent and directionality of the effects of dopamine (DA) on adaptive PE signaling for rewarding and aversive contexts is not known. To clarify this issue, we used 18F-fluorodopa (FDOPA) PET to obtain stable, trait measures of tonic DA neurotransmission (Vingerhoets et al. 1994) and studied the same individuals with fMRI during affective instrumental learning. We tested the hypothesis that presynaptic DA synthesis in the midbrain (a DA innervating site) will differentially influence PE signals for loss and gain outcomes, in line with the role of DA in modulating adaptive behavior.

**Methods:** Subjects & Learning Task: Sixty nine healthy adults (mean age = 34; 33 males) underwent probabilistic learning during event-related fMRI. Each trial consisted of: (1) an initial video cue showing facial expression (fear, happy, or neutral), (2) a subsequent choice cue that required selecting between two non-face pictures differing in the degree of emotional concordance to the initial face cue, (3) a feedback cue noting the picture they chose, and (4) an outcome cue indicating in 80% of trials (# of invalid outcomes = 20% of the trials), gain of a $1 for a concordant happy choice, loss of a $1 for a non-concordant neutral choice, or a blurred $1 cue indicating no gain or loss for a concordant neutral choice.

fMRI acquisition & analysis: Random effects analysis of BOLD (3T, TR = 1.95) modeled over the monetary outcome phase was carried out with SPM5 using the PE values of each individual as predictors of the fMRI time series across two runs (15 events for each of the 3 choice categories per run) at the first level, and by assessing the group signals derived from the PE signal analysis in a 1-sample t-test at the second level (at p < 0.05 FDR corrected).

Computational Modeling: To model aversive (loss) and rewarding (gain) PEs, each participant’s sequence of choices was fitted to a standard reinforcement-learning algorithm (Sutton & Barto 1998). For each pair of stimuli A (i.e. loss), the model estimates the expected values of choosing A (Qa), based on an individual’s sequences of choices and outcomes. This Q value is the expected reward or loss resulting from the particular action taken. These Q values were set at zero before learning, and after every trial t > 0 the value of the chosen stimulus (say A) was updated according to the rule Qa(t + 1) = Qa(t) + $\alpha$ * $\delta$(t), where $\alpha$ is the learning rate. The PE was $\delta$(t) = R(t) - Qa(t), where R(t) is defined as the reinforcement obtained as an outcome of choosing A at trial t. Thus the prediction error $\delta$(t) is the difference between the expected outcome [i.e. Q(t)] and the actual outcome [R(t)]. Thus, for the gain condition, the reinforcement magnitude R was +1 for $\$1$ outcome and 0 for ‘Nothing’ outcomes. Given the q values, the probability of selecting each action was estimated, e.g. for choosing A, P $\alpha$(t) = eQx(t)/$\beta$ (over) eQx(t)/$\beta$ + eQb(t)/$\beta$, where $\beta$ is choice fluctuation.

FDOPA PET acquisition & analyses: On a different day, 40 of the 69 fMRI instrumental learning participants received ~16 mCi of FDOPA preceding a dynamically acquired, 90-minute series of PET scans. The DA-midbrain and a cerebellar reference region were defined on each individual’s native space T1-weighted MRI image. After coregistration to the PET frames with SPM, time activity curves were generated for these regions. Patlak-Gjedde modeling was used to determined midbrain FDOPA Ki values, reflecting presynaptic DA synthesis. An ANCOVA using the FDOPA Ki values as predictors of the PE-associated signals was performed in SPM5 (at p < 0.005) to test for an effect of midbrain DA on aversive and reward PE learning signals separately.

**Results:** On average, participants avoided losing $1 in 78%; and gained $1 in 73% of the trials during instrumental learning. fMRI BOLD associated with loss PEs revealed clusters of positive correlation in the putamen, caudate and visual cortex, and negative correlation in anterior insulae and in frontal and visual cortices; whereas reward PEs showed positive correlation with nucleus accumbens, caudate, and frontal and visual cortices. Midbrain FDOPA Ki values positively predicted BOLD signals related to loss PE in the putamen, whereas negative correlations with reward PE BOLD were observed in the nucleus accumbens, prefrontal and cingulate regions.

**Conclusions:** A hallmark of adaptive behavior is the consistent ability to learn to avoid or minimize negative experiences and maximize the attainment of positive environmental outcomes. Our findings show that midbrain tonic DA neurotransmission predicts prediction error-related neural responses that were tuned by individuals’ adaptivity to impending challenges and rewards in the environment. The current results reflect dopaminergic modulation of instrumental choice-behavior and may provide a systems-level biomarker to examine dopamine-related dysfunctions manifested in cognitive, learning, and affective dysfunctions.

**Keywords:** Brain, Dopamine, Adaptive Behavior, Learning

**Disclosures:** Nothing to disclose.
T137. Psychological Factors, Hormones, and Brain Activity as Potential Biomarkers in Predicting Weight Loss following Bariatric Surgery: A Pilot Study


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Background: In the U.S., about 50% of overweight or obese adults are trying to lose weight, relying on a wide range of approaches, including lifestyle intervention, medication, and surgery. While many experience some initial success, most have difficulty maintaining clinically significant weight loss over the long term. Even for those who undergo bariatric surgery, well-established as the most effective treatment of severe obesity, resulting in an estimated 48–68% excess weight loss (EWL), approximately 30–50% of patients eventually regain considerable weight (many over 10% of their maximal weight loss), beginning 18–24 months after surgery (Magro et al., 2008; Shah et al., 2006). Given the cumulative cost associated with repeated treatments, matching the most effective therapy to each patient represents a significant challenge, with a critical need for more accurate prediction models. Efforts to characterize prognostic indicators in weight loss interventions, particularly for bariatric surgery, have highlighted psychosocial factors and neuroendocrine markers. For example, individuals with a history of mood dysregulation and poor emotion regulation have lower % weight loss, as daily life stressors may challenge their coping skills resulting in a return to pre-operative maladaptive eating behaviors. Additionally, alteration in hormone systems involved in appetite regulation may contribute to variable long-term efficacy in weight loss maintenance. More recent evidence from psychiatric neuroimaging supports the role of neural system functioning in prediction of treatment outcomes, which may inform the field of obesity treatment. Particularly pertinent to the bariatric surgery population, the neural circuits responsible for regulation of food cravings include mesocorticolimbic reward regions [ventral tegmental area (VTA), ventral striatum (VS), insula, medial orbitofrontal cortex (mOFC)] and cortical regions associated with cognitive regulation/inhibition [dorsolateral prefrontal cortex (DLPFC), dorsomedial prefrontal cortex (DMPFC)]. The goal of this pilot study was to assess the utility of these potential behavioral and biomarkers (mood, hormones, and brain activity) of treatment efficacy in bariatric surgery outcomes.

Methods: Nineteen patients (38.7 ± 9.6 years; 42.6 ± 4.1 BMI; 17 females) scheduled to undergo vertical sleeve gastrectomy were recruited for this study. Within one month prior to their procedure, participants completed a baseline study visit, including self-report measures of mood-related functioning [Beck Depression Inventory (BDI); State- Trait Anxiety Inventory - trait anxiety (STAI)] and eating behavior [Power of Food Scale (PFS), a measure of psychological impact of living in food-abundant environments and appetite for palatable foods], a fasting blood draw, and an fMRI scan with a food craving regulation task. This task required participants to utilize two strategies employed while viewing palatable foods (Enhance: upregulation of cravings; Regulate: cognitive reappraisal focusing on the positive benefits of not eating) in an event-related design. To date, a subset of 11 participants (at baseline: 42.8 ± 7.8 years; 42.8 ± 4.2 BMI; 9 females) additionally completed 6-month follow-up visits composed of a fasting blood draw and completion of self-report measures. Plasma hormones levels for glucose, insulin, and leptin were assessed using commercial immunoassy kits. fMRI data were analyzed using SPM8 (small-volume correction; p < 0.05, FWE-corrected), in a priori ROIs according to primary conditions of interest: Enhance (VTA, VS, insula, mOFC); Regulate (DLPFC, DMPFC). Individual percent signal change values were extracted from selected ROIs using REX.

Results: At baseline, participants demonstrated significant BOLD activity during Enhance in the VTA (z = 3.06; p = 0.005), left (L) insula (z = 4.47, p = 0.001), and mOFC (z = 3.69; p = 0.01), and during Regulate in the L DLPFC (z = 4.93; p < 0.001) and DMPFC (z = 5.17; p < 0.001). By 6 months following surgery, participants lost approximately 59.3 ± 11.4% of their excess weight. Comparison of mood-related functioning suggested significant improvement in BDI [t(10) = 3.41, p = 0.007] and PFS scores [t(10) = 5.60, p < 0.001], but no change in STAI trait anxiety [t(8) = 0.38, n.s.]. Levels of appetite-regulatory hormones decreased by 6 months [glucose: t(10) = 3.17, p = 0.009; insulin: t(10) = 5.57, p < 0.001; leptin: t(8) = 3.54, p = 0.008]. Exploring the relative association between baseline variables and primary outcome, there were no significant relationships between behavioral (BDI, STAI trait anxiety, PFS) and %EWL (r = 0.24-0.37, p = 0.33-0.51). Similarly, no significant associations emerged between baseline hormone levels and %EWL (r = 0.14-0.32, p = 0.68-0.37). BOLD activity during the Regulate condition at baseline was strongly associated with %EWL (L DLPFC: r = 0.69, p = 0.039; DMPFC: r = 0.74, p = 0.023).

Conclusions: These results suggest unique coupling between baseline variables and post-surgical weight loss, with data favoring neural activity during regulation of food craving as a potential biomarker relative to psychological factors (mood, anxiety) and hormone levels. Although preliminary, these trends identify potential mechanisms driving successful outcomes post-surgery, which we believe will ultimately contribute towards streamlining decision-making around therapeutic options in clinical settings.

Keywords: Mood, Appetitive Hormones, craving, Obesity, fMRI

Disclosures: Nothing to disclose.

T138. The Effects of Chronic Stress and HIV Disease on Hippocampal and Amygdala Shape Alterations and Verbal Memory Performance

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Background: Chronic stress can result in long-term or permanent brain changes, specifically within regions of the
hippocampus and amygdala. It is well-known that HIV-infection is associated with subcortical abnormalities. For example, studies comparing HIV+ individuals to seronegative controls have documented smaller volumes in the caudate, putamen (Becker et al., 2011), amygdala (Ances, Ortega, Vaida, Heaps, & Paul, 2012), thalamus and hippocampus (Jernigan et al., 2005). Three-dimensional shape analysis is an emerging method for structural analysis as it provides a more precise measurement of localized atrophy in comparison to volumetric measures (Apostolova et al., 2006 and Morra et al., 2009; Frisoni et al., 2010). Localized changes may provide more prognostic value in detecting early cognitive decline. The purpose of the current study was to examine the effects of chronic stress and HIV on hippocampal and amygdala shape alterations and memory functioning among a sample of community-dwelling adults.

Methods: Participants included HIV-seropositive (n = 30) and HIV-seronegative (n = 15) individuals. We used the Chronic Stress Burden scale (Gurung et al., 2004) to measure chronic stress (CS). Regions of the amygdala and hippocampus were extracted using automated segmentation procedures. For analysis of subcortical structures, we used 3D shape analysis, which examines the pattern of atrophy across the surface of the structure. This approach rests on the assumption that atrophy does not occur in a uniform fashion (Costafreda et al., 2011). We computed differences between groups (HIV+/HIV-) in amygdala and hippocampal shapes. Next, we examined relations of chronic stress and memory functioning with shape parameters. We expected to observe additive and interactive effects of HIV and CS on shape parameters and memory functioning.

Results: HIV+ individuals demonstrated greater reductions in the bilateral hippocampus and right amygdala than HIV- individuals. CS score were associated with shape changes localized to the right hippocampal head and tail, and bilateral hippocampal body. CS score was also associated with significant reduction of the dorsal anterior and ventromedial portion of the right amygdala. The interaction term (i.e., HIVxCS) was associated with reductions in the right hippocampal head and tail, right hippocampal body and the inferior surface of the left hippocampal body (all comparisons p < 0.003).

Verbal memory total and delayed recall T-scores were positively correlated with bilateral hippocampal shape. Specifically, higher verbal memory total recall T-score was associated with enlarged vertices in the lateral and inferior body and superior tail of bilateral hippocampi and sparse regions of the right dorsal hippocampal body. There were no significant correlations between verbal memory total recall T-score and amygdala shape. Higher verbal delayed recall T-score was associated with enlarged vertices in bilateral inferior hippocampal body, left hippocampal head and right hippocampal tail (all comparisons, p < 0.003).

Multiple regression results indicated that HIV status and CS score significantly predicted verbal memory total immediate recall score (p < .001). Standardized beta weights indicated that HIV status was a stronger predictor of verbal memory total recall score (B = .533, p < .0001) than CS score (B = .250, p = .03). CS score did not significantly add to the prediction of verbal memory delayed recall after accounting for HIV status (p > .10).

Conclusions: As expected, we observed significant shape alterations between HIV+ and HIV-negative participants such that HIV+ individuals demonstrated reductions in hippocampal and amygdala shape parameters. This is consistent with our knowledge of HIV effects on subcortical integrity. Further, chronic stress burden was also found to be associated with hippocampal and amygdala shape changes, and this was more pronounced in the HIV+ group. Memory performance was associated with shape parameters in hippocampal sub-regions of the body and tail, which have been previously linked to episodic learning and memory functioning. We found that HIV status and chronic stress were both predictive of memory functioning. Specifically, HIV+ individuals performed worse on verbal memory than HIV- individuals, and higher levels of chronic stress were associated with worse verbal learning performance. Together, our findings suggest that HIV+ individuals who experience chronic stress may be at greater risk for memory problems due to degeneration of hippocampal structures.

Keywords: chronic stress, Human Neuroimaging, HIV

Disclosures: Nothing to disclose.
predicted by the presence of ELS (B = .386, p = .045), as well as younger age (B = -.044, p = .013), and more co-existing medical disorders (B = -.0003, p = .004), respectively.

Conclusions: Early life stress predicted reduced effectiveness of a diabetes self-management intervention during followup. Certain aspects of such self-management interventions may be perceived as intrusive and unhelpful. Future studies will determine whether routine clinic practices and interventions might be tailored those who have suffered ELS in order to better optimize their glycemic control over time.

Keywords: functional capacity, diabetes, early life stress, hbA1c

Disclosures: Nothing to disclose.

T140. Increased Translocator Protein in the Brains of Active and Recently Retired NFL Players: A Pilot Study Using [11C]DPA-713 PET-Based Neuroimaging

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Background: Former National Football League (NFL) players have higher rates of delayed neurological, cognitive and affective impairments, including dementia with aging. Those impairments have been attributed to pathologic effects of repeated mild traumatic brain injury (mTBI), characterized by biomechanical shearing and inflammation of neuronal axons incurred over years of play. Autopsy studies of brains from a limited number of former athletes and PET-based neuroimaging in former NFL players collectively report pathologic tau deposition consistent with chronic traumatic encephalopathy (CTE). However, it is yet unclear whether the primary pathology of CTE is deposition of phosphorylated tau or whether dysregulated inflammation drives neurotoxic protein deposition. We recently showed that [11C]DPA PET is a promising tool to quantify increased expression of translocator protein (TSPO), a marker of brain injury and repair, in former NFL players. Thus, we sought to use [11C]DPA-713 PET-based neuroimaging in younger, active and recently retired NFL players to test for changes in binding to TSPO.

Methods: We quantified regional distribution of TSPO using [11C]DPA-713 PET-based neuroimaging data of nine, well-characterized active or recently retired NFL players and seven age-matched, athletic, healthy controls. All subjects were genotyped for the rs6971 TSPO polymorphism due to need to control for the effect of this common SNP on [11C]DPA-713 binding. Regional total distribution volume (VT) measurements were calculated using the Logan method from each subject’s 90-min dynamic PET data and their metabolite-corrected plasma input function. Regions of interest included several cortical, subcortical, and brainstem regions implicated in CTE. Bonferroni correction for multiple comparisons was applied to statistical analyses.

Results: Using two-way ANOVA with cohort (NFL players, controls) and TSPO genotype as fixed factors, [11C]DPA-713 VT values were significantly increased in several brain regions in the active and recently retired NFL players compared to the controls, particularly in right and left thalamus (P < 0.008), right and left temporal poles (P < 0.009) and brainstem (P < 0.006).

Conclusions: Our findings from this small pilot study of young NFL players support a model wherein microglial activation is an early, persistent response in select cortical, subcortical, and brainstem structures after repetitive, sports-related traumatic brain injury. [11C]DPA-713 PET in NFL athletes may prove useful in assessing whether this molecular response is reparative, or whether it is related to subsequent, pathologic tau deposition found in the brains of some former players.

Keywords: PET Imaging, TSPO, Mild Traumatic Brain Injury, [11C]DPA-713

Disclosures: Nothing to disclose.

T141. Understanding How CSF Tau Concentrations Reveal Alzheimer’s Disease

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Background: Tau protein in human cerebrospinal fluid (CSF) is the best characterized biomarker of neuronal injury and neurodegeneration available. Elevations in tau in the brain and CSF are a hallmark of later-stage Alzheimer’s disease (AD), and as tau tangles are thought to play a pathological role in the disease this molecule is a target of new AD drug discovery. It has been assumed that tau in CSF is a largely intact protein. However, recent reports indicate that it exists primarily in fragments, and it has been suggested that some of these fragments are in part responsible for the disease promoting ability of tau.

In order to better understand the nature of the tau protein in CSF, profiles were developed using RP-HPLC to separate tau species followed by ELISAs to detect different regions of Tau across the HPLC fractions. Tau protein profiles were produced from both cognitively normal (CN) individuals and AD patients.

Methods: Human lumbar CSF samples for CN vs. AD comparison were obtained from University of Gothenburg fluid banks; n = 20 patients from each group. The samples were prescreened by measuring Aβ1-42 and total tau (T-tau) via INNOTEST ELISAs. CSF Aβ1-42 levels were used for confirmation of clinical diagnosis. CSF Tau levels were used to enrich the sample pools for extremes of Tau pathology; AD samples utilized here had T-tau > 600 pg/ml; CN samples utilized here had T-tau < 400 pg/ml.

RP-HPLC purification and separation of tau species from CSF was performed via injection of 0.5 ml CSF denatured using 6M guanidine HCl on a C18 column (Agilent#770995-902) run at 60°C at 1.7 ml/min with gradient elution using 0.1% TFA/80% acetonitrile/HPLC water (0-100% over 52 minutes). Fractions were collected every 1 mL in deep
well blocks, spiked with GuHCl to 10 mM, and dried overnight in an Explorer 220 Savant SpeedVac (Thermo Scientific). Fractions were then reconstituted in 500 µl of 0.1%Tween/0.1%BSA/150mM Tris/pH7.8 with shaking at room temperature for 5 minutes before ELISA analysis.

Three tau sandwich ELISAs were used for sample analysis, based on recognizing N-to-mid-region tau (hT12 and Tau5 antibodies), mid-region (hT7 and Tau5 antibodies), or C-terminal tau fragments (hT25 and HT27). hT7 and Tau5 antibodies are commercially available, all others produced at Janssen R&D. All the assays recognize full length recombinant Tau equivalently.

Results: An RP-HPLC method for separation of tau species in CSF was developed, using recombinant human Tau441 protein as a marker of elution for full length material (38 minutes). This method purified tau from most CSF proteins as seen by UV absorbance. Analysis of individual HPLC fractions by ELISA showed no detectable endogenous tau present at the elution time (38 minutes) of recombinant tau with any of the assays. However, tau was detected at earlier time points (23-30 minutes) using the N-to-mid-region and mid-region assays, suggesting fragmentation of the tau protein to yield intact N-terminal half of the protein. In contrast the C-terminal ELISA did not detect any tau in the CSF fractions, suggesting loss of that fragment in CSF. The tau fragmentation pattern was not different between CN and AD patients, although total levels of any given fragment were higher in AD patients, as expected, given the elevation in CSF T-tau in AD.

Conclusions: CSF tau assays had been generally regarded as a measurement that quantified the amount of intact tau protein that had moved into the lumbar CSF from the brain. Recent evidence has demonstrated using Western Blot and ELISAs of the total tau pool that there are actually fragments of tau in CSF, and suggested lack of significant amount of full length tau (Meredith et al 2013). The data presented here confirms this using RP-HPLC to physically separate tau species, followed by analysis with fragment specific ELISA.

These findings may have an impact on how we measure and understand CSF-tau as a biomarker of neurodegeneration. For instance, cleavage of tau between antibody epitopes could make high levels of tau invisible to detection. Additionally, a phosphorylated form of tau could be very abundant but remain undetected if a capture antibody epitope was missing or altered. Finally, the far C-terminal of tau has been suggested to be the toxic/pathogenic form of tau, and if it is not in CSF it could be due to retention in the brain.

The distribution of tau fragments in a sample is dependent on protease pathways and these could differ between disease and non-disease environments. The pattern of tau protein fragments could be a disease-specific biomarker. The current technique using RP-HPLC provides the high sensitivity and specificity of ELISA detection, but the resolution of tau fragments is low. It will be important in future studies to dissect the fragment pool more completely so that it can be determined if tau fragmentation in AD is the same as in healthy individuals but accelerated, or if the disease produces new tau fragments. Either increased concentrations of specific “normal” tau fragments or the appearance of new tau fragments could be central to the propagation of neurodegeneration. It will also be important to examine tauopathies other than AD, e.g., progressive supranuclear palsy and some forms of frontotemporal dementia. Additionally, any disease-related tau fragments would offer a highly informative biomarker for disease and may provide a tau biomarker that is more AD-specific.

Keywords: Alzheimer’s Disease, tau, Biomarker, neurodegeneration, neurodegenerative

Disclosures: Gallen Triana-Baltzer is an employee of Janssen R&D, a pharmaceutical company of Johnson and Johnson.

T142. Examining the Effect of a Pathway-Selective Mutation on Communication from Thalamus to Cortex in a Mouse Model of Autism

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Background: Autism affects an increasingly large proportion of the population: 1:68 children, and as many as 1:42 males (Developmental Disabilities Monitoring Network Surveillance, 2014). The emotional and financial costs to families and society are immense, and there are few treatment modalities that address the brain changes central to autism. Central to the lack of treatment modalities is the lack of clinical targets for intervention. This project investigates a recently developed genetic mouse model of autism that shares both electrophysiological and neuroanatomical properties with certain other models of autism, and with the disorder in humans: a Tuberous Sclerosis 1 knockout mutation targeted to thalamic relay cells in a mosaic pattern sufficient to yield a behavioral phenotype that has characteristics of autism and obsessive compulsive disorder (Normand et al., 2013). The cortical network in this animal has never been extensively studied, and we hypothesize that an imbalance between inhibition and excitation exists, and yields stronger activity in the cortex of these animals relative to controls.

Methods: This ongoing project seeks to characterize functional changes at the level of individual neurons as well as neural networks by simultaneously imaging a brain slice to obtain network-level data using Flavoprotein Autofluorescence Imaging, as well as recording from individual neurons to characterize changes at the level of individual cells in cortex. All experimental protocols 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: While experimenters remain blinded to the direction of our results, there appears to be a significant difference in both the number of spontaneous large-scale events (likely up-states) generated in wild-type vs. mutant mouse brain slices. Preliminary data also suggest a difference in the threshold thalamic stimulus current...
required to elicit a large-scale network activation of cortex. A caveat to these results is that very few animals have been tested (two wild type, two mutant). Finally, there appears to be a difference in the spatial organization and extent of these large-scale activations in cortex.

**Conclusions:** Preliminary data suggest a difference in cortical network excitability between mutant and wild-type mouse brain slices. If these preliminary data hold true, and generalize to other mouse models of autism in which genes that are highly penetrant for autism in humans are knocked out, they would suggest clinical targets and directionality for noninvasive targeted treatment interventions in humans (e.g., Transcranial Magnetic Stimulation, Focused Ultrasound, transcranial direct/alternating current stimulation). Such an approach has the potential to rapidly identify treatment targets in animal models that could be used to inform studies utilizing these relatively new noninvasive, and non-harmful treatment modalities in humans.

**Keywords:** Autism, Obsessive Compulsive Disorder, electrophysiology, imaging

**Disclosures:** Nothing to disclose.

**T143. PET Reveals Interactions of Dopamine and Serotonin Systems in Subtypes of Tourette’s Syndrome and Obsessive-Compulsive Disorder**

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**Background:** Tourette’s syndrome (TS) is a complex neuropsychiatric disorder characterized by persistent motor and vocal tics and is highly co-morbid with Obsessive Compulsive Disorder (OCD). Previously, we reported elevated intravenous amphetamine-induced dopamine release (DArel) (measured by displacement of [11C]raclopride binding) and decreased [11C]McN5652 (serotonin transporter, SERT) in TS and TS + OCD. Subjects with the Forbidden Thoughts, Contamination, and Hoarding. To calculate DArel, 2 PET scans were performed with [11C]raclopride (RAC), preceded by intravenous saline or 0.3 mg/kg amphetamine. A subgroup of 10 TS and TS + OCD completed both RAC and [11C]McN5652 scans for serotonin transporter (SERT).

**Methods:** Using [11C]RAC, we studied a total of 18 healthy controls (HC), 15 TS only, 15 TS + OCD and 10 OCD only subjects. An additional 11 HC, 11 TS, 10 TS + OCD and 5 OCD only subjects were scanned with [11C]McN. Specific OCD subtypes, which may explain the heterogeneity in clinical presentations of OCD, were used to further subdivide subjects: Symmetry/Ordering, Forbidden Thoughts, Contamination, and Hoarding. To calculate DArel, 2 PET scans were performed with [11C]raclopride (RAC), preceded by intravenous saline or 0.3 mg/kg amphetamine. A subgroup of 10 TS and TS + OCD completed both RAC and [11C]McN5652 scans for serotonin transporter (SERT).

**Results:** Briefly, our major findings included: 1) a significant effect of OCD subtype group on DArel (p = 0.004, using ANOVA); 2) Subjects with the Forbidden Thoughts subtype had higher DArel than other subtypes (p < 0.05, post-hoc contrasts); 3) reduced [11C]McN binding in TS and OCD only groups; and 4) a positive correlation between SERT and DArel in a subset of TS and TS + OCD subjects (r = 0.78, p < 0.05).

In our subject pool there were 28 TS and TS with OCD who also reported current or lifetime depression.

**Conclusions:** The current results both confirm and expand on previous work, and is consistent with the hypothesis that TS+/OCD have higher DArel than controls in the posterior caudate and VS. Taken together, our data provides evidence that OCD interacts with Tourette’s syndrome and thus combinations of the conditions lead to differences in both the dopamine and serotonin systems. Future directions to be presented will include relationship of the PET findings to depression status/scales for some of the TS with OCD and TS alone.

**Keywords:** Human Neuroimaging, Positron Emission Tomography, Tourette syndrome, Obsessive Compulsive Disorder, Neuroreceptor imaging

**Disclosures:** Dean Wong: Consultancies Dartneuroscience and Piramal; Honoraria Dartneuroscience; Grants Addex, Avid, Dartneuroscience, Intracellualr, J + J, Lundbeck, Pfizer, Roche, Takeda; Research funds received for projects through Johns Hopkins University, not directly to Dr. Wong.

**T144. Decoding Brain Epigenome Maps with Broad H3K4me3 Signals: Discovering Functional Epigenetic Patterns and Their Dynamics in Gene Regulatory Networks**

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**Background:** Trimethylation of histone H3 at lysine 4 (H3K4me3) is a chromatin modification known to mark the transcription start sites (TSS) of active gene promoters. Regulators of H3K4me3 mark are significantly associated with the genetic risk architecture of common neurodevelopmental disease, including schizophrenia and autism. Here, through integrative computational analysis of epigenomic and transcriptomic data based on next generation sequencing, we investigated H3K4me3 landscapes of sorted neuronal and non-neuronal nuclei in human postmortem, non-human primate and mouse prefrontal cortex (PFC), and blood. We explored that H3K4me3 mark is spreading over several kilobases of genes and enriched in cell-type specific functions, not restricted to small domains around TSS by using an unbiased manner with an innovative approach on 41+12 ChIP-seq and RNA-seq datasets.

**Methods:** We employed a broad range of bioinformatics approaches on next generation sequencing-based transcriptomes and epigenomes from FACS sorted neuronal and non-neuronal nuclei from PFC gray and white matter and, for comparison, peripheral mononuclear blood cells (PMBC). First, our ChiPseq pipeline was applied to identify broadest H3K4me3 domains and to compare across species. Bayesian network (BN) is used to construct gene networks based on a previously described dataset of gene expression profiles from 173 PFC samples from non-demented healthy individuals using the RIMBANET package. We utilized the resulting PFC Bayesian gene expression network to
We characterized the broadest H3K4me3 peaks disorder. developmentally regulated H3K4me3-enriched loci in PFC enhancers, and with previously published datasets on with functional elements including promoters and super enhancers, and with previously published datasets on developmentally regulated H3K4me3-enriched loci in PFC neurons from controls and subjects on the autism spectrum disorder.

**Results:** We characterized the broadest H3K4me3 peaks from human PFC in the context of cell-type specific regulation, association with neuronal and non-neuronal gene expression and potential implications for normal and diseased development. We first addressed the occurrence and the biological significance of the broadest H3K4me3 peaks in three different cell types, including NeurN+ PFC neurons, NeuN- PFC cells, and nucleated blood cells. We identified novel regulators of these three different cell types by focusing on top 5% broadest H3K4me3 peaks (length in base pairs). The broadest NeurN+ H3K4me3 peaks in the present study were enriched for genes regulating neuronal connectivity and signaling, including many ion channels, and synaptic plasticity and learning and memory. Broadest H3K4me3 peaks in non-neuronal PFC cells showed enrichment for oligodendrocyte and other glial-related genes, in contrast to nucleated blood cells in which broadest peaks were associated with immune functions. Interestingly, cross-species comparison of broadest H3K4me3 peaks in NeurN+ neurons of the adult cortex identified many genes regulating excitatory glutamatergic neurotransmission and dopaminergic pathways with a conserved broadest peak profile in human, non-human primates and mouse. According to our network analysis, the broadest domain H3K4me3 peaks are centrally located in a network of 7000 genes associated with PFC function in control (‘healthy’ because non-demented) subjects.

**Conclusions:** This present study compares histone methylation landscapes across 4 different species and 3 different cell types (including neuronal nuclei collected from human brain), and provides novel insights into an unusual type of histone H3 lysine methylation and the resulting implications for gene regulatory networks and neuronal function. A more detailed analyses of specific histone modification profiles, including spread and breadth of histone H3K4 and other lysine methylation markings in specific cell types, bears promising potential to deliver valuable insights into epigenetic mechanism of normal and diseased brain development and aging. Such type of approaches, in the ‘Big data era’ of functional genomics with NIH sponsored consortia such as PsychENCODE charting brain epigenomes and transcriptomes in hundreds of specimens across the lifespan, are likely to provide critical insights into the neurobiology of psychiatric disorders such as autism and schizophrenia.

**Keywords:** Epigenetic, autism Spectrum Disorders, DL-PFC, Histone, Bioinformatics

**Disclosures:** Nothing to disclose.
scores measuring the impact of PBA were significantly improved from baseline (-3.1 [3.2], P < 0.001). Overall mean (SD) PHQ-9 scores were also significantly improved from baseline (-5.6 [6.2], P < 0.001), as were overall mean (SD) MMSE scores (0.59 [2.97], P = 0.002. On CGI-C (clinician-rated) and PGIC (patient/caregiver rated) 72% (189/261) and 77% (200/261) of patients, respectively, were rated as “much” improved or “very much” improved compared with baseline. In total, 75% (197/261) reported being “somewhat” or “very” satisfied with DM/Q treatment. AEs were reported by 132 (36.0%) patients; most were of mild or moderate intensity. Though treatment emergent AEs occurred in 36% of patients, AEs deemed by the investigators to be treatment-related occurred in 15% patients. The most common AEs were diarrhea (5.4%), headache (4.1%), urinary tract infection (2.7%), and dizziness (2.5%). Serious AEs (SAEs) occurred in 23 (6.3%) patients; none were deemed by the investigator to be related to study medication. The only SAEs reported in >1 patient were urinary tract infection (n = 3; 0.8%) and fall (n = 2; 0.5%).

**Conclusions:** The open-label PRISM II study adds to controlled evidence of efficacy and tolerability available thus far on the use of DM/Q for the treatment of PBA, and expands the clinical dataset to include the effect of DM/Q treatment for PBA secondary to dementia, stroke or TBI. DM/Q was well tolerated and was associated with clinically meaningful improvements in PBA symptoms as measured by CNS-LS score reduction accompanied by improvements in global measures of clinical change (CGIC, PGIC), QOL and treatment satisfaction. The significant improvements observed in the overall PRISM II study population were consistent with results across the three individual etiology cohorts (dementia, stroke, and TBI), and also consistent with improvements observed in previously completed phase 3 trials of PBA secondary to ALS or MS, therefore supporting the effectiveness of DM/Q for PBA irrespective of etiology.

**Keywords:** Pseudobulbar affect, Stroke, Traumatic Brain Injury, Dementia, Dextromethorphan/Quinidine

**Disclosures:** Study supported by: Avanir Pharmaceuticals, Inc. and Other; SU. Royce Lee* serves on the Scientific Advisory Board, Speaker's Bureau, and/or Investigator’s Advisory Board for: Merck, Dompé, and other companies.

**T146. A High Density EEG Study of Shared Attention and Visual Perspective Taking**

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**Background:** The study of psychiatric disorders such as borderline personality disorder characterized by dysfunctional social behaviors requires novel, reliable tools to examine brain function during social interaction. To this end, we adapted a visual perspective taking (VPT) task for use during high density EEG recording that has previously been reported to detect modulation of the face sensitive N170 event related potential (ERP) by shared attention and/or visual perspective taking.

**Methods:** All subjects provided written, informed consent using IRB approved consent forms. 18 normal control subjects (9 male and 9 female) were studied during performance of the VPT task. EEG was recorded with a Biosemi Active 128-electrode array while subject viewed faces presented on a computer screen pointed towards the ceiling. There were three conditions in which subjects viewed face stimuli on a computer screen resting flat on a table: 1. sitting alone; 2. sitting next to a confederate; 3. sitting opposite from a confederate. Comparisons of EEG data between social conditions were conducted in the time and frequency domain.

**Results:** For mean amplitude of the N170 in occipitotemporal electrodes, RM-ANOVA revealed a significant interaction of social condition x hemisphere, such that viewing faces next to or opposite a confederate is associated with right greater than left hemispheric asymmetry relative to the alone condition. In the frequency domain, permutation testing revealed differences in the low Beta frequency range (14 – 22 Hz) in the latency of the N170 (150 – 200 ms) in comparisons of the next-to vs. opposite condition. Exploratory beamformer analyses localized the beta suppression effect to the motor cortex.

**Conclusions:** Discussion: Shared attention was associated with increased right vs. left N170 amplitude. An effect of visual perspective taking was found on beta desynchronization, such that viewing faces next to a confederate was associated with enhanced beta suppression. The results confirm the hypothesis that social context modifies brain response to face stimuli in two ways. The first is an enhancement of bottom-up processing as reflected in the right hemisphere N170. The second is via beta suppression,
which has previously been linked to action observation and imitation. These novel findings should be applied to the study of personality disorder, which is associated with deficits in cognitive empathy.

**Keywords:** social neuroscience, EEG biomarkers, Borderline Personality Disorder

**Disclosures:** Royce Lee has received research funding from Azevan Pharmaceuticals. Emil F. Coccaro is a scientific advisor for Azevan Pharmaceuticals.

**T147. WITHDRAWN**

**T148. The Computational Role of Hippocampus in Social Dysfunction**

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**Background:** Over the last two decades, efforts to uncover the "social brain", a set of brain structures specialized in processing social information, have propelled the rapid evolution of social neuroscience. The constituents of the social brain remain elusive, however, as social cognition abilities refuse to neatly map onto anatomically defined brain regions. Research thus far has associated large parts of the cortex (including parietal, temporal, prefrontal and cingulate) with various social processes (such as social perception, theory of mind, impression formation, and self-reflection), but the delineation of the social brain is blurry and the specific computations it performs are obscure. Here we examined the neural and behavioral correlates of a geometric model of social relationships, and whether variations in the geometric representation of social space may provide a marker for social skills and personality traits.

**Methods:** We developed a “choose-your-own-adventure” game where participants played the lead role and interacted with six characters during functional neuroimaging (fMRI). The participants made choices throughout the game that shaped their relationships with the characters along the two main factors that influence relationships: power and affiliation. We calculated a geometric proxy of social relationships by drawing a hypothetical vector between the participant and each character at each social interaction, within the two-dimensional space of power and affiliation. The character’s location was determined by accumulation of the choices made by the participant during power and affiliation interactions. The angle (orientation) of this vector represents the relative balance between power and affiliation, and the vector length represents the combined magnitude of power and affiliation. Together, the vector angle and length describe the specific location of each character in the game’s theoretical social space. Next, we identified the neural tracking of the vector angle and length at each social interaction throughout the game, which could reflect a neural mechanism for navigation in social space. Following the fMRI task, participants filled out questionnaires assessing social anxiety, social effectiveness, and personality traits. We examined the correlation between the participants’ behavioral scores, geometric variables, and the beta-weights of their vector regressors.

**Results:** Eighteen participants completed the experiment. We found that during social interactions, the hippocampus represents people we interact with as locations within a two-dimensional space framed by power and affiliation. Specifically, we show that the hippocampus computes a spatial metric, the orientation of a vector representing the distance between the participant and each character. This metric reflects the balance between the power and affiliation each character has relative to the participant at each social interaction. Interestingly, participants who reported better social skills had better hippocampal tracking of these geometric social representations. Moreover, a number of geometric variables correlated robustly with the questionnaire scores. For example, the vector angle early in the game correlated with social fear and avoidance (Pearson’s \( r = -0.67, p = 0.003 \); \( r = -0.66, p = 0.003 \); respectively), indicating that participants with social anxiety tended to give less power (modulated by affiliation) to the characters at first impression. Furthermore, participants who reported less social avoidance tended to show increased social “exploration” indicated by widely spread characters’ locations in social space (\( r = -0.52, p = 0.0256 \)); and participants that had larger social space at the end of the game reported higher general self-efficacy (\( r = 0.60, p = 0.008 \)).

**Conclusions:** Our results demonstrate that the concept of social space is akin to physical space. Spatial descriptions of social situations such as “climbing up the social ladder” or having a “tight social circle” are perhaps more than mere metaphors, but rather indicate specific positioning of others using geometric computations. Our findings further suggest that the neural representation of social space, similar to knowing where we are in physical space, is important for psychological wellbeing. We propose that geometric modeling of social relationships may provide a possible new diagnostic tool for social dysfunction.

**Keywords:** Hippocampus, Social, Geometry, Anxiety

**Disclosures:** Nothing to disclose.

**T149. Prefrontal Functional Connectivity and Hostility in First-Episode Schizophrenia**

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**Background:** Aggressive behavior can be observed in some patients with schizophrenia, yet the underlying neural circuitry remains unknown. Previous studies have described abnormal links between prefrontal and limbic regions that correlate with aggression in chronic patients with schizophrenia (Hopman et al., 2010, 2014). To date no studies have been conducted in first-episode patients with schizophrenia that examine aggression. In the present study we aimed to use resting state functional connectivity to investigate neural circuits implicated in aggression in this population.

**Methods:** We examined 24 patients with first-episode schizophrenia who were treated for 12 weeks with second generation antipsychotic agents as a part of a large clinical
trial. Patients predominantly had resolution of their psychotic symptoms, which were measured by the BPRS. Patients underwent resting state fMRI imaging at the 12 week timepoint within the trial. Following standard preprocessing, whole-brain functional connectivity maps were calculated for each region of interest (ROI) within a predefined network of limbic and prefrontal regions implicated in aggression (Stein et al., 2007). The hostility rating from the BPRS at the 12-week point was extracted and entered as a regressor in group level analyses with maps for each ROI. Results were considered significant at p <0.05, corrected for false discovery rate.

Results: Of the 12 ROIs independently examined, the right orbitofrontal cortex (OFC) resulted in a suprathreshold result at the group level. Patients with increasing hostility showed decreased functional connectivity between OFC and a region of the superior frontal gyrus.

Conclusions: Our results support the hypothesis that abnormal prefrontal functioning is involved in the mechanism of aggression in schizophrenia. Our finding in the superior frontal gyrus is consistent with previous evidence implicating this region with response inhibition (Dambacher et al., 2014), and aggressive behavior in lesion studies (Paradiso et al., 1996). Reduced connectivity between superior frontal gyrus and orbitofrontal cortex may reflect abnormal “top down” processing of limbic regions in patients with increased aggression. Additional studies are required to further characterize this finding.

Keywords: aggression, first-episode schizophrenia, Resting State Functional Connectivity, violence

Disclosures: Dr. Sarpal has received research support from Janssen Pharmaceutical. Dr. Robinson has served as a consultant for Asubio, Otsuka, and Shire and has received grants from Bristol-Myers Squibb, Janssen, and Otsuka. Dr. Malhotra has served as a consultant for Forum Pharmaceuticals and has served on a scientific advisory board for Genomind.

T150. Dopamine D2 Receptors Availability in the Dorsal Caudate and Learning in Patients with Schizophrenia: A Prospective PET [11C]-Raclopride Study

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Background: Dopaminergic neurotransmission in the dorsal caudate is associated with learning. Antipsychotics occupy the dopamine D2 receptors in the dorsal caudate and, therefore, could contribute to learning deficits observed in treated patients with schizophrenia. Thus, in this first prospective Positron Emission Tomography (PET) study, we assessed the impact of reducing the dose of antipsychotics on learning, D2 receptors availability in the dorsal caudate, and their relationship among clinically stable patients, 50 years and older, with schizophrenia.

Methods: Thirty-four clinically stable subjects with schizophrenia underwent baseline clinical, cognitive, and [11C]-raclopride PET to assess D2 receptors availability. This was followed by up to 40% reduction in their olanzapine or risperidone dose. After the gradual dose reduction, they underwent follow-up clinical, cognitive, and [11C]-raclopride PET.

Results: Following the dose reduction, change in D2 receptors availability predicted change on learning ability of subjects with schizophrenia. Further, at follow-up and 65% occupancy, D2 receptors availability was associated with performance on learning tasks, but not at baseline when occupancy was at 73%.

Conclusions: Reducing antipsychotics dose changes D2 receptors availability in the dorsal caudate and related cognitive function. It also uncovers a relationship between D2 receptors availability and learning suggesting that high antipsychotics dosages were preventing dopaminergic neurotransmission from contributing to cognitive function. Thus, reducing antipsychotics dosages to a specific D2 occupancy target could optimize response to cognitive enhancing interventions.

Keywords: Antipsychotic agents, Memory and Learning, PET, dorsal caudate

Disclosures: Nothing to disclose.

T151. Memantine’s Acute Effects on Neurocognition in Schizophrenia (SZ) as a Predictor of Neurocognitive Benefits from Compensatory Cognitive Training (CCT)

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Background: CCT reduces symptoms and improves cognition and functional capacity in SZ. Little is known about the use of drugs to predict or augment the effects of CCT in SZ. It is possible that the cognitive response to an acute drug challenge might help identify patients most likely to benefit from CCT, or that drugs that enhance specific components of neurocognition, e.g., working memory (WM), might more specifically, and perhaps synergistically, enhance the clinical benefits of CCT. We are assessing ways to enhance the benefits of CCT in SZ via the use of pro-cognitive agents in biomarker-identified sensitive patients. In 2009, we reported that a single dose of the NMDA antagonist, memantine (MEM), significantly increased prepulse inhibition (PPI) of the startle reflex in healthy subjects (HS). PPI is a laboratory-based measure of sensorimotor gating that is positively associated with global neurocognition and specifically with WM in HS. We more recently (2015) demonstrated that MEM significantly enhanced PPI as well as mismatch negativity (MMN) in SZ patients; these patients were then invited to participate in a controlled 12-week trial of CCT. We now report on 2 issues addressed in this newer study: 1) the feasibility of using a drug challenge lead-in to a CCT trial to generate potential biomarkers to guide treatment assignment; and 2) the predictive value of these laboratory-based biomarkers on treatment outcome.
Methods: 82 subjects (HS = 41, SZ = 41) completed laboratory screening, and full test days involving administration of either placebo (PBO) or MEM (10 or 20 mg po), followed by neurocognitive testing (MATRICS Consensus Cognitive Battery; MCCB) as well as PPI and MMN. Of the 41 SZ subjects, 30 (73%) completed pre-screening for the treatment phase; 24 were randomized to CCT (manipulated 1h/wk x 12 wks; n = 14) vs. goal-focused supportive contact (GFSC; n = 10).

Results: We previously reported age-dependent MEM-potentiated PPI and MMN in these SZ patients (Swerdlov et al. 2015). Overall, neither 10 nor 20 mg MEM significantly enhanced neurocognition in HS or SZ patients. While 10 mg MEM produced some modest increases in MCCB performance scores in HS in certain domains (e.g. verbal learning (VerL); d = 0.2), 20 mg impaired performance in HS in speed of processing, WM, VerL and visual learning (d’s = 0.4–0.8). For the 10 mg MEM dose, age was positively correlated with greater MEM effects on Attention/Vigilance (AV; SZ patients: p < 0.008; all subjects: p < 0.016), and for the 20 mg dose, age was positively correlated with greater MEM effects on VerL (SZ patients: p < 0.06; all subjects p < 0.025). Baseline (screening) PPI strongly predicted MEM effects on VerL (p < 0.002) and Social Cognition (SC; p < 0.05), and PPI sensitivity to MEM correlated significantly with MEM effects on AV and SC (both p’s < 0.02).

Of the SZ patients randomized to group assignment and who began treatment, 16 (67%; CCT n = 11; GFSC n = 5) completed 3 months of treatment and post-treatment neurocognitive and clinical assessment. The completion rate difference (79% vs. 50%) was not statistically significant between CCT and GFSC groups, nor were treatment-related improvements in neurocognitive, functional or clinical outcome measures. No robust correlations were detected between either baseline or MEM-modified levels of PPI and MMN and treatment response. By contrast, acute MEM effects (ME) on several neurocognitive domains appeared to modestly or robustly predict changes in neurocognitive performance with treatment. In simple regression analyses, 6 out of 6 pair-wise correlations were positive (e.g. ME for a given domain vs. [month 3 minus baseline] value for the same domain), with statistically significant correlations noted for AV (p < 0.036), WM (p < 0.002) and VerL (p < 0.029) when all subjects (CCT and GFSC) were included, and with stronger correlations when groups contrasts were limited to CCT subjects.

Conclusions: A treatment model incorporating acute drug challenge and laboratory biomarker assessments in advance of treatment is feasible with SZ patients. In this small sample, we found some evidence that the neurocognitive response to an acute challenge with MEM predicted the subsequent neurocognitive benefits from CCT. Conceivably, acute drug effects on cognition provide a “read-out” of available cognitive resources that can be engaged by other therapeutic modalities such as CCT. Future studies will determine whether these or other biomarkers might predict sensitivity to pharmacologic enhancement of cognitive training effects.

Keywords: memantine, schizophrenia, compensatory cognitive-training

Disclosures: Dr. Light has served as a consultant for Astellas, Forum Pharmaceuticals, Boehringer Ingelheim and Neuroverse.

T152. N-Methyl-D-Aspartate Receptor (NMDAR)-based Enhancement of Auditory Plasticity

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Background: Schizophrenia is associated with profound social cognitive deficits, including auditory emotion recognition (AER). Auditory deficits (pitch-processing) correlate with AER, suggesting that remediating sensory-level dysfunction may lead to significant improvement AER. This two-phase study piloted effects of D-serine, a N-methyl-D-aspartate receptor (NMDAR) agonist in the enhancement of plasticity during an auditory frequency discrimination task, along with its neurophysiological correlates.

Methods: In Experiment-one, 40 schizophrenia/schizoaffective patients and 42 healthy controls completed one session of auditory plasticity, while in Experiment-two, 21 schizophrenia patients received D-serine/placebo prior to three auditory-plasticity sessions separated by ~1 week. Electrophysiology was measured immediately pre/post [mismatch negativity (MMN)] and during [quantitative EEG (QEEG)] the auditory task.

Results: In experiment-one, patients showed significantly reduced plasticity vs. controls (p = 0.001), which highly correlated with AER and reading ability. In healthy controls, improvements in auditory plasticity were associated increases in alpha power. In experiment-two, receiving D-serine 2 days in a row (e.g. D-serine in session 1 and 2 or sessions 2 and 3) as opposed to at least one placebo day led to a highly significant treatment effect (p < 0.001), with the D-serine leading to a 40% improvement in pitch-processing threshold, while placebo led to a 25% worsening. Moreover, a significant overall D-serine effect (p < 0.05) for both MMN to frequency deviants in theta and beta power/intertrial coherence was seen. A relationship between improvements in MMN, theta power and auditory plasticity were seen.

Conclusions: This study serves as a permissive first-step in utilizing D-serine in cognitive-emotion retraining, and are consistent with low dose D-cycloserine findings in cognitive-behavioral therapy.

Keywords: schizophrenia, cognitive remediation, mismatch negativity

Disclosures: Dr. Javitt holds intellectual property rights for use of NMDA modulators in treatment of neuropsychiatric disorders.

T153. Altered Ca2+ Channel Function in the Fast-spiking Interneurons of an Nmdar Hypofunction Mouse Model for Schizophrenia

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Background: Converging evidence suggests that cortical GABAergic interneurons are a prime target for NMDAR
to be small regardless of the genotypes. Next, we conducted whole cell patch clamp recording to confirm the down-regulation of Cav2.1 channels in GluN1-deleted FS neurons. The ω-agatoxin IVA-sensitive Ca2+ currents were diminished in the mutant FS neurons (n = 10) compared to the controls (n = 6), whereas compensatory increase in nimodipine-sensitive currents was observed in the mutants. Since Cav2.1 protein level in the mutant PV neuron somata was unchanged to that of control PV neurons, Cav2.1 channel function may be compromised by GluN1 deletion in PV neurons. To further assess which alteration, down-regulation of Cav2.1 channel or either up-regulation of L-type or potential R-type Ca2+ channel is crucial for impaired synchronized perisomatic inhibition of pyramidal cells, we conducted paired recordings from visually identified layer II/III pyramidal cells to assess the spontaneous IPSC event synchrony. Bath application of nimodipine (L-type Ca2+ channel blocker) does not rescue the spontaneous IPSC event synchrony deficit in the mutants. On the other hand, in vivo spike synchrony events of S1 cortex pyramidal cells were largely defective in a novel conditional KO mouse line where Cav2.1 channel ablation selectively targeted to ppp1r2-cre neurons positive for PV. Taken together, the data suggests that down-regulation of Cav2.1 channels in NMDAR-deleted PV neurons is crucial for impaired synchronized perisomatic inhibition, which may result in spike synchrony deficit in the mutant mice.

**Methods:** Medial prefrontal cortex slices were prepared from Ppp1r2-cre (+/-)/GluN1 (f/f) mutant/YFP (f/f) mice or their GluN1 (f/f) control littermates (male and females mixed, 4-6 weeks old). Pyramidal neurons in layers 2-3 were visually identified using infrared transmitted illumination in Olympus microscope. About a half of YFP-positive interneurons fired at high frequency with no accommodation (fast-spiking) in response to somatic current injection. We conducted whole cell voltage clamp recordings and synaptically connected pairs were identified during simultaneous dual whole cell recordings. Once the presence of a synaptic connection was established, single stimulus was applied every 10-20 s to assess the short-term inhibitory synaptic dynamics. Since the abnormal Ca2+ channel function was suspected in the mutants, the evoked IPSP amplitudes were recorded from mPFC layer 2-3 pyramidal neurons to pharmacologically identify the voltage-gated Ca2+ channels-mediating GABA release from FS neurons. To block the presynaptic voltage-dependent Ca2+ channels, nimodipine (L-type blocker), peptide toxins ω-conotoxin-GVIA (N-type blocker), ω-conotoxin-MVIIC (N- and P/Q-type blocker) and ω-agatoxin-IVA (P/Q-type blocker) were administered by bath application. Paired recordings were also made from visually identified layer II/III pyramidal cells to assess the spontaneous IPSC event synchrony. Data are given as mean ± SEM. Statistical comparisons have been performed with the unpaired Student’s t-test, unless otherwise noted.

**Results:** In the control mice (n = 7), the IPSP amplitude declined to near 0 mV after application of ω-conotoxin MVIIC (1 μM), but not after ω-conotoxin GVIA (1 μM) or nimodipine (1 μM). This suggested an involvement of Cav2.1 (P/Q-type) channels in the GABA release at the FS neuron to pyramidal cell synapses. In the mutant mice (n = 7), this Cav2.1 component largely disappeared whereas the nimodipine-sensitive component (L-type) and remaining component (perhaps R-type) insensitive to ω-conotoxin-GVIA or ω-conotoxin-MVIIC were augmented. Contribution of Cav2.2 (N-type) channel appeared

**Conclusions:** NMDA receptor ablation in cortical fast-spiking neurons leads to a down-regulation of P/Q-type Ca2+ (Cav2.1) channels, which may result in impaired synchronized perisomatic inhibition of pyramidal neurons in murine medial PFC.

**Keywords:** NMDA Receptor, schizophrenia, GABAergic interneurons

**Disclosures:** Nothing to disclose.

**T154. Self-assessment of Social Cognitive Functioning in Patients with Schizophrenia vs Healthy Controls**

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**Background:** Patients with severe mental illnesses manifest substantial deficits self-assessment, which has been shown to impact on everyday functioning. In our recent research we have shown that mis-estimation of an individual’s level of cognitive impairment has an impact on everyday functioning that is at least as great as the cognitive impairments themselves. In this study, we expand these efforts to self assessment of social cognitive functioning, comparing people with schizophrenia to healthy individuals on both their social cognitive performance and their assessment of that performance.

**Methods:** Patients with schizophrenia (n = 55) and healthy controls (N = 35) were examined with the Bell-Lysaker Emotion Recognition Test (BLERT). The BLERT is a computerized assessment of emotion recognition with 21 items; this test was recently found to have excellent psychometric characteristics. Participants were asked after they completed each item to rate their confidence in their correctness on a 0-100 scale. Dependent variables included
comparisons of performance on the test, confidence in performance, and time to response for each item.

**Results:** Patients with schizophrenia performed more poorly on the BLERT than healthy controls (HC), as expected. Both patients and HC were more confident on items that they correctly answered than for items with errors, with patients being less confident overall. When items were examined in terms of their difficulty for HC, the HC responded more rapidly to easy items (p < .05) and were more confident in their responses (P < .001). In contrast, patients responded at the same speed to hard and easy items and were no more confident for easy items than hard ones. In fact, for patients there was an extremely high correlation (r² = .64) between confidence for easy and hard items while this correlation was much lower in HC (r² = .17); similar results were found for response times to hard vs. easy items in patients (r² = .64) vs controls (r² = .44).

**Conclusions:** Schizophrenia patients appear to have difficulty judging the level of difficulty of social-cognitive tests and had difficulty adjusting their efforts accordingly. These data suggest impairments in assessing situational demands and appear to be consistent with previous reports of impairments in self-assessment of cognitive functioning and functional skills. These results are convergent with recent research suggesting that schizophrenia patients fail to adjust their effort in response to variations in rewards, suggesting that self-assessment may be interacting with reward sensitivity in order to produce performance that fails to adapt to situational demands.

**Keywords:** schizophrenia, social cognition, neurocognition

**Disclosures:** Philip D. Harvey has served as a consultant to: Abbvie; Boehringer Ingelheim, Forum Pharma; Genentech; Lundbeck Pharma; Otsuka America, Roche Pharma, Sanofi, Sunovion, and Takeda Pharma in the past 3 years.

**T155. Progressive Reduction of Auditory Evoked Gamma in First Episode Schizophrenia but Not Clinical High Risk Individuals**

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**Background:** The early auditory-evoked gamma oscillation (AGO) may serve as an index of the integrity of fast recurrent inhibition and/or synaptic connectivity in the auditory cortex, where abnormalities in individuals with schizophrenia have been consistently found. The AGO has been rarely investigated in first episode schizophrenia patients (FESZ) and individuals at clinical high risk (CHR) for schizophrenia, and never been compared directly between these populations nor evaluated longitudinally. Here we examined the AGO in FESZ, CHR, and matched healthy controls (HC) at Baseline and 1-year Followup assessments to determine whether the AGO was affected in these clinical groups, and whether any AGO abnormalities changed over time.

**Methods:** The electroencephalogram was recorded with a dense electrode array while subjects (18 FESZ, 18 CHR, and 40 HC) performed an auditory oddball task. Event-related spectral measures (phase locking factor [PLF] and evoked power) were computed on Morlet wavelet-transformed single epochs from the standard trials.

**Results:** The average reference method enabled us to dissociate the AGO into frontal and temporal subcomponents. FESZ showed a progressive reduction of fronto-central AGO PLF from Baseline to Followup compared to HC. AGO evoked power at temporal electrodes in FESZ was significantly increased relative to HC at Baseline, but reduced relative to HC at Followup. No effects were found on fronto-central AGO evoked power. AGO measures did not differ between CHR and HC, nor between CHR and FESZ. Longitudinal effects on the AGO were not found in CHR or HC.

**Conclusions:** The frontal and temporal components of the AGO were differentially affected in the early course of schizophrenia. These patterns may reflect the abnormal maturation of gamma generating circuits in frontal and temporal cortical areas, possibly as a result of changes in glutamatergic and/or GABAergic receptor systems. FESZ showed changes in gamma generation during a one-year period, while no changes were observed in a sample of CHR who did not convert to psychosis.

**Keywords:** gamma oscillation, first-episode schizophrenia, clinical high risk, EEG

**Disclosures:** Nothing to disclose.

**T156. Intact Sensitivity to External Performance Feedback in Schizophrenia: Electrophysiological and Temporal Stability**

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**Background:** Schizophrenia is a debilitating disorder characterized by functional impairment and diminished engagement in motivated behavior (Barch & Dowd, 2010; Blanchard et al., 2011). Accurately monitoring and integrating internal and external performance feedback is necessary for guiding motivated behavior (e.g., Holroyd & Coles, 2002). These two aspects of the performance-monitoring system have been investigated through event-related potential (ERP) measures in healthy controls. Research in schizophrenia has shown robust impairments in internal feedback monitoring as indexed by the error related negativity (ERN), but few studies have investigated external performance feedback in schizophrenia. The feedback negativity (FN) indexes sensitivity to external performance feedback and is thought to reflect a generic error monitoring system (Miltner et al., 1997). Specifically, the FN is a negative deflection in the ERP that differentiates feedback indicating favorable (e.g., monetary gain, correct feedback) from unfavorable outcomes (e.g., monetary loss, error feedback). The few studies comparing patients with schizophrenia and healthy controls on external feedback
processing have indicated comparable FN differentiation between reward and non-reward feedback using a gambling task and a time estimation task (Horan et al., 2012; Morris et al., 2011). The goal of the current study was to replicate these findings in a larger sample, determine whether the FN was related to individual differences in negative symptoms and motivation, and establish the temporal stability of the FN.

**Methods:** In our study, the FN was examined using a time estimation task in 74 outpatients with schizophrenia and 55 healthy controls. This study also assessed the test-retest reliability of the FN for 63 patients over a 4-week retest interval. In the time estimation task (modified from Miltenor et al., 1997), participants estimated a 1-s interval (via button press) after an auditory tone and they received feedback about their performance in the form of a happy face (positive feedback condition) or a sad face (negative feedback condition). In order to maintain an approximate success rate of 50%, the 1-s window was dynamically adjusted throughout the task for each participant. Further, the Positive and Negative Syndrome Scale (Kay, 1987) and the Clinical Assessment Interview for Negative Symptoms (Krulger et al., 2013) were used to assess negative symptoms and motivation, and the Role Functioning Scale (Mapers, 1984) assessed community functioning in patients.

**Results:** Results indicated no significant differences between patients and controls on positive and negative feedback, but both groups received more negative compared to positive feedback. The FN was significantly more negative for the negative feedback condition than for the positive feedback condition across groups. The overall mean of the FN amplitude was generally smaller in patients than controls across conditions; however the FN difference wave (the difference between negative and positive feedback conditions) did not significantly differ between groups, indicating comparable levels of neural sensitivity to external feedback. Among patients, the FN difference wave was not significantly associated with negative symptoms, motivation, or community functioning. Analyses with patients indicated statistically acceptable test-retest reliability for grand mean FN amplitudes, $r = .66$ for the positive feedback condition and $r = .76$ for the negative feedback condition. Test-retest reliability for the difference wave was smaller, as expected, but also significant, $r = .30$.

**Conclusions:** The results of our study converge with prior findings to suggest that external performance feedback monitoring, as indexed by FN, is an intact aspect of reward processing in schizophrenia. Together with other findings indicating deficient internal feedback processing as indexed by the ERN (Horan et al., 2012; Mathalon et al., 2009, Morris et al., 2011), our findings help clarify components of error monitoring and reward processing that are differentially impaired and intact in schizophrenia. Furthermore, this is the first study to evaluate the test-retest reliability of external performance feedback monitoring as indexed by the FN in patients.

**Keywords:** ERP, Motivation, Schizophrenia, Feedback negativity

**Disclosures:** Nothing to disclose.
measure. In the treated patients, chlorpromazine equivalents (CPZEs) were not related to choline/Cre or choline/water levels. Also, the 17 patients studied off all meds and again on a single antipsychotic no change in choline levels was detected.

**Conclusions:** We confirm the moderate elevation of choline levels in the anterior cingulate of medicated patients with psychosis observed by Bustillo et al. (2014), with very similar effect size. Choline levels in the dorsal anterior cingulate were elevated primarily in patients undergoing treatment with several medications. A smaller elevation was seen for Cho/water in the untreated group compared to controls. Unaffected siblings of patients with psychosis showed no difference from controls. Inferences about unaffected siblings and untreated patients remain limited due to their small sample size. Further evidence available from studies on versus off a single antipsychotic and the lack of relationship to CPZEs in the medicated group, do not support the hypothesis that these findings were driven primarily by antipsychotic treatment. This is consistent with MRS studies of neurexopteramy in rodents, which have shown no effect on choline levels (Bustillo et al. 2006). There was a trend for anticholinergic medications to increase Cho/Cre ($p < 0.06$) and Cho/water ($p < 0.1$), but the complexity of treatment (only 28% of the sample were treated exclusively with an antipsychotic) in the medicated patients precluded further interpretation of this finding.

**Keywords:** schizophrenia, choline, magnetic resonance spectroscopy, genetics, Antipsychotics

**Disclosures:** Nothing to disclose.

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**T158. Translational Optogenetic Modeling of Spindle Deficit in Schizophrenia**

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**Background:** One of the few, perhaps only, consistently replicated sleep abnormality in schizophrenia (Sz) is a deficit in sleep spindles, which are waxing and waning EEG events occurring during light NREM sleep. Spindle abnormalities are not present in bipolar patients taking neuroleptics, but are present in medication-naive early course Sz patients and the first degree relatives of Sz patients. This spindle deficit in schizophrenia is associated with impaired sleep-dependent memory consolidation. However, since clinical research cannot define the neural circuitry associated with spindles and accurately model the potential sources of the abnormality and hence treatments, we have embarked on a systematic effort to model in mice the genesis of spindle abnormalities.

We hypothesize spindle deficits result from reduced activity of thalamic reticular nucleus (TRN) GABAergic neurons containing the calcium-binding protein parvalbumin (PV neurons), neurons for which converging evidence is highly suggestive of their potential role in Sz spindle abnormalities. Postmortem studies have shown cortical GABA/PV neurons are abnormal in Sz and in the methylazoxymethanol (MAM) model of Sz which also shows spindle abnormalities. Cav3.3 calcium channels are responsible for the burst firing of TRN PV neurons needed for spindle generation; they have been shown to be are the major calcium channel subtype responsible for spindles. Importantly, the most recent major GWAS study (Nature 2014) reported association of the gene encoding the alpha subunit of Cav3.3 channels with Sz. Here, we use optogenetic techniques that permit selective excitation or inhibition of TRN PV neurons to test the role of TRN PV neurons in control of sleep spindles. Using optical inhibition we test the effect of reducing activity of TRN PV neurons on spindles and memory consolidation in the novel object recognition (NOR) and object place recognition (OPR) tasks, providing a link between changes in spindle density and memory consolidation performance.

**Methods:** Experiments were performed in mice that express Cre recombinase in PV neurons (PV-Cre). The selective activation or inhibition of TRN PV neurons was performed using channelrhodopsin2 (ChR2) or archaeorhodopsin (ArchT), respectively. Adeno-associated virus (AAV)-ChR2-EYFP or AAV-ArchT-GFP were bilaterally injected into TRN in PV-Cre mice, implanted with optical fibers targeting TRN, and instrumented to record sleep. Histology confirmed transduction of TRN PV neurons. Blue 473 nM laser light was applied at least three weeks after injection of AAV for optogenetic excitation or green 532nM light illumination for optogenetic inhibition. To assess memory, mice were tested with the NOR and OPR tasks which consist of two phases; the familiarization phase and the recall phase for the novel object or the novel placement. These tasks are based on mice’s natural tendency to investigate a novel object or a new placement of an object. To decrease spindles, inhibition of TRN PV neurons was performed via laser illumination (1-min on, 4-min off) during the 4-hr retention interval between the familiarization and recall phases, which is commonly accepted as a memory consolidation period. Memory performance and spindles were compared between inhibition (laser on) and control (no laser) conditions. All animal studies complied with VA, HMS and NIH guidelines.

**Results:** ChR2 excitation of PV TRN neurons at 10Hz ($n = 4$) consistently produced cortical EEG spindles when the mouse was in NREM sleep. Concomitantly, there was an increase (~30%) in NREM sleep ($n = 4$) while wake decreased. ArchT inhibition of TRN PV neurons consistently blocked ongoing spontaneous trains of spindles for 4s ($p < 0.0001$; vs. no laser); and increased wake time (30%) ($n = 5$). ArchT inhibition of TRN PV neurons also enhanced the cortical response to 40 Hz auditory stimulation, suggesting an enhancement of sensory transmission. In the memory consolidation NOR task, ArchT inhibition of histologically localized TRN PV neurons in the 4h between familiarization and recall produced less novel object preference (49%, near chance 50%) vs. control (laser off, 90% novel object preference; $p = 0.01$). Moreover, spindle density in the 4h memory consolidation period was correlated with NOR performance, approaching significance even in this small group of $N = 4$ ($rho = 0.68, p = 0.06$). In the OPR task, ArchT inhibition of TRN PV neurons in the 4h between familiarization and recall produced less preference for the novel place (48%) vs. control (laser off,
77%, \( p < 0.05 \)). Spindle density in the 4h memory consolidation period was correlated with OPR performance, approaching significance in this group of \( N = 3 \) (\( \rho = 0.77, p = 0.07 \)).

**Conclusions:** Our data indicate that TRN PV neurons generate cortical spindles and influence NREM sleep. ArchT inhibition of TRN PV neurons impairs spindle-associated sleep-dependent memory in both NOR and OPR tasks. Overall we believe these findings strongly support our mouse model of reduced activity of TRN PV neurons as being responsible for Sz spindle abnormalities and associated memory deficits. These mechanistic insights are ultimately important for understanding the underlying neural circuitry in order to develop a targeted approach to correct sleep abnormalities of spindle and associated memory consolidation deficits in Sz.

**Keywords:** optogenetics, schizophrenia, mouse model, parvalbumin interneurons, sleep spindles

**Disclosures:** Nothing to disclose. Supported by: VA Merit (RWM) and CDA (JTM); NIMH MH039683-29 (RWM).

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**T159. Genetic Variation in GRM5 is Associated with Cognition, Hippocampal Volume and Schizophrenia**

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**Background:** GRM5 is the gene encoding for the metabotropic glutamate receptor type 5 (mGluR5), a postsynaptic G-protein coupled receptor involved in modulation of glutamate signaling. mGluR5 is implicated in hippocampal-dependent cognitive functions that are disrupted in schizophrenia, and we recently provided evidence of aberrant expression of mGluR5 protein in the postmortem schizophrenia hippocampus. Here, we set out to investigate the association between allelic and genotypic frequencies of two novel single nucleotide polymorphisms (SNPs) in GRM5 with schizophrenia, as well as the effects of these genetic variants on cognitive function and hippocampal volume in a well-selected schizophrenia case-control cohort.

**Methods:** rs60954128 \([C>T] \) and rs3824927 \([G>T] \) were genotyped by Sequenom MassARRAY in 268 schizophrenia and 268 control Caucasian individuals, using DNA from the Australian Schizophrenia Research Bank (ASRB). All participants completed a standard set of neuropsychological tests, and 78 controls/103 cases had high-resolution T1-weighted anatomical scans available (MPRAGE, Siemens Avanto 1.5 Tesla). Scans were processed and hippocampal volumes extracted using Freesurfer v5.1. Chi-squared analyses were used to determine associations between allelic/genotypic frequencies and schizophrenia. The effects of diagnosis, genotype, and their interactive effects were examined for each cognitive measure and hippocampal volume using mixed design MANCOVA.

**Results:** Males with schizophrenia were more likely to carry minor alleles for rs60954128 than male controls (\( p = 0.011 \)). Genetic variants for this SNP also affected intelligence and delayed memory in males, and working memory in females with schizophrenia (\( p < 0.042 \)). rs60954128 variants significantly interacted with diagnosis in schizophrenia males affecting hippocampal volumes, with carriers of the minor allele showing significantly reduced right hippocampal volume relative to major allele homozygotes and controls (\( p = 0.013 \)). For rs3824927, schizophrenia males carrying the minor allele showed reduced intelligence, working and delayed memory scores (\( p < 0.025 \)), although allelic frequencies for this SNP were not different in schizophrenia compared to controls.

**Conclusions:** These findings converge with previous reports from animal models and postmortem brain studies to implicate GRM5 variants in the cognitive symptoms seen in patients with schizophrenia, with these effects potentially mediated by anatomical integrity of the hippocampus, and possibly occurring in a sex-specific manner. Further studies are now required to determine the functional and molecular consequences of these genetic variants, and the molecular pathways by which they operate to impact on hippocampal integrity, cognitive function and the genetic vulnerability to schizophrenia.

**Keywords:** metabotropic glutamate receptor, schizophrenia, fMRI/imaging genetics, Cognition

**Disclosures:** Nothing to disclose.

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**T160. Deficiency of Neurogranin, a Susceptibility Gene for Schizophrenia, Confers Multiple Molecular and Behavioral Phenotypes Related to Schizophrenia**

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**Background:** Large-scale genome-wide association studies have identified risk variants for schizophrenia in the gene encoding neurogranin (N RG N; Stefansson et al., Nature, 2009; Schizophrenia Working Group of the Psychiatric Genomics Consortium, Nature, 2014). Neurogranin is a neuron-specific calmodulin binding protein abundantly expressed in brain regions implicated in schizophrenia pathophysiology, such as the hippocampus and cortex. Nrgn knockout (KO) mice exhibit aberrant behavioral phenotypes involving deficits in cognitive functions and abnormal emotional behaviors (Pak et al., PNAS, 2000; Miyakawa et al., Hippocampus, 2001; Huang et al., J. Neurosci., 2006). In this study, we sought to determine whether Nrgn KO mice exhibit additional behavioral abnormalities, in particular those relevant to schizophrenia. We also investigated whether the mutant mice have schizophrenia-related molecular alterations in the brain, including decreased expression of parvalbumin (PV), a marker of fast-spiking interneurons, activation of glial cells, and pseudo-immaturity of granule cells in the dentate gyrus (DG).

**Methods:** We subjected adult (>20 weeks old) Nrgn KO mice to a comprehensive battery of behavioral tests. These tests cover various behavioral domains such as learning and memory, sensory-motor function, and emotion. Using immunohistochemical methods, we examined expressions of PV, GFAP and Iba1 (markers for astrocytes and...
microglia, respectively), and maturation markers of granule cells in the hippocampus and frontal cortex of 1-month-old adult mice. We measured genome-wide gene expression levels in the DG of these mutant mice by mRNA sequencing (RNA-seq; >20 weeks old). Quantitative RT-PCR analyses were performed to examine maturity of DG granule cells of young (<10 weeks old) and older (>20 weeks old) adult mutant mice.

**Results:** Nrgn KO mice exhibited hyperactivity in the open field test, abnormal anxiety-like behavior, decreased immobility in the porsolt forced swim test, decreased social behavior, impaired working memory in the T-maze spontaneous alteration task and decreased prepulse inhibition, demonstrating that these mutant mice display a series of schizophrenia-related behavioral abnormalities. Histological analyses revealed decreased number of cells expressing PV in the hippocampus and frontal cortex of these mutant mice, which is also observed in the brain of patients with schizophrenia. Regarding glial activation, in the hippocampus of the mutant mice, we found increased number of Iba1-expressing cells, and confirmed increased number of GFAP-expressing cells (Huang and Huang, Genes Brain Behav., 2012), which suggest inflammatory conditions in the brain region. Furthermore, we found decreased expression of calbindin, a marker of mature granule cells, in the DG of the mutant mice, suggesting pseudo-immaturity of granule cells. RNA-seq analyses revealed that the gene expression patterns of the DG of the mutants are significantly similar to those of normal young mice and of several other strains of mutant mice showing “immature dentate gyrus” phenotype, such as zCaMKII heterozygous knockout mice, Schnurri-2 knock-out mice, and mutant SNAP-25 knockin mice. Interestingly, while mRNA expressions of immature and mature granule cell markers were not changed in almost any young (<10 weeks old) mutant mice, older (>20 weeks old) mutants showed increased expression of immature granule cell markers and decreased expression of mature granule cell markers, suggesting that mature granule cells are reversed to a pseudo-immature status as the mutants get older and that Nrgn KO mice might be an animal model recapitulating the fact that typical onset of schizophrenia occurs during late adolescence or early adulthood.

**Conclusions:** Nrgn KO mice exhibited a series of behavioral abnormalities that resemble those of schizophrenics, including hyper-locomotor activity and impairments in working memory, social behavior and sensorimotor gating. The mutant brain also demonstrated multiple schizophrenia-related molecular phenotypes, including signs of inflammation, decreased number of PV-expressing cells, and pseudo-immaturity of DG granule cells, all of which have been implicated in schizophrenia. These observations demonstrate that Nrgn KO mice represent a novel animal model of schizophrenia that has excellent face and concept validity. Relatively late onset of their phenotypes would occur during late adolescence or early adulthood.

**Disclosures:** Nothing to disclose.
Results: Consenting, clinically stable outpatients with SZ (DSM IV criteria) \( n = 286 \) were randomized to a single blind study into three arms YT \( n = 103 \), PE \( n = 90 \) and TAU \( n = 92 \). A total of 238 (83.5\%) completed the study \( \text{YT} = 86, \text{PE} = 75, \text{TAU} = 77 \). A mixed model repeated measures analytical method was used to test the results after 21 days, 3 and 6 months. Compared to TAU, significant cognitive improvements were observed with adjunctive YT or PE at all follow-up time-points for several cognitive domains; profiles differed for YT/PE and by time \( p < 0.05, \text{effect sizes} 0.26-0.60 \). YT had a sustaining effect in speed compared to accuracy in several cognitive domains.

Conclusions: Relatively short term, adjunctive YT, as well as PE can enhance cognition in patients with SZ, with differing patterns of improvement. The lack of improvement in the TAU group also suggests that the improvements noted with TAU and PE cannot be attributed solely to practice effects. Non-pharmacological treatment can benefit key aspects of SZ disability.

Keywords: schizophrenia, Cognition, Yoga

Disclosures: Nothing to disclose.

T163. DNA Sequencing in Multiplex Families with Schizophrenia and Affective Disorder

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Background: Much of the current focus in genetic studies of major psychiatric disorder is on multiple common risk alleles detected through very large GWAS analyses. Yet families do exist, albeit rare, that have multiple affected members who appear to have a similar inherited cause to their illnesses. In addition, the diagnoses within families seem to cross conventional psychiatric diagnostic boundaries. We hypothesize that within these families there may be rare highly penetrant mutations that segregate with illness within families, but that each family may have a different mutation that leads to a similar clinical presentation across families. If enough of these mutations are detected, they may converge on specific pathways to a common underlying neurobiological basis for illness. Thus, a new focus returning to multiplex families may lead to new progress.

Methods: Families with a minimum of 3 available affected relatives and 3 available unaffected relatives had blood samples collected. Thus far, 83 individuals from nine of the families had whole genome sequencing performed (2 x 150 BP, 20-40x median coverage). Variant calling and genotyping was performed after a series of data clean-up measures that included indel realignment and base recalibration. Basic quality control filters were applied to find rare variants that alter protein sequences and were transmitted within families with affected status. Only those variants present in all affected family members were examined.

Results: Within one family, 7 male siblings with schizophrenia or schizoaffective disorder each carried a novel private missense variant within the SHANK2 gene, which
has been associated with schizophrenia in recent population studies. The variant lies within the consensus SH3 protein-binding motif by which SHANK2 may interact with postsynaptic glutamate receptors. In a separate family, 3 male siblings with schizophrenia and 1 female sibling with schizoaffective disorder and their unaffected mother each carried a novel private predicted-damaging missense variant in the SMARCA1 gene on the X chromosome, a chromatin remodeling factor associated with differentiation of dopaminergic neurons. Compelling monogenic candidate variants were not identified within the 7 remaining families, indicating potential multigenic and/or regulatory contributions to the phenotype.

**Conclusions:** These results suggest that in some cases an individual missense variant may confer high risk for schizophrenia spectrum disorders. In addition, these initial data exemplify the importance of going back to the evaluation of high density affected families for uncovering private mutations within each family. Taken together these new uncovered mutations can lead to an understanding of the biological underpinnings of serious mental illness, as well as lead to potential new treatment targets.

**Keywords:** DNA sequencing, Multiplex families, schizophrenia, bipolar disorder, missence mutations

**Disclosures:** Amgen funded this project but did not influence the design or analysis.


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**Background:** Neurocognitive deficits in people with schizophrenia contribute to abnormalities in emotion regulation—a process by which people alter the intensity, onset, and expression of positive and negative emotions. Deficits in emotion regulation capacity may lead to aversive social behaviors such as reactive aggression when the emotional appraisal of stressful situations overwhelms cognitive control circuits in the prefrontal cortex and anterior cingulate regions. Executive function deficits have been shown to contribute to poor impulse control, problem solving, decision making, and other abilities necessary to cope with stressful situations. The current study examined if neurocognitive profiles can predict hostility, aggression incidents, and violent offending in people with schizophrenia. The study also examined if cognitive remediation can contribute to reductions in hostility and aggression in people with schizophrenia.

**Methods:** The study randomized 78 people with schizophrenia or schizoaffective disorder to receive cognitive remediation versus computer games control activities. Participants were receiving services at a state hospital and included 43 individuals classified as violent offenders. Participants were extensively characterized with regard to age, sex, ethnicity, education diagnoses, duration of hospitalization, criminogenic history, and other clinical characteristics. Study staff administered the following measures to participants at baseline: the Wechsler Abbreviated Scale of Intelligence (WASI-II); the MATRICS Consensus Cognitive Battery (MCCB) as measure of neurocognition; the Positive and Negative Syndrome Scale (PANSS) as a measure of psychopathology; and the Overt Aggression Scale (OAS) to access aggression incidents. The MCCB, PANSS, and the OAS were also completed at posttreatment following 50 hours of cognitive training or computer games activities. The cognitive remediation group completed auditory and visual training activities from the Posit Science Brain Fitness Program 2.0 and the Insight respectively. Control games were drawn from the Viva Media 101 Premium Games. First offenders and non-offenders were compared on neurocognition, excitement/agitation, and aggression incidents. Next, the cognitive remediation and control groups were compared on the outcome measures.

**Results:** Multivariate comparisons of participants at baseline by offender status revealed significant differences in neurocognition. The offender group demonstrated greater deficits in working memory and verbal learning relative to the non-offender group controlling for sex, IQ, and years of education. A binary logistic regression model prioritized working memory, verbal learning, and overall neurocognition as predictors of violent offender status. The three neurocognitive variables had 77.2% accuracy at classifying offenders and non-offenders in the sample. In the entire sample, working memory, verbal learning, and overall cognition had small to moderate associations with the PANSS excitement/agitation factor, and aggression incidents.

Cognitive remediation was associated with improvements in several neurocognitive domains. People assigned to the cognitive remediation group demonstrated greater reductions in the excitement/agitation factor of the PANSS.

**Conclusions:** Neurocognitive deficits contribute to the phenomenology of negative emotionality and aggression in people with schizophrenia. Cognitive remediation may enhance efforts at decreasing negative emotionality and reactive aggression in people with schizophrenia. Aspects of cognition including emotion recognition, theory of mind, and emotional awareness are even more proximal to emotion abnormalities and social behaviors. Thus targeted social cognitive training may provide even greater benefits on emotion regulation and reactive aggression.

**Keywords:** neurocognition, cognitive remediation, emotion regulation, schizophrenia

**Disclosures:** Nothing to disclose.
T165. Transcription Factor MEF2C, Associated with Neuronal Epigenome Alterations in Schizophrenia, Improves Cognition and Working Memory

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**Background:** Dysregulated neuronal gene expression in the prefrontal cortex (PFC) is a critical building block in the neurobiology of schizophrenia (SCZ). The underlying molecular pathology likely includes broad changes in the transcriptome and epigenome of PFC neurons, and is likely to involve many cis-regulatory elements at gene proximal promoters and distal enhancers, often at sites harboring common polymorphisms implicated in heritable liability for SCZ and directly affecting gene expression. Surprisingly, however, to date there is very little knowledge on the therapeutic potential of transcriptional regulators associated with chromatin aberrations in SCZ.

**Methods:** We compared, at base pair resolution on a genome-wide scale, open chromatin-associated histone methylation landscapes and transcription factor signatures in prefrontal cortex (PFC) neurons of subjects diagnosed with schizophrenia and controls.

**Results:** Hypermethylated nucleosomes in diseased neurons were enriched for myocyte-specific enhancer factor 2C, MEF2C, recognition sites, including promoters and broad stretches of enhancer chromatin ('super-enhancers') tethered into chromosomal loopings governing the regulation of MEF2C-sensitive genes critical for neuronal signaling. Localized MEF2C chromatin occupancies were linked in vivo to transcriptional regulation in adult PFC via methyl-adenine footprinting of neuronal nuclei. Furthermore, short-term (days) and long-term (weeks) neuronal Mef2c expression up-regulation in juvenile and adult mouse PFC enhanced cognitive performance at baseline and under a pharmacological challenge with NMDA receptor antagonist drugs.

**Conclusions:** MEF2C carries strong therapeutic potential for treating neuronal dysfunction and cognitive disorders.

**Keywords:** transcription factor, working memory, Epigenetics

**Disclosures:** Nothing to disclose.

T166. Conditioning Illusory Percepts: Testing a Predictive Coding Model of Hallucinations

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**Background:** Psychosis involves a disconnection from reality; patients perceive stimuli that did not occur and believe things that are often bizarre and patently false. These hallucinations and delusions have thus far defied explanation in terms of their phenomenology and underlying cognitive or neural mechanisms. In recent years much progress has been made toward an explanation of delusion formation and maintenance in terms of translational neuroscience and formal animal learning theory. Similar theories have been proposed to explain hallucinations, but empirical tests are lacking. Here we utilize a predictive coding framework for perception in which we learn and update our expectancies most from situations that confound our predictions, taking advantage of a long history of sensory conditioning research meant to elicit hallucinatory experiences via traditional learning mechanisms.

**Methods:** Four groups of subjects were recruited for participation in a functional imaging paradigm: 1) those who have a diagnosis of a psychotic disorder who experience hallucinations; 2) those who have no diagnosable psychotic disorder who experience hallucinations; 3) those who have a diagnosis of a psychotic disorder who do not experience hallucinations; and 4) those who do not have a diagnosis of a psychotic disorder and do not experience hallucinations. Each participant underwent full clinical interviews and hearing screening followed by a functional imaging session. During this session, participants performed a simple task meant to elicit perception of an auditory event without an accompanying physical stimulus via learning of auditory-visual event contingencies. To this end, first each individual’s threshold for detection of a faintly-presented auditory stimulus in white noise is determined and participants are then required to judge the presence or absence of said stimulus presented at threshold when paired with a salient visual stimulus. Functional imaging data were acquired during the performance of both of these tasks. Data analysis is focused first on the behavioral indicators of the illusory perception of an auditory event, including detection percentage, rate of learning, and confidence measures. Imaging analysis consists of initial processing of event-related BOLD changes associated with illusory auditory perception followed by effective connectivity analysis via dynamic causal modeling (DCM) aimed at uncovering top-down vs. bottom-up dynamics within an identified network of regions involved in expectancy and auditory perception.

**Results:** Results indicate an increased propensity to experience conditioned hallucinations in individuals who experience spontaneous hallucinations at baseline. Additionally, a very similar network to that shown to be active during symptom-capture approaches to spontaneous hallucinations appears to be active during conditioned hallucinations, and this network appears to be more active in individuals who experience spontaneous hallucinations.

**Conclusions:** Taken together, this unique methodological approach and these promising initial findings may form the basis for a measurable and reproducible biomarker for hallucinatory experiences both at illness onset and in response to treatment.

**Keywords:** Auditory hallucinations, psychosis, Predictive Models, functional neuroimaging

**Disclosures:** Nothing to disclose.
T167. Reduced CYFIP1 in iPSC Derived Human Neural Progenitors Results in Donor Specific Dysregulation of Schizophrenia and Epilepsy Genes

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Background: Deletions spanning a four gene region on Chromosome 15 (BP1-2 at 15q11.2) increase risk for schizophrenia and epilepsy, but only a subset of carriers have either disorder. Mechanisms remain unclear and existing models fail to capture key features of disease.

Methods: To investigate the role of CYFIP1, a gene within the BP1-2 region, we performed knockdown experiments in neural progenitor cells (NPCs) derived from human induced pluripotent stem cells (iPSCs). NPCs were prepared from iPSC lines from three unrelated BP1-2 copy number neutral donors, transduced with a CYFIP1-specific or control shRNA, and subjected to RNA sequencing. Gene ontology analyses were carried out on differentially expressed genes (DEGs) and qPCR, western blot, and immunofluorescence used to investigate functional outcomes. Overrepresentation of disease and disease-related pathway genes among DEGs was evaluated, and a machine learning algorithm used to identify DEGs showing unexpected similarity to established epilepsy genes. Finally, towards personalized treatment of deletion carriers, we tested the ability of a known anti-convulsant to increase levels of CYFIP1.

Results: Analysis of RNA-seq data determined that transcripts involved in cytoskeletal remodeling are downregulated in response to CYFIP1 knockdown in human NPCs. Consistent with these molecular findings, knockdown resulted in a reduction in WAVE1/2 protein levels, less polymerized actin, and cells with larger nuclei. FMRP targets and postsynaptic reduction in WAVE1/2 protein levels, less polymerized actin, with these molecular findings, knockdown resulted in a response to CYFIP1 knockdown in human NPCs. Consistent involved in cytoskeletal remodeling are downregulated in

Conclusions: We describe a new model system for study of BP1-2 deletion-related schizophrenia and epilepsy and demonstrate that disease-related biological signatures are evident prior to neuronal differentiation. We show that knockdown of CYFIP1 in NPCs results in consistent dysregulation of cytoskeleton remodeling genes, FMRP targets, PSD genes, and genes resembling those known to cause epilepsy. We also determine that CYFIP1 knockdown results in the dysregulation of known schizophrenia and epilepsy genes, but not genes implicated in other disorders. The presence and magnitude of these disease effects varied as a function of donor line, recapitulating the clinical heterogeneity seen in BP1-2 deletion carriers. Lastly, incubation of cells with a widely prescribed anti-convulsant increases levels of CYFIP1, raising the possibility of personalized treatment of deletion carriers.

Keywords: Induced pluripotent stem cells (iPSCs), 15q11.2, FMRP, in vitro cellular model

Disclosures: Nothing to disclose.

T168. Evaluating Cannabis Use on Cortical Inhibition Prior To - and following 28-day Abstinence Period in Patients with Schizophrenia: Preliminary Findings from a Tms-Eeg Study

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Background: Cannabis is the most commonly used illicit drug among patients with schizophrenia. Cannabis use is associated with symptom exacerbation and reduced function in this disorder. The effect of cannabis is associated with the downregulation of gamma-aminobutyric (GABA)ergic inhibitory activity mediating cortical inhibition — a neurophysiological mechanism whereby inter-neurons selectively inhibit the activity of other neurons. Using combined transcranial magnetic stimulation (TMS) and electroencephalography (EEG), dysfunctional cortical inhibition has been demonstrated in the dorsolateral prefrontal cortex (DLPFC) among patients with schizophrenia. Given that cannabis inhibits the release of GABA, it is therefore possible that cannabis use may further impair cortical inhibition in patients with co-morbid cannabis dependence. Moreover, it is possible that cannabis abstinence may improve such deficits at baseline among schizophrenia. The objective of this study was to index cortical inhibition from the motor and DLPFC using TMS-EEG in cannabis dependent patients with schizophrenia compared to non-psychiatric controls prior to- and following a 28-Day abstinence period.

Methods: In an on-going study, cortical inhibition from the left motor and DLPFC was indexed in 6 (mean age 28.5 ± 10.7 SD) patients with schizophrenia or schizoaffective disorder and 7 (mean age 32.1 ± 8.2 SD) non-psychiatric control males with cannabis dependence. TMS induced cortical evoked activity was indexed with concurrent EEG recording acquired with a 64-channel Synamps2 DC-coupled EEG system (Compumedics) recorded DC at 20 kHz sampling rate and 100 Hz low-pass filter. Cortical evoked activity was then Hilbert transformed and the ratio of the area under curve for the conditioned to the unconditioned curve was calculated for the electrodes under the site of stimulation for the motor and DLPFC prior to and following 28-Day abstinence period.

Results: Our preliminary findings demonstrate reduced cortical inhibition among patients with schizophrenia (n = 6) compared to non-psychiatric controls (n = 7) that was selective to the DLPFC (t = 3.566, df = 11, p = 0.004; Cohen’s d = 1.99), while no difference was found in the motor cortex at baseline (t = 0.849, df = 11, p = 0.414). Following a 28-Day biochemically verified abstinence period,
Disclosures: Nothing to disclose.

T169. Small Molecule Antagonists of the VPAC2 Receptor as a Novel Direction for Schizophrenia Therapeutics

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Background: Schizophrenia affects 1% of the population. Most therapeutics already in use target the dopamine system, have many off-target effects, primarily treat the positive symptoms of the disease and show collectively only a 15% success rate (Robinson et al., 2004). It is crucial, therefore, to improve our understanding of disease mechanisms and identify novel drug targets to provide improved therapies for the future. Rare, highly penetrant mutations such as Copy Number Variants (CNVs) play a major role in the susceptibility to neurodevelopmental disorders, cognitive dysfunction, and psychiatric illness. One of the most recurrent CNVs significantly associated with schizophrenia involves genomic gains in the Vasoactive intestinal peptide receptor 2 (VPAC2) genetic locus. Such gains were found in 0.3% of patients compared with 0.03% of controls (Levinson, 2011; Vacic et al., 2011; Yuan et al., 2014). The VPAC2 receptor is expressed in multiple brain regions, including those implicated in schizophrenia, and is a GPCR acting via adenylate cyclase to increase cAMP levels. Our goal was to develop novel compounds that act as inhibitors of the VPAC2 receptor.

Methods: In order to develop and test new antagonists for the VPAC2 receptor we developed an assay to screen for elevations in cAMP levels specifically activated by the VPAC2 receptor. We utilised the highly robust Bioluminescence Resonance Energy Transfer (BRET) technique to measure cAMP levels. In brief, the YFP-Epac-RLuc8 (CAMYEL) BRET sensor together with the VPAC2 receptor were introduced into CHO cells lines to detect cAMP elevation in response to VPAC2 receptor activation. We then generated stable cell lines expressing these two constructs to allow rapid and medium throughput drug testing. The compounds developed were based on compound C1 identified by Chu et al., (Chu, Caldwell, & Chen, 2010). To improve potency and central nervous system penetration we initiated several strategies for the generation of new compounds from C1 including, but not limited to, substitutions or removal of hydroxyl groups, substitutions at aromatic rings, or substitutions with isosteres. We then tested these new compounds using our BRET assay. Finally, we mutated targeted positions in the VPAC2 receptor to identify sites of the VPAC2 receptor/antagonist interaction.

Results: We successfully developed a cell-based assay for the medium throughput testing of compounds acting as inhibitors or activators of the VPAC2 receptor. We created and screened over 150 different compounds and through this process obtained new small molecules demonstrating improved affinity and specificity for the VPAC2 receptor compared with currently available antagonists. Our compounds are predicted to have enhanced blood brain barrier penetration based on reduced molecular weight, polarity, improved lipophilicity and increased stability. We further reveal pharmacological properties of their interaction demonstrating the affinity profiles and regions required for the interaction of the small molecules with the VPAC2 receptor.

Conclusions: A number of agonists and antagonists have been reported for the VIP receptor family though none show high specificity for the VPAC2 receptor. Furthermore available compounds have issues with stability and penetration of the blood brain barrier. Here we develop a new screening assay for VPAC2 receptor antagonists and show the successful generation and characterization of novel VPAC2 receptor antagonists. Furthermore, we demonstrate their interactions and address their use in a physiological setting. We hope in the future that these molecules will provide the skeleton or be applicable themselves to pre-clinical studies to expand potential therapeutics for this devastating illness.

Keywords: schizophrenia, novel therapeutics, Pharmacology

Disclosures: Nothing to disclose.

T170. Long-Term Cariprazine Treatment for the Prevention of Relapse in Patients with Schizophrenia: Additional Analysis from a Randomized, Double-Blind, Placebo-Controlled Trial

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Background: Cariprazine is a dopamine D3/D2 receptor partial agonist atypical antipsychotic, with preference for D3 receptors. Cariprazine has a unique pharmacokinetic profile, with 2 active metabolites, desmethyl cariprazine and desdemethyl cariprazine; the effective half-life for the total active moiety, which takes into account cariprazine and the 2 metabolites, is approximately 1 week. This long half-life may confer some additional protection against relapse in cases of nonadherence. Cariprazine has demonstrated efficacy in 6-week, randomized, placebo-controlled trials in patients with acute exacerbation of schizophrenia. This study was conducted to evaluate the efficacy, safety, and tolerability of cariprazine versus placebo in the prevention of relapse in patients with schizophrenia (NCT01412060).
Additional analyses were conducted to specifically explore the time to onset of relapse following discontinuation of cariprazine treatment in patients randomized to the placebo arm.

**Methods:** This was a multinational, randomized, double-blind, placebo-controlled, parallel-group study in adult patients with schizophrenia; the total study duration was up to 72 weeks. Schizophrenia symptoms were stabilized during 2 open-label phases: an 8-week, flexible-dose, run-in phase and a 12-week, fixed-dose, stabilization phase with cariprazine 3-9 mg/d. Patients who completed the 20-week open-label treatment phases were randomized to continue cariprazine (3, 6, or 9 mg/d) or switch to placebo for up to 72 weeks of double-blind treatment. The primary efficacy parameter was time to relapse, defined as worsening of symptom scores, psychiatric hospitalization, aggressive/violent behavior, or suicidal risk. Clinical measures were implemented to identify early signs of relapse and ensure patient safety in the event of relapse. Time to relapse between the placebo and cariprazine groups was compared using the log-rank test and hazard ratio (HR) with 95% confidence interval (CI); the cumulative distribution function of time to relapse was estimated by Kaplan-Meier curves. Additional efficacy parameters included change in Positive and Negative Syndrome Scale (PANSS) total score and Clinical Global Impression-Severity (CGI-S) score. Safety assessments included adverse event (AE) reports, clinical laboratory parameters, vital signs, and extrapyramidal symptom (EPS) scales.

**Results:** A total of 264/765 (35%) patients completed the open-label treatment phase; 200 met eligibility criteria and were randomized to double-blind placebo (n = 99) or cariprazine (n = 101) treatment. The time to relapse was significantly longer in patients who continued cariprazine than in patients who switched to placebo (P = .0010 [log-rank test]). Relapse occurred in nearly twice as many placebo-treated (47.5%) as cariprazine-treated patients (24.8%); the HR (95% CI) was 0.45 [0.28, 0.73]). The Kaplan-Meier analysis estimated that separation of the cariprazine and placebo curves started at almost 50 days. At week 2, few placebo- or cariprazine-treated patients had relapsed (two [2%] each group); at week 4, only 3 (3%) placebo-treated patients had relapsed despite discontinuing cariprazine treatment. The placebo group showed greater mean worsening in PANSS and CGI-S scores relative to the cariprazine group during double-blind treatment. The most common AEs (≥10%) during open-label treatment were akathisia (19.2%), insomnia (14.4%), and headache (12.0%). There were no treatment-emergent AEs that occurred at an incidence of ≥10% in the cariprazine group during the double-blind treatment phase.

**Conclusions:** Long-term cariprazine treatment demonstrated significantly greater efficacy than placebo for the prevention of relapse in patients with schizophrenia. The low relapse rate during the first few weeks of double-blind treatment in patients switched to placebo may suggest a sustained treatment effect for cariprazine that is related to the long effective half-life. The safety profile of cariprazine in this long-term study was comparable to the safety profile observed in the acute cariprazine studies.

**Keywords:** relapse prevention, schizophrenia, cariprazine
T172. fMRI of Aversive Face Conditioning in Clinical Risk and Schizophrenia

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Background: The amygdala and its circuitry are critical for social-emotional processing, particularly in the context of aversive learning. We hypothesize that in schizophrenia and those at clinical risk, amygdala dysfunction drives aberrant aversive social conditioning, contributing to negative symptoms and social deficits. Here we report the design and preliminary results of an ongoing 3T fMRI study testing this hypothesis using passive aversive conditioning of face stimuli. The study is part of an integrated translational Conte program that also includes a parallel human EEG study, human postmortem brain tissue and genetics, and rodent models.

Methods: fMRI participants (n = 47 analyzed to date) are youth or young adults in three groups: schizophrenia (SC, n = 17), clinical risk (CR, n = 13) and healthy control (HC, n = 17). During fMRI subjects performed aversive conditioning and reversal paradigms, as well as a resting state scan. Conditioned stimuli (CS) were two neutral male faces. The aversive unconditioned stimulus (US) was a loud scream, which accompanied one face (CS+) 50% of the time, but not the other face (CS-). The preliminary fMRI analyses reported here included voxelwise examination of differential conditioning as CS+ vs. CS- differences in the linear change of activation over time. In addition, we applied a model-based analysis to percent signal change extracted on a trial-by-trial basis from the amygdala ROI. This model-based analysis applied a Rescorla-Wagner model (RW) to fit CS-related value and US-related prediction errors to amygdala activation across conditioning and reversal.

In the RW model, the expected value of the CS is updated based on associated US outcomes according to the equation: Change in Value = Learning Rate x Prediction Error, where Prediction Error = Actual Outcome – Expected Outcome. Pupil dilation responses and changes in subjective ratings of CS also indexed conditioning. Negative and positive symptoms were assessed using the CAINS, SANS, SAPS, and SIPS, and anxiety was assessed using the STAI.

Results: Behavioral data indicated that psychosis was associated with impaired discrimination between CS+ and CS- during conditioning. HC subjective ratings of the CS+ face shifted more negatively after conditioning while ratings of CS- were unchanged. In contrast, CR and SC ratings shifted negatively for both CS+ and CS-. Across all subjects, a differential conditioning effect was observed in the left amygdala, driven by increasing activation over time in response to the CS+ but a decrease over time to the CS-. The striatum, frontal pole, and pre-genual cingulate showed a similar differential conditioning effect. The ventromedial prefrontal cortex showed a greater reduction in activation over time for the CS+ than the CS-, and this vmPFC effect was significantly stronger in HC than the combined clinical group (SC and CR; cluster-corrected p < 0.05). The RW model-based ROI analysis showed that amygdala activity correlated significantly with trial-by-trial variation in RW prediction errors (p < 0.001 across all subjects). Amygdala responses exhibited a lower model-derived learning rate in SC than HC, driven by the reversal phase; CR learning rate was intermediate. Learning rate was negatively correlated with symptom severity across SC and CR groups (SANS total: r = -.54, p < .01; CAINS total: r = -.46, p < .05).

Conclusions: These preliminary results provide initial support for aversive conditioning abnormalities in early and established psychosis. Behaviorally, SC and CR appear...
to overgeneralize aversive conditioning to “safe” stimuli, a finding we also observe in our EEG data from the same subjects. fMRI psychosis-related abnormalities in differential conditioning responses are found within the ventromedial prefrontal cortex, a region that is known to be strongly interconnected to the amygdala and regulate amygdala responses to emotional stimuli. Abnormal modulation of amygdala activation during aversive conditioning was seen in the model-based analysis, with greater abnormalities relating to negative symptom severity. As the sample increases we will have greater power to characterize group differences and dimensional symptom correlations, and will integrate fMRI analysis with pupillometry and EEG to provide a broad neurophysiological characterization of aversive conditioning abnormalities.

**Keywords:** schizophrenia, clinical high risk, Fear conditioning, fMRI, Amygdala

**Disclosures:** Nothing to disclose.

### T173. Effects of Past Moderate Cannabis Use on Cognition in First Episode Schizophrenia and Typically Developing Adolescents: A Multimodal Analysis of Brain Structure and Function

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**Background:** Cannabis (CN) use has been repeatedly linked to psychosis onset, however, the relationship of CN use to brain structure and function abnormalities in first episode schizophrenia (SZ) patients is less well understood. While CN use in healthy subjects is linked to detrimental effects on cognition and brain structure, there is evidence for higher performance in CN users with SZ on executive functioning measures. Further, the relationship between brain structure and CN use in this population is unclear. Therefore, we used multi-modal analyses to investigate the relationship between past CN use and measures of brain function, structure, and behavioral performance in SZ and healthy controls.

**Methods:** First episode SZ patients with a history of moderate CN use (n = 33) and patients with no history of use (n = 24) were identified from referrals to the UC Davis EDAPT clinic using the SCID-I. Healthy control participants with (n = 19) and without (n = 30) a history of cannabis use were also recruited. All participants urine-screened negative for CN on the date of scan and did not meet criteria for CN abuse or dependence. Images were obtained on a 1.5-T GE scanner and processed using Freesurfer 4.3 and SPM8. Cortical thickness and fMRI data were FWE cluster corrected (p < .05). The expectancy AX Continuous Performance Test (AX-CPT) was used as a measure of cognitive control and all volumetric structural analyses included intracranial volume, age, and gender as covariates.

**Results:** Analyses of behavioral data revealed higher performance on the AX-CPT in past schizophrenia CN users versus nonusers (d’-context and BX accuracy, p < .05). Additionally, schizophrenia CN users showed greater brain activity during the AX-CPT as well as thicker cortex in the parietal lobe. In terms of total cerebral cortex volume, past CN users showed significantly greater gray matter volume in both hemispheres with no significant differences in white matter volume. Finally, of the a priori subcortical regions examined, only the left accumbens was significantly larger in patients with past CN use compared to patients with no past CN use. The control subjects showed no significant effect of past CN use on any measure, although control subjects with past cannabis use showed comparable behavioral performance to past schizophrenia CN users.

**Conclusions:** These findings highlight the complex relationship between CN use and the structural, functional, and behavioral deficits repeatedly identified in SZ, particularly given the small effect of past CN use in the healthy control group. While patients with a history of moderate CN use showed better cognitive control performance, BOLD response, larger whole-brain gray matter volume, and larger nucleus accumbens, CN use may or may not be the primary causal factor. While it is possible that some cannabinoids may have procognitive or neuroprotective effects it is also possible that patients with premorbid CN use may represent a subgroup with better premorbid adjustment and potentially greater cognitive reserve. Additional research focusing on the effects of specific cannabinoids on brain structure and function in human subjects as well as in animal models systems are required to resolve these questions.

**Keywords:** cannabis use, first episode schizophrenia, fMRI, Structural MRI, cognitive control

**Disclosures:** Nothing to disclose.

### T174. Association of a Schizophrenia Risk Variant at the DRD2 Locus with Antipsychotic Treatment Response in First Episode Psychosis

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**Background:** Findings from the Psychiatric Genomics Consortium genome-wide association study (GWAS) showed that variation at the DRD2 locus is associated with schizophrenia risk. However, the functional significance of rs2514218, the top DRD2 single nucleotide polymorphism in the GWAS is unknown. Dopamine D2 receptor binding is a common mechanism of action for all antipsychotic drugs, and DRD2 variants were related to antipsychotic treatment response, larger whole-brain gray matter volume, and larger nucleus accumbens, CN use may or may not be the primary causal factor. While it is possible that some cannabinoids may have procognitive or neuroprotective effects it is also possible that patients with premorbid CN use may represent a subgroup with better premorbid adjustment and potentially greater cognitive reserve. Additional research focusing on the effects of specific cannabinoids on brain structure and function in human subjects as well as in animal models systems are required to resolve these questions.

**Methods:** The present study examined whether rs2514218 genotype could predict antipsychotic response, including efficacy and adverse events, in a cohort of patients with first episode of psychosis treated with either risperidone or aripiprazole for 12 weeks. Subjects were genotyped using the Illumina Infinium HumanOmniExpressExome array platform. After standard quality control, data from 100 subjects (49 randomly assigned to treatment with aripiprazole and 51 assigned to risperidone) was available for analysis. Subjects were assessed for psychotic symptomatology and medication-related adverse events weekly for 4 weeks, then biweekly for 8 weeks.
Results: Linear mixed model analysis revealed that the homozygotes for the risk (C) allele at rs2514218 had significantly greater reduction in positive symptoms during 12 weeks of treatment compared to the T allele carriers. In the aripiprazole group, C/C homozygotes also reported more akathisia than the T allele carriers, while in the risperidone group, male T allele carriers demonstrated greater prolactin elevations compared to male C/C homozygotes.

Conclusions: These findings suggest that the schizophrenia risk variant at the DRD2 locus (or another variant in close proximity) is associated with observable differences in response to treatments which reduce striatal dopamine signaling.

Keywords: pharmacogenetics, Antipsychotics, DRD2 locus

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T175. Cognitive Function in Individuals with Psychosis: Moderation by Adolescent Cannabis Use

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Background: In healthy individuals, there is convincing evidence that acute use of cannabinoids negatively impacts cognition with some evidence of residual cognitive deficits but of unclear duration. Studies of adolescent cannabis users, however, find that cognitive impairment is longer lasting, with a recent prospective study finding that adolescent-onset, but not adult-onset, cannabis use was associated with persistent cognitive deficits. Given this effect in the otherwise healthy brain, one might expect an incremental worsening of cognition in individuals with psychosis who have a history of cannabis use, particularly those with adolescent use. On the contrary, there is evidence suggesting that ACU in schizophrenia is associated with better performance on specific cognitive tasks. In this study, we sought to determine if the association between ACU and cognitive function extends beyond schizophrenia to other psychotic disorders. We compared neuropsychological performance of individuals with schizophrenia, schizoaffective disorder or bipolar I disorder with psychosis, grouped together as “Psychosis”, to Control participants. Cognitive function was tested using the BACS (Brief Assessment of Cognition in Schizophrenia) battery, a validated and reliable tool widely used in schizophrenia research. Psychosis and Control groups were each divided into three subgroups: cannabis non-users, adolescent-onset cannabis use before age 18 (ACU), and later-onset cannabis use commencing at or after age 18. We tested the hypothesis that Psychosis with ACU group would show better cognitive performance compared to Psychosis non-users. Next, we tested this hypothesis in schizophrenia/schizoaffective and bipolar I with psychosis groups separately to determine if adolescent cannabis use had similar or different effects on cognitive performance between the two psychosis groups.

Methods: Participants were recruited at the Dallas site of the Bipolar-Schizophrenia Network on Intermediate Phenotypes (BSNIP) consortium. Participants included 97 volunteers with psychosis and 64 controls. Based on cannabis use information obtained in a semi-structured manner, participants were grouped into non-users, adolescent cannabis users (initiated before age 18) and late cannabis users (initiated at or after age 18). Global neuropsychological performance as measured by the Brief Assessment of Cognition in Schizophrenia (BACS) neuropsychological battery. The a priori hypothesis tested was that individuals with psychosis and a history of adolescent cannabis use will have better cognitive performance compared to those with psychosis and no cannabis use history.

Results: BACS composite scores were significantly higher in individuals with psychosis who have a history of adolescent cannabis use compared to individuals with psychosis and no prior cannabis use. Post hoc analyses reveal that this effect was driven by schizophrenia/schizoaffective disorder groups and not those with bipolar I disorder with psychosis. Exploratory analyses suggest that, within the schizophrenia/schizoaffective disorder group, adolescent cannabis users demonstrate better performance in specific domains, namely executive function and verbal memory, compared to other cases in the schizophrenia/schizoaffective groups. Adolescent cannabis use did not influence cognitive function within the bipolar I with psychosis group.

Conclusions: Adolescent cannabis use is associated with the relative sparing of cognition in individuals with schizophrenia/schizoaffective disorder compared to affected individuals without a cannabis use history. Adolescent cannabis use is not, however, associated with cognitive function in the bipolar psychosis group. There are no treatments for cognitive deficits available to individuals with psychotic illnesses and these findings suggest that a history of adolescent cannabis use may need to be factored in the design of clinical trials of cognitive enhancers in schizophrenia/schizoaffective disorder.

Keywords: neurocognition, cannabis use, schizophrenia, moderators

Disclosures: Nothing to disclose.
T176. White Matter Abnormalities Associated with Psychotic-Like Experiences Predict Later Social Competency in Children and Adolescents

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Background: Although often defined as a dichotomous characteristic for clinical purposes, several independent lines of evidence suggest that psychosis exists on a continuum and can be examined in general population samples. Indeed, population-based epidemiological studies have found that psychotic-like experiences (PLE’s) are quite prevalent, peaking in late childhood and adolescence, with rates as high as 40-60%, before decreasing to an adult prevalence of ~ 7%. The high prevalence of PLE’s in general population samples provides unique opportunities to examine hypotheses about the developmental trajectory and pathophysiology of psychotic disorders such as schizophrenia. Data from our group and others have identified both continuities and discontinuities in clinical, cognitive and imaging markers between otherwise healthy individuals who exhibit PLE’s and patients with psychotic disorders. To date, however, few studies have examined whether these markers may be useful in predicting longitudinal outcome. Thus, the present study examined whether abnormalities in white matter (WM) associated with PLE’s in children and adolescents at a baseline assessment, are predictive of social functioning at a 12-month follow-up.

Methods: Healthy children and adolescents ages 8-18 (N = 57) were recruited from the community and received a diffusion tensor imaging (DTI) exam on a GE Signa HDx 3.0 T system and comprehensive clinical exams at baseline and 12-months post-scan. Voxel-wise statistical analysis of fractional anisotropy (FA) using tract-based spatial statistics and probabilistic tractography were initially used to identify WM abnormalities associated with PLE’s at baseline. These abnormalities were then examined for association to social competency and social problems, as assessed with the Childhood Behavior Checklist, at a 12-month follow-up.

Results: Reduced FA in regions proximal to the right corticospinal tract, left inferior fronto-occipital fasciculus and bilateral superior longitudinal fasciculus (SLF) were significantly associated with high levels of PLE’s at baseline (all FWE corrected p values < .05). Moreover, baseline FA in the SLF, but not the severity of PLE’s at baseline, was significantly predictive of social competency at a 12 month follow-up, accounting for 24% of the variance. In contrast, severity of PLE’s at baseline, but not FA at baseline, predicted social problems at 12-month follow-up, accounting for ~40% of the variance.

Conclusions: These findings suggest that alterations in WM integrity, which are associated with symptoms of psychosis that are below the threshold of clinical significance, have substantial ramifications for later development. Moreover, these findings suggest that imaging markers, when combined with clinical indicators of risk, may be useful in efforts aimed at early identification and intervention. Finally, the present data add to a growing evidence base suggesting that the examination of PLE’s in non-help seeking samples may provide novel insights into the neurodevelopmental origins of psychotic disorders such as schizophrenia.

Keywords: Subclinical psychosis, DTI, Social Competence

Disclosures: Nothing to disclose.

T177. Effects of the Antioxidant N-Acetyl Cysteine on Behavioral and Neurophysiological Deficits Induced by Developmental NMDA-R Antagonism and Their Relationship to Mitochondrial Dysfunction

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Background: The NMDA-hypofunction theory of schizophrenia suggests that schizophrenia is associated with a loss of NMDA receptors, specifically on corticobasal parvalbumin (PV)-expressing GABAergic interneurons, leading to disinhibition of pyramidal cells and cortical desynchronization. We recently characterized the behavioral and physiological effects of a developmental NMDAR antagonism model in which mice receive ketamine (KET) injections on postnatal days (PND) 7, 9 and 11 (Jeevakumar and Kroener, Cereb. Cortex, 2014; Jeevakumar et al., Behav. Brain Res. 2015). We found that sub-chronic developmental KET treatment results in a loss of PV expression in the medial prefrontal cortex (mPFC); reduced cognitive flexibility, changes in the excitation-inhibition balance as measured by recordings of spontaneous and miniature EPSCs and IPSCs, respectively, and an unexpected homeostatic upregulation of NMDAR responses in PV expressing interneurons in layer 5. The loss of PV expression following subchronic NMDA antagonism appears to be the result of oxidative stress. Here we investigated whether the antioxidant N-acetyl cysteine (NAC) can prevent the KET-induced loss of PV expression, as well as the resultant behavioral and physiological changes, and how these changes are related to mitochondrial dysfunction.

Methods: Groups of ketamine- and saline-treated transgenic PV GFP+ animals (G42 line) were given subcutaneous injections of either NAC or saline between PND 5-21. After weaning, animals in the NAC treatment groups continued drinking NAC in their drinking water through adulthood. We then characterized immunohistochemical, behavioral and electrophysiological changes in adult (>90 PND) animals. Animals were trained in an attentional set-shifting task that required them to switch from an egocentric response strategy to a cue-based ( allocentric) response strategy to navigate a t-maze to obtain food rewards. Sections of the mPFC were stained for PV to assess changes in the number and distribution of fast-spiking GABAergic interneurons. We also used whole cell patch-clamp recordings in layers 2/3 from acute slices of the mPFC to analyze changes in synaptic transmission at pyramidal neurons and GFP+ interneurons. Finally, we examined the effect of NAC treatment on KET-induced mitochondrial dysfunction. Mitochondrial membrane potential and reactive oxygen species (ROS) levels were measured in brain slices from...
saline- or KET-treated mice with or without NAC pre-treatment by using staining with tetramethylrhodamine, methyl ester (TMRM) and MitoSox Red, respectively. To determine the influence on mitochondrial dynamics and motility, we used confocal imaging in primary cultured cortical neurons and exposed cultured neurons to 0.1 μM ketamine for 24 hours in the presence or absence of various concentrations (100 to 500 μM) of NAC. In addition to the staining of TMRM and MitoSox Red to determine mitochondrial membrane potential and ROS, neuronal mitochondria were visualized by Mitotracker Red. The length and density of neuritic mitochondria were then assayed to reflect the status of neuritic mitochondrial dynamics under the various treatment conditions.

**Results:** In adult NAC-treated animals PV expression in the mPFC remained at control levels, whereas the numbers of PV+ cells in animals that received saline injections (and no NAC in their drinking water) after KET treatment sharply declined. NAC also significantly improved performance on the attentional set-shifting task in KET-treated animals. In agreement with our previous findings, developmental KET treatment decreased the frequency of both spontaneous and action-potential independent miniature IPSCs at layer 2/3 pyramidal cells, suggesting long-lasting changes in GABAergic inhibition. The reduction in IPSCs onto pyramidal cells in KET-treated mice was mirrored by an increase in spontaneous excitatory postsynaptic currents (sEPSCs) onto PV+ interneurons in the same layer, further suggesting persistent cortical disinhibition. All of these KET-induced changes in synaptic transmission were prevented by the NAC treatment. Acute treatment of ketamine in vivo and in vitro induced a significant decline in mitochondrial membrane potential with a drastic elevation in mitochondrial superoxide levels. Moreover, ketamine induced a significant reduction in neuritic density in cultures. These KET-mediated mitochondrial changes were considerably more pronounced in GFP+ interneurons when compared to pyramidal neurons from the same cultures. NAC treatment significantly mitigated the KET-induced mitochondrial defects in a dose-dependent manner.

**Conclusions:** Developmental KET treatment induces persistent schizophrenia-like symptoms in behavior and alters the physiology of the mPFC. These changes correlate with specific mitochondrial deficits that are exacerbated in fast-spiking PV+ interneurons. Concurrent NAC treatment either in-vitro, or throughout development in-vivo prevents these changes, thus preserving normal prefrontal cortical function and cognitive abilities.

**Keywords:** schizophrenia, glutamate GABA, interneuron

**Disclosures:** Nothing to disclose.
expression levels were detected in clozapine-treated vs. control mice exposed to PRS (F = 11.7, df = 1, 8, p < 0.01).

**Conclusions:** These data indicate that prenatal stress induces long term effects on the RNA editing of GluA2 in the hippocampus. These molecular changes are correlated with reduced social interaction behavior in the PRS mice. Both these molecular and behavioral effects of PRS are reversed by clozapine but not haloperidol. Clozapine may increase social interaction in PRS mice by modulating GluA2 RNA editing in the hippocampus, perhaps through altered ADAR3 expression. Therefore PRS may result in long-term changes in AMPAR function in the hippocampus, which could contribute to the long-term behavioral consequences of PRS. GluA2 RNA editing is predicted to alter AMPAR assembly and trafficking, and is developmentally regulated (Greger et al, 2006). Our data indicate that GluA2 RNA editing in the hippocampus is a marker of prenatal stress, and may alter the development of the hippocampus. Therefore GluA2 RNA editing could contribute to the pathophysiology of stress-induced psychiatric disorders.

**Keywords:** glutamate, hippocampus, RNA splicing, gene expression, haloperidol

**Disclosures:** Nothing to disclose.

**T179. HDAC2 but not HDAC1 Transcript Levels are Reduced in the Postmortem DLPFC from Schizophrenia Patients Compared to Non-Psychiatric Controls**

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**Background:** Epigenetic mechanisms have emerged as central components in various brain disorders. Among these, aberrant expression of histone deacetylase (HDAC) subtypes HDAC1 and HDAC2 have been reported postmortem studies of schizophrenia, bipolar disorder, depression and recently, Alzheimer’s Disease (Benes et al. PNAS, 2007; Sharma et al. Schizoph Res, 2008; Covington et al. J.Neurosci, 2009; Anderson et al. PLoSOne, 2015). Disease-specific changes have been reported in the frontal cortex (Sharma, 2008), nucleus accumbens (Covington, 2009) and GABAergic cells of the hippocampus (Benes, 2007), which has led to investigation of the role of epigenetics in disease etiology and its potential in developing biomarkers and therapeutics for diverse brain diseases. Here, we took advantage of the large collection of meticulously curated postmortem human brain tissues and cDNA libraries at the Human Brain Collection Core (HBCC) in order to better understand the relationship of HDAC 1 and 2 in brain disease and to evaluate disease-related covariates.

**Methods:** Methods: cDNA libraries representing >700 donor dorsolateral prefrontal cortex (DLPFC) samples were allocated by the HBCC. These samples represented ages ranging from prenatal (second trimester before birth) through ≥80 years. Samples ≥ 18 years old comprised individuals diagnosed with schizophrenia (n = 176); bipolar disorder (n = 61); major depressive disorder (n = 138) and unaffected controls (n = 210). TaqMan quantitative PCR (qPCR) was used. Probes were selected for HDAC1 and HDAC2 (LifeTechnologies assayIDs Hs00606262_g1, Hs00231032_m1) and cDNA from DLPFC was assayed for the full sample set described. The same probes were used to assay cDNA prepared from caudate in a subset of samples. ABI Prism qPCR machines (Applied Biosystems) measured a fluorescence intensity cycle threshold (CT) from each probe in triplicate for each sample. Relative quantification was used to transform per-sample average CT values into relative gene expression with levels of HDAC 1 and 2 normalized to the geometric mean of three housekeeping genes (actin, b2microglobulin and glucoronidase beta). Specific details were tabulated on patient demographics (e.g. age, race, sex), medical history (e.g. diagnosis, smoking history, duration of illness), drug toxicology at time of death and archived sample characteristics (e.g. tissue pH, post mortem interval (PMI), RNA integrity number (RIN)). Statistica and GraphPad Prism software were used to remove outliers ± 3 standard deviations from mean and compare diagnosis group-averaged values for HDAC 1 or HDAC2 levels. Statistical comparisons were controlled by ANCOVA for variables correlating with HDAC expression.

**Results:** We found that in DLPFC from SCZ patients, HDAC2 levels were reduced by 34% compared to controls. Age, PMI, pH and RIN were each correlated with HDAC2 levels and were included in ANCOVA analysis which demonstrated a highly significant difference between SCZ and Controls (F(4,546) = 30.485, p < 10-4). HDAC2 was not significantly altered in the DLPFC from BP or MDD. We further found that HDAC1 was significantly elevated by 16% in the DLPFC from patients with MDD compared to controls, (F(4,539) = 4.495, p = 0.0014), with no significant changes identified for SCZ or BP. In the total complement of controls age prenatal through adult (n = 326), HDAC1 and HDAC2 levels were both dramatically higher in prenatal samples as compared with the rest of the lifespan. These correlations were significant (HDAC1: r = -0.11, p = 0.0027; HDAC2: r = -0.45, p < 10-4 ) and consistent with developmental profiles of HDAC 1 and 2 from previous large-scale transcript studies (Brain-Cloud, Coulantoni et al. Nature 478:7370, 2011).

Investigating disease-relevant correlations with HDAC2 expression in DLPFC, sample records clarified no significant effects of smoking, nicotine/cotinine levels, antidepressant medications, antipsychotics, duration of illness, manner of death, body mass index, or diagnosis group subdivision on the basis of toxicity reports indicating treatment with the antidepressant drugs sertraline, escitalopram or fluoxetine or the antipsychotic drugs aripiprazole, risperidone, olanzapine or haloperidol. Among 34 samples reflecting treatment with valproic acid/Depakote, a compound with demonstrated potential in developing biomarkers and therapeutics for diverse brain diseases, we took advantage of the large collection of meticulously curated postmortem human brain tissues and cDNA libraries at the Human Brain Collection Core (HBCC) in order to better understand the relationship of HDAC 1 and 2 in brain disease and to evaluate disease-related covariates.

**Methods:** Methods: cDNA libraries representing >700 donor dorsolateral prefrontal cortex (DLPFC) samples were allocated by the HBCC. These samples represented ages ranging from prenatal (second trimester before birth) through ≥80 years. Samples ≥ 18 years old comprised individuals diagnosed with schizophrenia (n = 176); bipolar disorder (n = 61); major depressive disorder (n = 138) and unaffected controls (n = 210). TaqMan quantitative PCR (qPCR) was used. Probes were selected for HDAC1 and HDAC2 (LifeTechnologies assayIDs Hs00606262_g1, Hs00231032_m1) and cDNA from DLPFC was assayed for the full sample set described. The same probes were used to assay cDNA prepared from caudate in a subset of samples. ABI Prism qPCR machines (Applied Biosystems) measured a fluorescence intensity cycle threshold (CT) from each probe in triplicate for each sample. Relative quantification was used to transform per-sample average CT values into relative gene expression with levels of HDAC 1 and 2 normalized to the geometric mean of three housekeeping genes (actin, b2microglobulin and glucoronidase beta). Specific details were tabulated on patient demographics (e.g. age, race, sex), medical history (e.g. diagnosis, smoking history, duration of illness), drug toxicology at time of death and archived sample characteristics (e.g. tissue pH, post mortem interval (PMI), RNA integrity number (RIN)). Statistica and GraphPad Prism software were used to remove outliers ± 3 standard deviations from mean and compare diagnosis group-averaged values for HDAC 1 or HDAC2 levels. Statistical comparisons were controlled by ANCOVA for variables correlating with HDAC expression.

**Results:** We found that in DLPFC from SCZ patients, HDAC2 levels were reduced by 34% compared to controls. Age, PMI, pH and RIN were each correlated with HDAC2 levels and were included in ANCOVA analysis which demonstrated a highly significant difference between SCZ and Controls (F(4,546) = 30.485, p < 10-4). HDAC2 was not significantly altered in the DLPFC from BP or MDD. We further found that HDAC1 was significantly elevated by 16% in the DLPFC from patients with MDD compared to controls, (F(4,539) = 4.495, p = 0.0014), with no significant changes identified for SCZ or BP. In the total complement of controls age prenatal through adult (n = 326), HDAC1 and HDAC2 levels were both dramatically higher in prenatal samples as compared with the rest of the lifespan. These correlations were significant (HDAC1: r = -0.11, p = 0.0027; HDAC2: r = -0.45, p < 10-4 ) and consistent with developmental profiles of HDAC 1 and 2 from previous large-scale transcript studies (Brain-Cloud, Coulantoni et al. Nature 478:7370, 2011).

Investigating disease-relevant correlations with HDAC2 expression in DLPFC, sample records clarified no significant effects of smoking, nicotine/cotinine levels, antidepressant medications, antipsychotics, duration of illness, manner of death, body mass index, or diagnosis group subdivision on the basis of toxicity reports indicating treatment with the antidepressant drugs sertraline, escitalopram or fluoxetine or the antipsychotic drugs aripiprazole, risperidone, olanzapine or haloperidol. Among 34 samples reflecting treatment with valproic acid/Depakote, a compound with demonstrated properties as an HDAC inhibitor, we found no change in HDAC1 and no additional change in HDAC2 expression.

We investigated the expression of HDAC1 and 2 in DLPFC from SCZ patients using a subset of the samples (SCZ = 53; BP = 35; Ctrl = 70) and clarified that no significant differences were associated with diagnosis in this region.

**Conclusions:** Discussion: Prior results have shown that HDAC dysregulation may be an important component of brain disease. The closest comparator to our study, Sharma reported in 2008 that HDAC1 was upregulated among PFC microarray results from a comparably small set of schizophrenia samples (n = 16) vs controls (n = 27). Our results
demonstrate the likelihood that HDAC2 expression is decreased in postmortem DLPFC of SCZ compared to controls. The effect – a highly significant 34% decrease – was not impacted by smoking history, therapeutic drug status or medication, and supports the notion that we have identified a robust, disease-relevant change in an epigenetic enzyme. The magnitude and consistency of our results supports follow-up studies to quantify protein-level differences using tools for postmortem (LC-MS/MS) or in vivo human brain research (PET imaging).

**Keywords:** HDAC, Postmortem Brain Tissue, schizophrenia

**Disclosures:** Nothing to disclose.

T180. Cortical D1 Tone Predicts Network Dynamics of Working Memory: A Simultaneous PET-fMRI Investigation

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**Background:** Working memory deficits comprise a core symptom of schizophrenia and other neuropsychiatric disorders. The reliance of working memory performance on prefrontal activation, and on prefrontal dopamine D1 receptor signaling, have been repeatedly demonstrated in human and nonhuman primate studies. However, recent work suggests that the neural substrate of working memory extends beyond prefrontal physiology, involving multiple cortical networks. Further, characterization of D1 receptor distribution across the human cortex has been limited to large regions-of-interest that are anatomically rather than functionally derived, and the relationship between cortical D1 signaling and network transitions underlying working memory remains largely unexplored. The present study used [11C] NNC112, a marker of D1 receptor binding potential with reliable cortical and subcortical signal, in conjunction with functional MRI conducted at rest and during working memory performance. Our primary goals were (1) to map variation in D1 receptor tone across functionally derived cortical networks and (2) to clarify the relationship between D1 tone and working memory-related network changes.

**Methods:** 27 healthy, right-handed adults (mean age 30.6, 11F/16M) underwent simultaneous PET-MRI using a Siemens BrainPET scanner with a 1-channel CP transmit/8-channel receive head coil. Immediately after bolus injection of NNC112, subjects underwent a 6-minute resting MRI scan followed by a 24-minute working memory task (Sternberg Item Recognition Paradigm, SIRP) using standard sequences. PET acquisition continued for a total of 90 minutes, generating 28 dynamic frames. In addition to the standard corrections (i.e., normalization, randoms, attenuation, scatter, dead-time and radioactive decay), MR-assisted motion and partial volume corrections were performed. D1 receptor binding potential (BPND), was mapped across the cortical surface and within the brain volume through pixelwise kinetic modeling, using the Logan reference tissue model with the cerebellar cortex (which is devoid of D1) to control for nonspecific binding. Variation in D1 tone across functional cortical networks was compared using the canonical 7-network parcellation of Yeo and colleagues. Working memory load-dependent activation, determined using FreeSurfer, was used to localize key nodes in frontaloparietal control (FPCN) and default mode (DMN) networks, which were strongly activated and deactivated (respectively) during SIRP performance. Connectivity within and across networks, both at rest and during SIRP performance, was computed using FreeSurfer, and relationships between network changes and D1 tone were evaluated with linear regression.

**Results:** D1 receptor BPND varied substantially among cortical networks, with the highest signal occurring in the DMN (p's<.001 versus every other network), followed by other association networks and then primary sensory-motor networks. While BPND also varied substantially among individuals, BPND levels among networks were strongly correlated (R2=.75 to .97); accordingly, a single mean cortical BPND value was assigned for each subject for subsequent analysis. Connectivity between FPCN and DMN dropped precipitously between resting and task state (p<10-10), indicating strong task-related decoupling of these networks. Mean cortical BPND strongly predicted between-network decoupling (R2=.46, p=.0003) of the right FPCN and DMN, but was unrelated to within-network changes in connectivity. Mean cortical BPND was also a stronger predictor of network decoupling than was caudate or putamen BPND (R2 changes>.2, p's<.005).

**Conclusions:** Through simultaneous MRI and PET imaging, the present study has revealed new anatomical and functional insights into cortical dopamine signaling. The prominence of D1 receptor density in default network territory, coupled with the reliance of cross-network connectivity changes on D1 tone, suggest that D1 mediation of working memory does not exclusively reflect prefrontal cortical mechanisms. Rather, the present results argue that cortical D1 signaling may orchestrate changes among task-positive and task-negative networks that are necessary for the brain to shift from a resting to active state. These findings may be relevant for development of dopamine-based therapeutics for working memory impairment.

**Keywords:** Dopamine, PET, fMRI Functional Connectivity, working memory, default network

**Disclosures:** Nothing to disclose.

T181. An Epigenetic Model of Schizophrenia

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**Background:** Exposure to environmental factors during gestation affects the epigenome. This can lead to permanent alterations in gene expression of genes that are crucial for the normal development and lead to increased susceptibility to certain diseases in adults. Maternal methyl-donor diet influences the DNA methylation and leads to permanent changes in offspring genes. DNA methylation is a critical
epigenetic factor that modifies expression of genes involved in neuronal functions such as neurogenesis and differentiation. The DNA methylation rate depends in part on methionine levels since methionine is the primary methyl donor. Over the years abnormal DNA methylation has been implicated in schizophrenia. More recently, the results from genome-wide epigenomic profiling studies show about 100 loci with DNA methylation changes in patients with major psychosis.

**Methods:** To modulate DNA methylation during development, we examined the effect of repeated methionine administration during the gestational stage E14-20. Mice were bred in house. Eight female and four male mice were used in four breeding cages. Each male was allowed to mate with two females. On day 14 after conception the two females were separated and injected subcutaneously twice a day with either saline or methionine (750mg/kg) until parturition day. The male pups (MET pups) were tested in a battery of behavioral experiments starting at age 6 weeks with a week gap between every two tests. Tests were conducted in a sequence ranging from the least to the most stressful: Stereotypy, social interaction, sucrose preference, novel object recognition, T maze, and finally prepulse inhibition. We tested the typical (haloperidol) and atypical (clozapine) antipsychotic drugs on the behavioral responses of the MET pups. Antipsychotic drugs were administered as acute doses on the day of the experiments. To determine which genes’ expressions are affected by the methionine treatment, we carried out an mRNA microarray analysis covering around 28000 genes. To confirm the results, we performed quantitative real-time PCR (QT PCR).

**Results:** Methionine injections did not produce defects in gross development. MET pups show normal weight, food consumption, motor coordination and nociceptive responses. Furthermore they displayed normal anxiety- and depression-related behaviors. However, the MET pups exhibited higher level of stereotypy. They displayed significant social deficits as reflected by the decrease in amount of time interacting with the unfamiliar mice in the social interaction assay or the new control in the social novelty assay. The MET pups also display memory and learning deficits. In the novel object recognition they displayed significant decrease in discriminating new and old objects. In the spontaneous alternation task which relates to spatial working memory, MET pups exhibited a significant decrease in correct responses when compared to pups of saline-treated mothers. Finally, the MET pups displayed pre-pulse inhibition deficit which reflects an inability to filter non-relevant sensory information. A single injection of haloperidol (0.1 mg/kg) and clozapine (1 mg/kg) reversed the stereotypic behavior found in MET pups. Clozapine (1 mg/kg) but not haloperidol improved the social deficits in MET pups. Clozapine (1 mg/kg) reversed the impairment of cognitive functions in MET pups as observed in novel object recognition assay. Both clozapine (2.5 mg/kg) and haloperidol (0.25 mg/kg) reversed the PPI deficits in MET animals. 5 genes displayed changes in expression level that were considered of importance (>1.5 fold). Neuronal PAS domain protein 4 (Npas4), Activity regulated cytoskeletal-associated protein (Arc), c-Fos and early growth response 2 (Egr2) showed a decrease in gene expression. Fibroblast growth factor 1 (Fgf1), was upregulated by two fold. All these genes are known to play important roles in a variety of cellular processes including neurodevelopment, neurotransmission, neuronal plasticity, and learning and memory. The results of the QT PCR experiments confirmed the microarray findings.

**Conclusions:** Our data show that methionine administration to pregnant mice at E14-20 produces, in the adult offspring, a behavioral phenotype that mimics schizophrenia spectrum disorder symptoms, and that antipsychotics can reverse the methionine induced symptoms. The behavioral phenotype was accompanied with prominent changes in expression of five genes that are involved in neurodevelopment and neural activity, and have been directly implicated in the pathophysiology of schizophrenia. Our approach can serve as an epigenetic model for schizophrenia.

**Keywords:** Methionine, Schizophrenia, Animal Model, Developmental, Antipsychotics

**Disclosures:** Nothing to disclose.

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**T182. Serotonergic Hallucinogens Preferentially Activate Subsets of Cortical Neurons, Interneurons, and Glial Cells in the mPFC, Somatosensory Cortex, and Claustrum, and Induce Rapid Redistribution of 5-HT2A Receptor Protein in Neurons**

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**Background:** The classic serotonergic hallucinogens (i.e. psychedelics) are known to produce their behavioral effects primarily through activation of cortical serotonin 5-HT2A receptors (5-HT2AR). These effects can include hallucinations, delusions, and detachment from reality. Several recent reports have informed on the neural circuitry underlying these effects, but the key molecular and cellular mechanisms still remain unknown. To gain a better understanding of the effects of psychedelics on cellular, molecular, and genetic aspects of brain function we treated rats with the drug DOI, followed by an analysis incorporating cell sorting of cortical cells by flow cytometry, gene expression analysis, and immunofluorescence experiments.

**Methods:** We have refined and further developed the process of cortical cell dissociation and sorting by flow cytometry and are the first to report here being able to separate highly purified individual cellular populations from adult cortex that include neurons, different subtypes of interneurons, glia and astrocytes, and the use of FACS to identify and sort a subset of 5-HT2AR expressing neurons. Significantly, with our protocols we are able to isolate intact cells that include cytoplasm and plasma membrane, which allows for sorting based on a range of cellular markers. We treated rats with either saline or DOI (6.0 mg/kg, i.p.) for 105 minutes then the medial prefrontal and somatosensory cortices were separately dissected, dissociated, immunostained, and sorted for the presence of various markers using fluorescence-activated cell sorting (FACS). Sorted populations were processed for RNA extraction and subjected to qRTPCR for gene expression analysis. Immunofluorescence on cortical brain slices was subsequently performed to validate gene expression results from activated cellular populations, and to determine 5-HT2AR protein expression relevant to certain distinct cellular populations.
Results: DOI was discovered to strongly induce expression of c-Fos (a marker of neuronal activity) in a small, previously unrecognized heterogeneous population of cells in the cortex, including pyramidal neurons, interneurons, and astrocytes. These activated neurons (~3-5% of the total number of neurons), however, display a ten-fold higher expression of 5-HT2AR mRNA than the non-activated population. Because c-Fos expression in neurons has been correlated with depolarization, we hypothesize that the neurons directly activated by DOI represent a “trigger” sub-population that is directly depolarized by psychedelics and necessary to initiate the reported effects on cortical network connectivity. We also found that DOI activates inhibitory somatostatin and parvalbumin GABA interneurons, as well as glial populations, including astrocytes. Analysis of 5-HT2AR expression by immunofluorescence revealed that DOI induces a rapid redistribution of receptor protein into perinuclear intracellular compartments, even in cells that do not appear to be directly activated with respect to cFos expression. Interestingly, high levels of both 5-HT2AR protein expression and cellular activation were observed in the claustrum.

Conclusions: Psychedelics like DOI directly activate only small subpopulations of both excitatory and inhibitory neurons, as well as glial cells, including astrocytes. High levels of activation were observed in the claustrum, one of the least understood brain regions, which recently was suggested to function by segregating attention between modalities and has been proposed to be the “seat of consciousness.” Psychedelics also induce dramatic 5-HT2AR redistribution in both c-Fos-activated and non-activated cells. The effects of psychedelics at the cellular level are, therefore, mediated by a complex interaction not only between diverse cellular types but also discrete regions of the brain that together give rise to the observed behavioral effects. A better understanding of these interactions will likely inform on the etiology of several psychiatric diseases, such as schizophrenia, which have some symptoms that can resemble the effects of psychedelics. Finally, we are the first to demonstrate that FACS can be used directly to function by segregating attention between modalities and has been proposed to be the “seat of consciousness.”

Keywords: serotonin 2A, Medial Prefrontal Cortex, claustrum, Fluorescence-activated cell sorting, gene expression
Disclosures: Nothing to disclose.

T183. The Effects of NMDA Receptor Co-agonist Availability on Reward Processing Using a Mouse Model of Anhedonia: Implications for Co-morbid Schizophrenia and Substance Dependence
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Background: Substance abuse shares substantial co-morbidity with schizophrenia, possibly due to a shared neural substrate, N-methyl-D-aspartate receptor (NMDAR) dysfunction. NMDAR hypofunction is a core feature of schizophrenia, and NMDARs have been implicated in the aberrant synaptic plasticity that is characteristic of addiction. A common symptom in these disorders is anhedonia, an impaired capacity to experience pleasure. In order to investigate the shared pathophysiology and symptomology of these disorders, our laboratory has developed two transgenic mouse lines with contrasting levels of NMDAR function. The first line is a constitutive knockdown of glycine transporter 1 (GlyT1), which regulates synaptic glycine, a NMDAR co-agonist. Heterozygous mutants (GlyT1+/−) exhibit NMDAR hyperfunction. The second line is a constitutive knockout of serine racemase (SR), the enzyme that produces D-serine, the predominant NMDAR co-agonist in the forebrain. Null mutants (SR−/−) exhibit NMDAR hypofunction. An intracranial self-stimulation (ICSS) paradigm was implemented to investigate the role of NMDAR function in reward processing.

Methods: Monopolar electrodes were implanted into the brains of wildtype (WT), GlyT1 +/−, and SR−/− mice in the right medial forebrain bundle (MFB) at the level of the lateral hypothalamus. Following recovery, mice began training in operant chambers where responses were rewarded with electrical pulses. Current was adjusted until responding reached a rate of 1 reward per second. Then, stimulation threshold and maximum response rate were calculated for each animal from responses made across 15 descending frequencies. The ability of cocaine to facilitate responding for brain stimulation was tested using a within-subject design. Each animal was tested with 3 doses of cocaine (3, 6, and 12 mg/kg i.p.) and saline.

Results: As expected, WT mice exhibited a dose-dependent leftward shift in stimulation threshold in response to cocaine (i.e., cocaine facilitated responding for brain stimulation at frequencies that were previously too low to maintain responding). However, cocaine treatment had no effect on maximum response rate. GlyT1 +/− mice behaved similarly to WT mice (i.e., they also exhibited a dose-dependent leftward shift in stimulation threshold in response to cocaine, while cocaine treatment had no effect on maximum response rate). Notably, in SR−/− mice, cocaine had no effect on stimulation threshold. Maximum response rate also was not affected.

Conclusions: These results suggest that glutamatergic dysfunction, specifically, NMDAR hypofunction, may be a common neural substrate underlying substance abuse and schizophrenia, and highlight NMDAR modulation as a possible avenue of treatment for these co-morbid disorders.

Keywords: intracranial self-stimulation, NMDA receptors, cocaine, schizophrenia, Substance abuse
Disclosures: JTC – Abbvie, Novartis, En Vivo, a patent owned by MGH for the use of D-serine to treat serious mental illness could yield royalties.

T184. Protein Pathology of NKCC1 (SLC12A2) as a Marker in Chronic Mental Illness
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Background: Disturbed proteostasis is a major hallmark of dysfunctional neurons in chronic brain disorders. These can
be extreme as in neurodegenerative diseases leading to microscopically visible deposition of proteins, or more subtle as in schizophrenia, a chronic brain disorder, albeit without detectable degeneration. Identification of insoluble proteins specific for schizophrenia or in patient subsets thereof will help to biologically define the basic neuropathological changes underlying this disorder.

Methods: Insoluble proteome from pools of post mortem brains (schizophrenia, depression, bipolar disorder and normal controls) was prepared similar to a previously described methodology (Leliveld et al., 2008 J Neurosci 28:3839) from brain samples provided by the Stanley Research Medical Institute (SMRI) and the Lieber Institute for Brain Development and analyzed by mass spectrometry. Genetic analysis was applied to support significance of data.

Results: We identified a specific signature of insoluble proteins corresponding to a specific proteostasis defect in brains of patients with schizophrenia. As an example of one specific insoluble protein, we identified NKCC1, but not its counterplayer KCC2, as insoluble in the schizophrenia brain, which was confirmed by Western blotting of individual samples. Genetic analysis of a Northern Finnish birth cohort confirmed genetic association of NKCC1 with schizophrenia, depression, protein aggregation, and DISC1-dependent way.

Conclusions: Protein abnormalities undoubtedly are present in a severe chronic brain disorder such as schizophrenia. By linking genetic traits associated with this disorder to postmortem studies, a pathophysiological process, potentially targetable, can be identified. Abnormalities in NKCC1 appear to play a role in schizophrenia (Mortia et al. 2014, J Neurosci 34:4929). NKCC1 protein also has been demonstrated to interact with DISC1 (Kim et al, 2012, Cell 148:1051). Our demonstration of NKCC1 protein pathology supported by novel genetic findings in mental illness provides converging evidence for the significance of this membrane transporter protein for mental health.

Keywords: schizophrenia, depression, protein aggregation, proteostasis

Disclosures: Nothing to disclose.
Conclusions: The CMINDS can readily be employed in the assessment of cognitive deficits in neuropsychiatric disorders. The CMINDS requires little administrator training, has English and Spanish task versions, keeps track of alternate task versions presented in longitudinal designs, is HIPAA and 21 CFR Part 11 compliant, records all data collected electronically, and automatically calculates the most commonly used performance and response time scores. The CMINDS may be particularly useful in large-scale studies that may benefit most from its electronic data capture and other advantages over paper-and-pencil tasks.

Keywords: psychosis, neuropsychology, Cognition

Disclosures: Dr. Van Erp consulted for Roche Pharmaceuticals and has a contract with Otsuka Pharmaceutical Co., Ltd. (OPCJ). Dr. Bustillo consulted with Novartis and Otsuka Pharmaceuticals. Dr. Mathalon is a consultant for Bristol-Myers Squibb and consulted for Roche Pharmaceuticals. Dr. Preeda consulted for Boehringer-Ingelheim. Dr. Potkin has financial interests in Bristol-Myers Squibb, Eisai, Inc., Eli Lilly, Forest Laboratories, Genentech, Janssen Pharmaceutical, Lundbeck, Merck, Novartis, Organon, Pfizer, Roche, Sunovion, Takeda Pharmaceutical, Vanda Pharmaceutical, Novartis, Lundbeck, Merck, Sunovion and has received grant funding from Amgen, Baxter, Bristol-Myers Squibb, Cephalon, Inc., Eli Lilly, Forest Laboratories, Genentech, Janssen Pharmaceutical, Merck, Otsuka, Pfizer, Roche, Sunovion, Takeda Pharmaceutical, Vanda Pharmaceutical, NIAAA, NIBIB, NIH/NCRR, University of Southern California, UCSF, UCSD, Baylor College of Medicine. The remaining authors declare no potential conflict of interest. None of the authors of this manuscript are affiliated with or receive compensation from NeuroComp Systems, Inc., MATRICS Assessment, Inc., or Neurcog Trials, Inc.

Methods: QUALIFY was a 28-week, randomized, open-label, rater-blinded, head-to-head study (NCT01795547) of two atypical long-acting injectable anti-psychotics (LAIs), AOM 400 and PP (flexible dosing, per label, with 50-150 mg/month as paliperidone [EU and Canada], 78-234 mg/month as paliperidone palmitate [US]) in patients with schizophrenia. Included patients were age 18-60 years needing a change from current oral antipsychotic treatment and, in the judgment of the investigator, would benefit from LAI treatment. The primary endpoint of QUALIFY was change from baseline to week 28 on the Heinrichs-Carpenter Quality-of-Life Scale (QLS) total score[2]. The QLS comprises 21 items in 4 domains; interpersonal relations (8 items), intrapsychic foundations (7 items), and common objects and activities (2 items), rated from 0 (severe impairment) to 6 (no impairment) by a blinded clinician, and changes in QLS total scores of ≥5.3 points are considered clinically relevant[3]. Work readiness (Yes/No), was rated at baseline and at week 28 by a non-blinded clinician. The primary analysis of the study used a mixed model for repeated measures (MMRM) to assess changes from baseline to week 28 on QLS total scores. Logistic regression was used in the post-hoc analyses to compare relative odds for work readiness after AOM 400 and PP treatment adjusting for treatment. The primary endpoint of QUALIFY was change from baseline to week 28 (No to Yes, Yes to Yes, or No at week 28), and the changes in QLS total, domain, and items scores were compared with similar MMRM methodology as for the primary analysis.

Results: Analysis of the primary endpoint showed superior improvements with AOM 400 (n = 136) vs PP (n = 132) on QLS total score (least squares mean [LSM] difference between treatments: 4.67, 95%CI: [0.32;9.02], p = 0.036). At week 28, the odds of being rated ready for work were higher for AOM 400 vs PP (adjusted odds ratio: 2.67, 95%CI: [1.39; 5.14], p = 0.003), and 29/110 (26.4%) of AOM 400 patients changed from No to Yes in work readiness compared to 12/98 (12.2%) of patients in the PP group. Patients (independent of treatment) who shifted from No to Yes in work readiness (n = 41) showed a LSM change from baseline to week 28 (± SE) on QLS total scores of 14.3 ± 2.2 points, and this change was significantly greater than in the group of patients who were No at week 28 (n = 118) in work readiness (LSM change from baseline to week 28: 2.7 ± 1.4; LSM difference: 11.6 ± 2.6, 95% CI: [6.5;16.7], p < 0.0001). Patients with Yes to Yes in work readiness at baseline and week 28 (n = 49) also had improved QLS total scores compared with patients who were No at week 28 (LSM differences: 7.9 ± 2.7, 95%CI: [2.5; 13.2], p = 0.0045). QLS instrumental role domain scores were significantly improved in patients shifting from No to Yes in work readiness compared to patients who were No at week 28 in work readiness (LSM difference between groups: 3.3 ± 0.7, 95%CI: [1.8;4.8], p < 0.0001). In contrast, patients judged as ready to work both at baseline and at week 28 (Yes to Yes) did not show greater improvement on QLS instrumental role domain scores compared to patients who were No at week 28 in work readiness (LSM difference: 1.1 ± 0.8, 95%CI: [-0.4; 2.6], p = 0.150). On the specific items of QLS instrumental role (occupational role, work function-
ing, work levels, work satisfaction), LSM improvements of nearly 1 point on each item were found in the patients shifting from No to Yes in work readiness.

Conclusions: A 14-point improvement on QLS total score in patients shifting from No to Yes in work readiness after 28-week treatment with AOM 400 or PP suggests a strong association between shifts in work readiness and improvements on QLS. This association was particularly prominent in categories of QLS related to work functioning, highlighting consistent rating between two different scales assessed by two independent raters in the QUALIFY study (one blinded and one not blinded to the patient’s treatment). The association between QLS and WoRQ was irrespective of treatment, but significantly greater improvements in both scales were seen with AOM 400 vs PP treatment. Regardless of treatment assignment, the strong association between functional improvements in health-related quality of life and work readiness suggest that increasing patients’ capacity to work is a realistic and desirable goal in the treatment of schizophrenia.

Keywords: aripiprazole once-monthly 400 mg (AOM 400), paliperidone palmitate, head-to-head clinical trial, work readiness, health-related quality of life

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References:

T188. Divergent Transcription of the BDNF Locus: Relevance for Schizophrenia


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Background: Non-coding RNA (ncRNA) transcription by RNA polymerase II often initiates from bidirectional promoters that synthesize mRNA and ncRNA in opposite directions. These divergent ncRNAs may be particularly important in CNS function as cells of neural origin are highly transcriptionally active and show robust ncRNA expression. BDNF is a complex and highly regulated gene: several untranslated 5’ exons can be spliced with a 3’ coding exon providing bipartite or tripartite transcripts and multiple splice variants. Here we detected and analyzed previously unknown divergent transcription in this locus.

Methods: PolyA enriched RNA was extracted from post-mortem human (N = 412: 238 controls, 174 patients with schizophrenia) and mouse (N = 12) prefrontal cortex (PFC) and then purified and enriched with PCR to create a cDNA library for high throughput sequencing using the Illumina HiSeq2000. We used Race-PCR, followed by PacBio Sequencing of the PCR amplicons, in order to detect the structure of the divergent transcripts. We performed a KCl-induced depolarization experiment on cultured E14 mouse cortical neurons. A functional magnetic resonance imaging analysis of brain physiology in vivo was performed in 326 healthy volunteers, 56 patients with schizophrenia and 84 of their healthy siblings.

Results: In the human and mouse transcriptome, novel antisense divergent ncRNAs (divRNAs) are initiated from bidirectional promoters located in the region of BDNF exons I, II and III and - in humans - also from the known promoter of BDNF-AS, and have different 3’ terminations, located more than 50kb 5’ of BDNF. Expression of these divRNAs is low at baseline (<1 RPKM), but increases 20-fold following induced neural activity (N = 12, p < 0.01). In human DLPFC, the expression of the divRNAs is highly correlated with the protein-coding BDNF gene (N = 412, t = 11.7, p < 0.01), thus confirming that BDNF and its divergent transcripts are expressed in similar contexts, likely with different temporal dynamics. We found that genetic variants associated with expression of these divRNAs (N = 412, p < 0.01) show association with schizophrenia in the PGC2 dataset (p < 1.6e-4) and predict PFC activity measured with fMRI during working memory in patients with schizophrenia and also in their healthy siblings. Specifically, the genotype associated with increased expression of the divRNAs is associated with greater prefrontal cortex activity during working memory, i.e. lower efficiency, compared with the other genotype groups.

Conclusions: We demonstrate multiple antisense divRNAs in the BDNF locus, revealing an additional complexity in the biology of BDNF, which may be differentially regulated in schizophrenia as well as other disorders. Divergent transcription might contribute to the complex regulation of the BDNF gene, in human and in mouse, specifically after neuron depolarization. More in general, when mapping a phenotype to a certain genomic locus, it is necessary to consider ncRNAs and bidirectional promoters as causes for phenotypic variability. The knowledge of the specific role of divergent transcription has the power to inform several potential therapeutic applications including development of non-coding RNA based drugs, which could not only turn off, but also turn on target genes.

Keywords: BDNF, divergent transcription, schizophrenia, prefrontal cortex, neural activity

Disclosures: Nothing to disclose.
T189. Replication of Patterns in Gray Matter Abnormalities for Targeted Drug Development in Multi-Site Schizophrenia Datasets


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**Background:** Numerous studies of structural magnetic resonance imaging (sMRI) in schizophrenia have reported differences in Gray Matter Concentration (GMC). However, replication of the patterns across multisite datasets, using uniform pre-processing pipeline/methods has not been performed yet. This work addresses this issue and discovers reliable patterns of GMC deficit, which could be targeted in the development of drugs for schizophrenia.

**Methods:** Source based morphometry (SBM) a multivariate alternative to voxel-based morphometry (VBM) automatically combines information across different voxels and provides patterns of common GMC variation among subjects. This multivariate technique can improve sensitivity by parceling noise, scanner effects into separate components and reduces the number of corrections for multiple statistical tests. A total of 936 structural MRI images of healthy controls (HC) (mean age = 34.81, SD = 11.89, range: 13–80) and 784 patients with schizophrenia (SZ) (mean age = 36.65, SD = 11.62, range: 17–64) from 8 independent studies (several being multisite) formed the aggregated dataset.

Imaging data was processed in a standard VBM pipeline with age and gender regressed voxelwise. The number of components was set to 30 and we used ICASSO (20 runs) to determine the stability of the components. Site effect estimates could be noisy for small datasets, so we included them in the SPSS model after the decompositions. The SBM module of the GIFT Toolbox (http://mialab.mrn.org/software/gift/) was used to perform independent SBM decompositions on each of the 8 multisite studies and an aggregated dataset respectively.

A larger loading for an individual or group mean indicates that the spatial pattern is more strongly weighted in the data for that individual or group. However, the interpretation of the loading coefficient difference depends upon the spatial image of the component. If the spatial component map is predominantly positive, and if the group mean of loadings are greater in HC than in SZ, we infer that GMC is greater in HC for the spatial component under consideration.

We examined the differences in loading coefficients from SZ and HC participants using SPSS. A multivariate analysis of covariance (MANCOVA) model was used with SBM coefficients as dependent variables, diagnosis as a factor, site as a dummy-scored covariate and site by diagnosis as an interaction. A threshold of \( P < .05 \) corrected for multiple testing using the false discovery rate (FDR) method was used to find components showing a significant effect of diagnosis. To confirm these findings further we also performed independent SBMs on each of the 8 studies, to determine components that showed group differences at an uncorrected \( P < .05 \).

**Results:** SBM analyses of the aggregated dataset showed nine patterns with diagnostic differences. They comprised of separate cortical, subcortical, and cerebellar regions. Seven patterns showed greater GMC in HC than SZ, while 2 patterns (brainstem and cerebellum) showed greater GMC for SZ. The directionality of overlapping components observed in each of the eight multisite studies with the aggregated decomposition was same. We discuss four patterns (ordered on their effect sizes) to be consistent and replicable across various SBM decompositions as explained below. The top three components replicated in at least 5 of the 8 independent studies passing an uncorrected \( P < .05 \).

In the aggregated dataset, the greatest GMC deficit was in a single pattern comprising regions in the superior temporal gyrus, inferior frontal gyrus and medial frontal cortex (partial eta squared = 0.048). This replicated across all multisite datasets having the highest effect size. The group mean loading directionality was HC>Sz.

Regions of superior frontal gyrus, middle frontal gyrus and medial frontal gyrus had an effect size of (partial eta = 0.032) with the group mean loading directionality of HC>Sz. Pattern covering regions of brainstem (partial eta squared = 0.027) showed Sz participants had larger group mean of loading coefficients than did HC, implying that these regions showed more GMC in SZ than HC. Chronic exposure to dopamine D2-blocking therapeutic agents, the norm in our sample, may have resulted in miniscule compensatory increases in GMC around some brainstem regions.

Regions of inferior semilunar lobule and cerebellar tonsil (partial eta squared = 0.004) was ranked ninth in the aggregate decomposition based on effect size. The loading directionality was HC greater than Sz.

**Conclusions:** In summary, SBM is an effective technique delineating distinct and consistent anatomical brain regions that show differences between HC and SZ. We identified that cortical, brainstem and cerebellar areas of gray matter loss form highly replicable patterns across studies. These patterns of spatial components could serve as endophenotype for SZ, indicating regions which are affected similarly by common causes such as genetics, disease progression, or medication, which could in turn facilitate targeted drug development.

**Keywords:** schizophrenia, Structural MRI, multivariate, voxel-based morphometry (VBM)

**Disclosures:** Nothing to disclose.

T190. Do the Therapeutic-Like Effects of Acute Oxytocin Persist with Chronic Administration?

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**Background:** There is a great need for improved treatments for severe psychiatric disorders. One molecule that holds
out much promise molecule for addressing this need is oxytocin (OT), a neurohormone that plays a critical role in the regulation of a number of diverse CNS functions including social affiliation, reward and social cognition. Recent small clinical studies have produced evidence for OT’s efficacy in treating a wide variety of psychiatric abnormalities including psychosis and social cognitive deficits. Converging evidence from preclinical studies suggests that acute OT administration produces antipsychotic drug (APD)-like effects and facilitation of social cognition. When it comes to assessing the therapeutic potential of oxytocin, the preclinical effects of long-term OT treatment are more informative than the effects of acute treatment, given the chronic nature of pharmacological treatment of mental illness. To date, however, there have been very few studies that have investigated chronic OT in animal models relevant to psychiatric treatment and the findings from those studies suggest that pro-social effects of acute OT treatment do not persist with chronic treatment. However, those studies used normal animals and not animals that modeled deficits seen in psychiatric illness. Investigating the chronic effects of OT in deficit animal models is likely to be more relevant for evaluating the therapeutic potential of chronic OT. We therefore investigated the effects of chronic, peripheral OT administration in Brown Norway rats, a strain of rats with natural deficits in prepulse inhibition (PPI) and social discrimination (SD), preclinical paradigms used to evaluate the potential therapeutic effects of drugs for psychosis and social cognitive deficits, respectively. Methods: PPI study: Brown Norway rats were administered saline, 0.04, 0.2 or 1.0 mg/kg OT, subcutaneously, once/day for 22 days. On days 1 and 22, animals were tested in startle chambers 30 min after OT injection. In addition, on day 24, animals were treated with OT 30 min prior to PCP, and 7 days after the last OT injection animals were challenged with PCP alone to evaluate the enduring effects of chronic OT on PPI. Social Discrimination study: Same as the chronic OT injection schedule for Days 1 – 22 of the PPI study except that animals were put into SD chambers on days 1 and 22. In addition, animals were tested in SD chambers 24 hours and 7 days after the last OT treatment to evaluate the enduring effects of OT on SD. Results: PPI: Acute administration of the high OT dose facilitated PPI (P < 0.05) while chronic OT produced a facilitation of PPI quantitatively similar to that of acute OT that was no longer significantly different from saline. On day 24, the high OT dose reversed PCP-induced PPI deficits (P < 0.01) and one week subsequent to the last OT injection, the low and mid OT doses appeared to block these deficits. Social Discrimination: Acute treatment with OT reversed SD deficits (P < 0.05) while significant effects after chronic OT treatment were seen only with the mid OT dose (P < 0.05). In addition, there was evidence that OT-induced reversal of these deficits persisted at least seven days after the last OT injection. Conclusions: Acute OT reversed PPI and SD deficits in BN rats consistent with other reports suggesting acute OT administration produces APD-like and pro-social effects. Chronic OT produced similar effects to that of acute OT on PPI and SD suggesting that OT’s acute APD-like and prosocial effects persist with chronic treatment and these effects even persist at least one week after chronic OT treatment is discontinued. The enduring nature of these therapeutic-like effects after discontinuation of chronic treatment is consistent with lasting alterations in relevant brain circuits that go beyond the acute pharmacological effects of OT. Our findings stand in contrast to earlier reports that the effects of a single dose of OT on behavior were not detectable in normal animals after chronic OT treatment. The utilization of animal models with inherent deficits in this study, rather than intact animals used in previous studies of chronic OT, likely contributes to these discrepant results. The antipsychotic-like and social cognition facilitating effects of chronic OT suggest that OT may be an effective treatment for psychosis and social cognitive deficits.

Keywords: oxytocin, social recognition memory, psychosis, chronic, animal model

Disclosures: DF is inventor of a patent submitted by UCSD for use of oxytocin in psychiatric disorders.

T191. Feedback-Controlled Sleep Spindle Transcranial Alternating Current Stimulation Reveals the Functional Role of Sleep Spindles in Motor Memory Consolidation

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Background: Sleep in general and sleep spindles in particular are proposed to be involved in memory consolidation, a process that stabilizes and integrates new, labile memories acquired in the awake state [1]. Sleep spindles are transient EEG oscillation (10–16 Hz) with thalamo-cortical origin that are predominant during non-rapid eye movement (NREM) sleep [2]. Correlations between sleep spindles and sleep-dependent memory consolidation have been repeatedly reported yet proof of a causal role of sleep spindles in memory consolidation is still missing due to the lack of a tool to selectively modulate spindles in humans. We aimed at boosting sleep spindles based on the prevailing rhythms in the EEG and testing their causal role in memory consolidation using EEG feedback-controlled transcranial alternating current stimulation (FB-tACS).

Methods: Sixteen healthy, male participants signed written consent prior to participation. The study was approved by the UNC IRB. All participants first underwent a screening night and thereafter completed in a randomized, counter-balanced cross-over design two experimental nights, one with all-night spindle FB-tACS (verum) and one without stimulation (sham). During both sessions, participants performed a declarative word-pair associates and a procedural motor sequence tapping task in the evening and in the following morning to assess sleep-dependent memory consolidation. Participants were in bed for 8 hours with all-night polysomnographic recordings (21-channel EEG, EMG, EOG). Real-time NREM and spindle detection
was designed using participant-adapted thresholds based on spectral power values and spindle characteristics obtained during the screening night. Whenever a spindle was detected in real-time during NREMs sleep bi-frontal short epochs of alternating currents with a spindle-like waveform were applied during verum condition. No stimulation was applied during sham condition.

**Results:** Successful online spindle detection in all participants was reflected in an increased EEG instantaneous Hilbert amplitude in the spindle frequency range around the defined stimulation start (during sham night) compared to a baseline window (unpaired t-test, p < 0.001 for all 16 participants). Furthermore, participants were successfully blinded to the condition (0/17 reported stimulation for verum condition, 2/17 for sham condition). Spindle FB-tACS led to superior sleep-related motor performance improvements (increased speed for correct trials) compared to sham (robust linear mixed model analysis, factor stimulation condition: F(1,11.8) = 5.7, p = 0.035) but had no effect on declarative memory (F(1,11.8) = 0.00, p = 0.97). Our spindle stimulation resulted in a selectively enhanced spindle activity (11-16 Hz) in a short window directly after stimulation (paired t-test, n = 15, p < 0.05) without increasing other sleep oscillations or affecting the time spent in individual sleep stages (all p > 0.1). This spindle activity increase, especially in the fast frequency range (15 - 16 Hz), was related to the FB-tACS induced speed gains (reduction in response time) in the motor sequence memory task for a specific parieto-occipital cluster (Pearson correlation r (15) = - 0.65, p = 0.009).

Finally, spindle FB-tACS led to a decrease in delta-theta activity (2-9 Hz, paired t-test, n = 15, p < 0.05).

**Conclusions:** Our results demonstrate for the first time that sleep spindles play a functional role in sleep-dependent speed gains in a motor memory task. Thus, spindle FB-tACS might be a promising therapeutic approach to target motor memory impairments found in patients suffering from neurological or psychiatric disorders such as schizophrenia [3] and in older individuals [4]. Besides sleep spindles, slow waves (NREM sleep oscillations of 1 - 4.5 Hz) have been proposed to be involved in memory consolidation1. However, the delta-theta decrease found in our study indicates that slow waves might play a limited role in motor consolidation. Furthermore, our results extend the notion that sleep spindles and slow waves cannot be modulated independently and share a reciprocal relationship [2,5].

**Keywords:** sleep spindles, transcranial current stimulation, Memory, Neurmodulation, Cognition

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**References:**


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**Background:** Real world information is often abstract, dynamic and imprecise. Deciding if changes represent random fluctuations, or alterations in underlying contexts involve probability estimations about the changing environment that can be challenging. Dysfunction of the higher cognitive processes involved may contribute to erroneous, rigid beliefs, such as delusions. Here we examined brain function as subjects made inferences about context change from noisy information. We examined associated cortical-subcortical circuitry engaging anterior (APFC) and dorsolateral prefrontal cortex (DLPFC). We hypothesized that schizophrenia-related deficits in prefrontal function might overestimate context change probabilities, and that this more chaotic worldview may subsequently be over-reinforced with resultant influence on delusion severity. We relate these effects to more basic information processing biases against less familiar in favor of preconceived or familiar information patterns, with relevance to genetic risk for schizophrenia.

**Methods:** 24 normal controls (NC) and 17 schizophrenia patients (SZ) performed an event-related fMRI task in a 3T-MRI scanner. Subjects were presented with numerical information varying noisily about an underlying integer, which occasionally shifted up or down. Subjects were to respond when they believed the underlying numerical context had changed. We fitted Bayesian models to estimate probabilities associated with change inferences. Dynamic Causal Models were used to investigate how prefrontal, parietal and midbrain circuitry interacted during uncertain context inference, and with reduced uncertainty following more information. Genetic risk for schizophrenia associated
with these findings was explored in an independent sample of 36 NC and 35 unaffected siblings (SIB) of patients during processing of intuitive number sequences along the number line or counter to it.

**Results:** Models fitting subject behavior suggest that relative to NC, SZ over-estimated context change probabilities. Here, patients engaged APFC relatively less than healthy controls, in part driven by reduced effective connectivity from DLPF to APFC. In processing subsequent information indicating reduced uncertainty of their predictions, patients engaged relatively increased mid-brain activation, driven in part by increased DLPF to midbrain connectivity. These dissociable reduced and exaggerated prefrontal-parietal and subcortical circuit functions were accentuated in patients with delusions. Analogous dissociable reduced APFC and exaggerated MB engagement occurred in SIB when processing less intuitive versus more familiar number sequences.

**Conclusions:** Patients over-estimated ambiguous context change probabilities with relatively reduced anterior frontal engagement. Subsequent reduced uncertainty about contextual state appeared over-reinforced, potentially contributing to confirmation biases and a cascade of aberrant belief processing about a more chaotic world relevant to delusions. These opposing cortical-subcortical effects relate in part to dysfunctional imbalances in neural processing of less familiar versus preconceived or familiar information patterns, and are associated with genetic risk for schizophrenia.

**Keywords:** Delusions, probabilistic reasoning, anterior prefrontal cortex, dopamine, schizophrenia genetics

**Disclosures:** Nothing to disclose.

T193. Recollection and Familiarity of Social Recognition Memory in Schizophrenia: Performance and Relationship to Functional Outcome across the Phase of Illness


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**Background:** Although episodic memory impairment has been extensively studied in schizophrenia and is considered as a promising candidate for an endophenotype, less is known about how patients remember social relationships they encounter in everyday life. Specifically we do not know whether patients remember qualitative details of social relationships (i.e., recollection) or rely on the familiarity of social interactions (i.e., familiarity). Further we do not know how patients remember associated social context and whether social recognition memory changes over the course of illness. Thus, using 3 clinical samples across the phase of illness and a novel Social Remember-Know (RK) Task, this study investigated the following research questions: 1) do probands across phase of illness show impaired social recognition memory and, if so, do deficits occur on both recollection and familiarity memory? 2) Do probands across phase of illness show deficits in identifying social context? And 3) is impaired social recognition memory related to daily functioning?

**Methods:** Participants included 55 individuals with the prodromal syndrome for psychosis (Prodromal), 80 first-episode schizophrenia patients (First-episode) and 49 chronic schizophrenia patients (Chronic), and their demographically matched controls (n’s 42, 49, and 36, respectively). The Social RK Task was designed as an incidental memory task. During the encoding phase, participants watched 24 clips each lasting 20 seconds, which depicted diverse social relationship (e.g., married couple looking at family photos). After a 10-minute break, recollection versus familiarity-based memory was assessed for pictures of people shown in clips. For 24 “target” faces from the clips and 24 foil faces, they were first asked to decide whether the face was shown in a clip or not (i.e., OLD or NEW). For each face they identified as “OLD”, they were asked to decide whether they remembered specifics about a face in a clip (recollection memory) or did not remember specifics but the face looked familiar (familiarity memory). The main dependent variable was d prime for recollection and familiarity. To examine social relationship context, they also completed 24 forced-choice questions in which they were asked to decide for 24 faces which of 4 short statements best describes the social relationship that target was engaged in. The main dependent variable was the percent accuracy.

We administered the MATRICS Consensus Cognitive Battery (MCCB) for nonsocial cognition. Probands also completed the Brief Psychiatric Rating Scale (BPRS), the Brief version of the UC San Diego Performance-based Skills Assessment (UPSA), and the Role Functional Scale (RFS) for community functioning.

**Results:** For the Social R-K task, 2 (memory type: recollection versus familiarity) by 2 (group) by 3 (phase) repeated measures ANOVA showed a significant group effect and a significant memory by group interaction. Probands showed overall poorer recognition memory than controls; further, probands showed poorer recollection memory compared to controls, but comparable familiarity memory. Regarding social context identification, the 2 (group) by 3 (phase) ANOVA showed a significant group effect, a significant phase effect, and a significant group by phase interaction. Overall the Prodromal subjects performed best, the Chronic subjects the poorest, and the First-episode subjects showed intermediate performance. A significant group by phase interaction was explained because probands showed poorer performance than controls only in First-episode and Chronic samples.

Within the Chronic sample, better social recollection was related to better social functioning, and better social context recognition was associated with better role and social functioning. Within the First-episode sample, better social recollection and social context recognition were related to better functional capacity, but not current functioning. For both the Chronic and First-episode samples, these associations remained significant when controlling for general cognition or nonsocial recognition memory. Finally, there was no relationship between social recognition memory and functioning in the Prodromal sample.
Conclusions: Using relatively large samples of patients and controls, this study examined social recognition across the phase of illness. When examining recollection versus familiarity of social relationship, we found similar patterns of impairment – impaired recollection but intact familiarity memory – across the phase of illness. However, the prodromal probands showed better social context identification than first-episode or chronic patients. This overall pattern indicates that impaired social recollection may be an endophenotype marker, whereas impaired social context memory may be a marker for illness. The findings also suggest that, as the illness progresses, impaired social recognition memory may have a bigger impact on functional impairment in schizophrenia.

Keywords: schizophrenia, phase of illness, social recognition memory, recollection, familiarity

Disclosures: Dr. Nuechterlein has been serving as a consultant to Janssen Scientific Affairs, LLC, Otsuka America Pharmaceutical, Inc., and Janssen-Cilag and received research support from Janssen Scientific Affairs, LLC, Posit Science Corporation, and Genentech Inc. Dr. Bearden is a consultant to the Los Angeles County Department of Mental Health and Boehringer Ingelheim Pharmaceuticals and is a co-inventor on a pending patent for a blood-based predictive biomarker for psychosis. Dr. Cannon has been serving as a consultant to the National Health Research Institute (NHRI) dataset from 2001 to 2011. The relationship of full siblings was identified based on shared parents. Only two or more siblings retrieved from 3 one-million cohorts (2000, 2005, 2010 year) of Database were enrolled into the studying sibling cohort (n = 221,755) with a total of 112,910 pairs. The prevalence of SZ was calculated for the individuals with and without SZ siblings. Odds Ratios (ORs) with 95% CIs were also calculated using the ratio of the siblings with and without SZ to identify the sibling risk for major depression (MDD), bipolar disorder (BD) and SZ.

Results: Subjects, who has at least one sibling with SZ, were more likely to have 8-fold greater prevalence in having SZ disorder (prevalence rate, 4.69% vs. 0.54%). Moreover, the relative risk was extremely higher among the twin siblings (prevalence rate, 30.77% vs. 0.36%; RR = 85.7). The ORs were 9.0 (6.90-11.74) for developing SZ, 4.65 (3.14-6.89) for BD, 2.22 (1.61-3.07) for MDD and 2.57 (2.18-3.04) for major psychiatric disorders in total. In addition, the effect of the same-sex sibling was found to have greater risk to develop SZ. The ORs of SZ for the same-sex siblings were 10.24 (6.6-15.87) and 10.59 (6.15-18.24) for SZ male and female siblings, respectively; however, for different-sex siblings, the ORs were decreased to 7 (3.9-12.56) in male siblings and 7.6 (4.24-13.61) in female siblings. Despite that, the effect of gender was different among siblings to develop bipolar disorders. The ORs for male to develop bipolar disorders were around 5.8, regardless of SZ male or female siblings while the OR for female to develop BD was higher with SZ male siblings (OR = 5.9, 3.15-11.2) than with SZ female siblings (OR = 1.37, 0.34-5.5, p = NS), indicating that for female, there is no significant difference of prevalence rate of BD development with either male or female SZ siblings.

Conclusions: Conclusion: This is the first large-scale epidemiological study, which provided the evidence of a significant degree of risk in genetic transferring among the sibling of SZ focusing on SZ and BD estimate.

Keywords: schizophrenia, Bipolar Disorder, sibling, risk, population -based

Disclosures: Financial support from Taipei Veterans General Hospital E-Proposal.

T194. Sibling Risk of Schizophrenia Patients in Taiwan: First Large–Scale National Population-Based Study from 2001 to 2011

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Background: The degree of genetic transmission of schizophrenia (SZ) in Han Chinese appears to have a greater risk for developing major psychiatric disorders, but estimates of individual sibling risks are unclear. Hence we evaluate the sibling risk of patients with the diagnosis of SZ in Taiwan from 2001 to 2011.

Methods: Method: A cohort of individuals registered in the National Health Research Institute (NHRI) dataset from 1996 to 2011 was used. The relationship of full siblings was

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T195. Paliperidone Palmitate 3-month vs 1-month Formulation in Patients with Schizophrenia: A Randomized, Double-Blind, Noninferiority Study

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Background: The recently developed, 3-month (3M) injection-interval formulation of paliperidone palmitate (PP) offers a new option for the treatment of schizophrenia. PP3M provides a sustained release formulation of paliperidone, thereby permitting a significantly extended dosing interval, i.e. 4 doses per year. This study aimed to demonstrate the comparative efficacy (noninferiority) of PP3M to the previously marketed PP 1-month formulation (PP1M), in patients with schizophrenia, previously stabilized on PP1M.

Methods: This was a randomized, double-blind (DB), parallel-group, multicenter, phase-3 study (NCT01515423)
conducted between April 2012 and March 2015, at 199 sites across 26 countries. Men or women aged 18 to 70 years, diagnosed with schizophrenia DSM-IV-TR and a total Positive and Negative Syndrome Scale (PANSS) score between 70 and 120 at screening and baseline were enrolled. After screening (up to 3 weeks), patients entered a 17-week, flexible-dose, open-label (OL) phase to receive PP1M (day 1 [150 mg eq. deltoid], day 8 [100 mg eq. deltoid.], weeks 5, 9 and 13 [50, 75, 100, or 150 mg eq., deltoid/gluteal]). Patients who were clinically stable based on pre-defined criteria after the OL phase entered the 48-week DB phase and were randomized (1:1) to receive fixed doses of either PP1M (50, 75, 100, or 150 mg eq.) or a 3.5 multiple of PP3M (175, 263, 350, or 525 mg eq.), in the deltoid or gluteal muscle. Injections occurred every 4 weeks (from week 17 – 61); to maintain blinding, patients from the PP3M group received matched placebo injections monthly when they did not receive active medication.

The primary efficacy endpoint was the percentage of patients who remained relapse-free (based on Kaplan-Meier estimates) at the end of the DB phase. Secondary efficacy endpoints included the changes from baseline (DB phase) in PANSS total and subscale scores, Clinical Global Impression-Score (CGI) score, and Personal and Social Performance (PSP) score, during the DB phase. Safety, tolerability and pharmacokinetics were also assessed.

Results: Overall, 1,429 patients were enrolled and dosed during the OL phase, and 1,016 were randomized to receive PP3M (n = 504) or PP1M (n = 512) in the DB phase. Of 1,016 patients, 948 were included in the per-protocol analysis set (PP3M, n = 458; PP1M, n = 490) and 995 were included in the modified intent-to-treat DB (mITT-DB) analysis set (PP3M, n = 483; PP1M, n = 512). Overall, 842/1,016 (83%) patients completed the DB phase (including patients with relapse). In the OL phase, the majority of patients were men and white (both 55%), with a mean age of 38.4 (SD 11.86) years. Demographics and baseline characteristics were similar between the PP3M and PP1M groups in the DB phase. The primary efficacy endpoint (based on per-protocol analysis set) showed a similar rate of relapse in patients receiving PP3M (n = 37, 8.1%) and PP1M (n = 45, 9.2%). The Kaplan-Meier estimate of the difference between the PP3M and PP1M treatment groups who remained relapse free was 1.2% (95% confidence interval [CI], -2.7%, 5.1%), where the lower bound of the 95% CI was larger than the prespecified noninferiority margin of -15%. Secondary efficacy (based on mITT-DB analysis set) results were consistent with the primary efficacy findings showing similar efficacy for PP3M and PP1M.

Mean (SD) changes from DB baseline to DB endpoint (PP3M vs PP1M) were: PANSS total score: -3.5 (12.50) vs -4.3 (11.78); CGI-S score: -0.1 (0.84) vs -0.1 (0.75); PSP score: 1.3 (10.22) vs 1.9 (9.21). For change from DB baseline to DB endpoint (PP3M vs PP1M) the least square mean difference (95% CI) between PP3M and PP1M were: PANSS total score: 0.9 (-0.61, 2.34); CGI-S score: 0.0 (-0.05, 0.13); PSP score: -0.5 (-1.73, 0.64). Overall, in the OL phase, 59.2% (n = 846) of patients reported treatment-emergent adverse events (TEAEs) and 7.1% of patients reported 1 or more serious TEAEs. During the DB phase, a similar percentage of patients in the PP3M and PP1M groups experienced TEAEs (67.9% vs 66.4%). There were 6 deaths during the study (OL phase: n = 2 [arteriosclerosis and cardiac arrest; 1 each]; DB phase: n = 4, PP3M: n = 1 [hepatocellular carcinoma], PP1M: n = 3 [suicide attempt, toxicity to various agents, and bacterial meningitis; 1 each]). Of these, for 5 deaths the causality was not related or considered to be doubtfully related to study drug, whereas for one death (toxicity to various agents) the causality was not reported. The most common (>5% patients) TEAEs during the DB phase (PP3M vs PP1M) were increased weight (20.8% vs 21.3%), nasopharyngitis (7.1% vs 6.4%), anxiety (5.4% vs 4.7%), and headache (3.6% vs 5.1%). The TEAEs related to extrapyramidal symptoms, suicidality, agitation and aggression, somnolence and sedation, tachycardia, orthostatic hypotension, QT prolongation, potentially prolactin-related, and weight gain were also comparable between the PP3M and PP1M groups.

Conclusions: The results of this study demonstrate that the efficacy of PP3M was noninferior to PP1M in patients with schizophrenia previously stabilized on PP1M. PP3M had similar tolerability profile as PP1M, with no new safety signals detected.

Keywords: schizophrenia, long-acting-antipsychotic, relapse prevention, clinical trial, paliperidone

Disclosures: Dr. Fleischhacker is an employee of the Medical University Innsbruck; and reports research grants from Otsuka, Janssen Cilag and Lundbeck; advisory board honoraria from Lundbeck, Roche, Otsuka, Janssen Cilag, Takeda, Amgen, Teva and Targacept; speaker honoraria from Lundbeck, Janssen Cilag, Otsuka, Roche and Takeda; and own stocks of MedAvante. All the other authors are employees and/or shareholders of Janssen Research & Development or Janssen-Cilag Polska Sp. z o.o. All authors meet ICMJE criteria and all those who fulfilled those criteria are listed as authors. All authors had access to the study data and made the final decision about where to present these data.

T196. Cognitive Deficit in Schizophrenia: Specific or General?

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Background: Schizophrenia is characterized by a general cognitive impairment as reflected by mean IQ scores of 1-2 standard deviations below norms. There are many studies in the literature showing differences between patients with schizophrenia in different areas of functioning of the brain, including results of neuropsychological tests in the domains of memory, attention, visuo-spatial abilities, verbal abilities, executive functions, arithmetic abilities, processing speed, abstract thinking and social cognition, among others. However, many of these neuropsychological findings are affected by generalized cognitive abilities, and at least part of these findings in specific neuropsychological domains might be due to the generalized cognitive impairment present in schizophrenia, and not directly caused by the illness. The aim of the
The current study is to differentiate which of the documented findings in neuropsychology in schizophrenia are related to the generalized cognitive deficit, hence are expected to diminish and/or disappear when schizophrenic patients are compared to controls matched for IQ, age and gender. This, as opposed to those findings which will still be apparent when comparing cases to IQ matched controls, hence might be considered core deficits of the illness.

**Methods:** Subjects were 28 patients with schizophrenia, 28 controls matched for full scale WAIS-III IQ, age and gender, and an additional control group of 28 subjects matched for age and gender. In order to examine their neuropsychological profile, all groups underwent standardized neuropsychological testing using the NIMH approved MATRICS battery for schizophrenia (MCCB). In addition, all subjects underwent an assessment of their social cognition using the University of Pennsylvania Computerized Neuropsychological Test Battery (PENN-CNP). All subjects underwent a structural MRI and an fMRI N-back working memory task. The N-back task for letters had three different conditions: 0 back, 1 back, and 2 back.

**Results:** Schizophrenia patients had a higher Verbal IQ \((t(23) = 2.03, p < 0.05)\) and a lower Performance IQ \((t(23) = -3.27, p < 0.05)\) compared to non-matched controls. Schizophrenia patients had higher scores than their IQ matched controls on the verbal understanding index \((t(23) = 2.51, p < 0.05)\) and were lower on conceptual organization \((t(23) = 2.698, p < 0.05)\) and processing speed \((t(23) = -2.16, p < 0.05)\) indices. In the MATRICS domains scores, significant difference was found only on the processing speed domain: schizophrenia patients had lower scores \((M = 38.29, SD = 6.28)\) than both control groups \((M = 44.9, SD = 10.15)\); \((t(23) = -3.23, p < 0.01)\). No significant differences were found in the other MATRICS domains (verbal learning, visual learning, attention and vigilance, reasoning and problem solving, and working memory). The PENN-CNP emotion battery analyses revealed a significant difference between the schizophrenia group and both control groups in the emotion discrimination task. No significant differences were found for the other PENN-CNP tasks. The structural MRI revealed significant differences between the schizophrenia group and the non IQ-matched control group, showing that subjects with schizophrenia had smaller intracranial volume, total brain volume, and ventricles brain ratio \((p < 0.05)\). However, no significant difference was found between the schizophrenia group and the IQ matched controls in these parameters \((p > 0.05)\). The fMRI N-back working memory task showed that at the high vs. low memory load \((2\text{back} > 1\text{back})\) no significant differences were found in brain activations between schizophrenia patients and IQ matched controls. However, when compared to non-matched controls, schizophrenia patients presented significantly more wide-spread patterns of activation.

**Conclusions:** These results indicate that when matched for IQ, many of the differences often observed between schizophrenia patients and normal controls are attenuated or disappear. These data might indicate that many of the differences often observed between schizophrenia patients and controls are driven by differences in IQ and not by the illness itself.

**Keywords:** schizophrenia, cognitive deficit, neuropsychology

**Disclosures:** Nothing to disclose.

**T197. Transcriptome Profiling of Layer 3 Parvalbumin Neurons from the Dorsolateral Prefrontal Cortex of Schizophrenia Subjects**

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**Background:** Alterations in markers of cortical GABA neurotransmission are among the most widely replicated findings in postmortem studies of subjects with schizophrenia. Cortical GABA neurons containing the calcium-binding protein parvalbumin (PV) have reciprocal connections with excitatory pyramidal cells (PCs), forming a microcircuit thought to be important in generation of gamma oscillations, a process that is disrupted in schizophrenia subjects. We have previously used cell-type specific microarray profiling to examine transcriptome alterations in layer 3 PCs from the dorsolateral prefrontal cortex (DLPFC) of schizophrenia subjects and found differences in the expression of numerous genes representing a variety of biochemical pathways. In this study, we used a similar strategy to interrogate schizophrenia-related transcriptome alterations in layer 3 PV neurons from the DLPFC.

**Methods:** Using a novel immunohistochemical approach to label PV neurons (identified by the presence of the aggrecan-labeled perineuronal nets), coupled with laser microdissection and microarray profiling, we analyzed the transcriptome of layer 3 PV cells from the DLPFC in 36 matched pairs of schizophrenia and healthy comparison subjects. A random intercept model, which accounts for possible confounding effects of multiple various covariates, was used to identify differentially expressed transcripts. These transcripts were then analyzed using Ingenuity Pathway Analysis software to identify PV cell-specific biochemical pathway alterations which were then compared to our previous analysis of layer 3 pyramidal cells from the same subjects. In addition, gene coexpression network analysis was used to compare coexpression network structure between PV cells from healthy and schizophrenia subjects.

**Results:** Over 600 differentially expressed transcripts were identified in PV neurons from schizophrenia subjects, with the majority of these transcripts showing increased expression in the disease. Pathway analyses of the differentially expressed transcripts identified molecular pathways involved in mitochondrial function and energy production; pathways previously identified as altered in pyramidal neurons from the same subjects. Interestingly, several pathways identified in both cell types, including those involved in energy production, had significantly more differentially expressed transcripts in PCs compared to PV cells. Lastly, analysis of gene coexpression networks from PV cells identified alterations in other pathways in schizophrenia subjects, including several related to the extracellular matrix (ECM).
Conclusions: The identification of disrupted mitochondrial function and energy production in both PV interneurons and pyramidal cells suggests a hypoxic DLPFC in schizophrenia subjects. Furthermore, the identification of transcriptome and pathway alterations specific to either PCs or PV cells, including altered interactions between PV cells and the extracellular matrix, suggests cell-type specific alterations are also present in schizophrenia subjects. These findings may explain previously characterized alterations in the ECM around PV cells in schizophrenia subjects as well as point toward novel cell-type specific therapeutic targets.

Keywords: schizophrenia, parvalbumin, microarray, mitochondria

Disclosures: DA Lewis currently receives investigator-initiated research support from Pfizer and in 2012-2014 served as a consultant in the areas of target identification and validation and new compound development to Autifony, Bristol-Myers Squibb, Concert Pharmaceuticals, and Sunovion. J Corradi and A He were employees of Bristol-Myers Squibb during the conceptualization and data collection phases of this study.

T198. Disruption of Metabolic Coupling to Glutamate Uptake Systems Contributes to the Pathophysiology of Schizophrenia

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Background: Animal models of psychiatric disorders have provided evidence for a link between glutamate transporter expression, glutamate spillover and behavioral endophenotypes. Glutamate buffering, by the transporter EAAT2 via a bind and release or bind and transport mechanism, regulates synaptic levels of glutamate and can shape synaptic NMDA and AMPA receptor activation. Alterations in the localization of EAAT2 can result in defects in neuroplasticity, and EAAT2 is spatially and functionally coupled with metabolic factors, mitochondria, glycolytic enzymes and structural proteins. We have previously found changes the localization of EAAT2 in schizophrenia (SCZ), and we posit that these alterations are likely driven by defects of neuroenergetic processes. To explore this hypothesis, we investigated the interface between metabolic pathway constituents and the glutamate transport system in the frontal cortex in SCZ.

Methods: EAAT2 protein complexes were affinity purified from DLPFC tissue homogenate using agarose resin-conjugated rabbit anti EAAT2 antibody. Mass spectrometry (MS) was conducted using an ABSciez 5600 + TripleTOF mass spectrometer at the University of Cincinnati. Data independent acquisition was used to identify and quantify peptides and data was analysed using Protalizer. Bioinformatic analysis, using Ingenuity pathway analysis, identified biological processes and pathways in the EAAT2 interactome that are implicated in SCZ. Frontal cortex tissue was fractionated via serial density centrifugation resulting in glisome and synaptosome fractions to examine altered compartmentalization of EAAT2 associated proteins in glial and neuronal tissues. Astrocytes and pyramidal cells were cut from 14 μm thick DLPFC sections by laser capture microdissection. RT-PCR measured mRNA expression of metabolic targets, including the lactate transporter MCT1. Protein expression of targets was quantified using Western blotting in gliosome and synaptosome fractions. Immuno-gold electron microscopy (IEM) confirmed the ultrastructural changes in localization of EAAT2 and MCT1, in layers III and IV of the DLPFC. These changes were quantified by measuring the distance between immuno-gold labelled proteins and mitochondria and counting labelled mitochondria and synapses in astrocytes and neurons. The enzyme activity of lactate dehydrogenase was measured in brain homogenate and laser captured pyramidal cells and astrocytes using commercially available kits. Kinase activity was studied using PamiStation12 microarray. Akt expression and activity were measured by immunoblot and commercially available kits.

Results: 68 EAAT2-interacting proteins with ≥ 1.2 fold change in SCZ vs control subjects were identified using MS. Bioinformatic analysis implicated these proteins in processes including glycolysis, ATP synthesis/breakdown and protein scaffolding and cell junction regulation, suggesting remodeling of the astroglial-neuron interface in chronic SCZ. In cell-level studies of laser captured astrocytes and pyramidal cells, mRNA expression of MCT1 was significantly increased in SCZ. Ectonucleosidase ENTPD1 mRNA levels were significantly downregulated in astrocytes but not pyramidal cells in SCZ. These targets are key components of the lactate shuttle and adenosine signaling pathways implying disruption of these pathways in SCZ that are cell-type specific. Protein levels of metabolic enzymes lactate dehydrogenase and hexokinase, expressed in the EAAT2 interactome, were robustly expressed in synaptosomes and gliosome fractions. IEM studies examined spatial compartmentalization of glutamate transporters and mitochondria with metabolic factors, which is necessary for glutamatergic transmission. EAAT2 immuno-gold labelling was higher on astrocytes than neurons with some localization to mitochondria and post synaptic densities. The distance between asymmetric synapses and EAAT2 labelling was greater in SCZ subjects, indicating altered ultrastructural localization in this disorder. These results, combined with upregulated MCT1 mRNA levels, suggest the increase in MCT1 expression is compensatory. Interestingly, lactate dehydrogenase activity is significantly decreased in SCZ, suggesting a reduction in lactate synthesis, a primary metabolic defect most likely in astrocytes; this finding supports a compensatory role for the increased lactate transport capacity associated with MCT1 in gliosomes and synaptosomes. Kinome array analysis implicated altered Akt, Erk and Jnk signaling pathways in SCZ. Akt is a potent activator of EAAT2 but a significant reduction of phophoAKT levels, reduced overall Akt activation and increased specific kinase activity in SCZ suggests dysregulation of EAAT2 in this disorder.

Conclusions: In chronic SCZ there is evidence of pervasive metabolic disturbances and neuroplastic deficits that are not well understood. The EAAT2 protein-protein interactome provides a substrate where disruptions in the coupling of metabolic processes to structural elements by protein-protein interactions can be studied. These results demonstrate disruptions in neuroenergetics at region level, at cell-level with neurons possibly playing a compensatory role in promoting compensatory metabolic pathways to mitigate losses in glutamate uptake.
role in response to metabolic defects seen in astroglia, and at the ultrastructural level with changes in the spatial juxtaposition of EAAT2 localization with synapses. Taken together, these findings suggest that deficits of the interface between neuroenergetic processes which support synaptic function and astroglial glutamate reuptake systems contribute to the pathophysiology of SCZ.

Keywords: glutamate transport, mass spectrometry, post-mortem

Disclosures: My wife is a consultant for Janssen

T199. The Parvalbumin Interneuron-Enriched microRNA, miR-206, Regulates Cortical GABAergic Transmission and Schizophrenia-Related Behaviors in Mice

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Background: Schizophrenia (SZ) is a debilitating, heterogeneous psychiatric disorder characterized by positive symptoms such as psychosis and negative symptoms including social, emotional, and cognitive deficits. Parvalbumin (PV)-expressing interneurons regulate the oscillatory dynamics of cortex that are key for higher-order information processing. Schizophrenia is associated with decreased number and/or function of PV interneurons in prefrontal cortex (PFC), which may contribute to the behavioral and cognitive symptoms of the disorder. MicroRNAs are enriched in the nervous system and are being increasingly implicated in the regulation of neuronal function, dysfunction, and psychiatric disorders. Interestingly, microRNA-206 (miR-206) is highly expressed in PV interneurons in neocortex. Human genetics data suggest that allelic variation in the miR-206 gene increases vulnerability to schizophrenia. Also, gene expression data from dorsolateral PFC of human schizophrenia patients shows that miR-206 levels are correlated with psychosis. These findings suggest that miR-206 may regulate the structure and function of cortical PV interneurons and thereby influence behaviors relevant to schizophrenia.

Methods: miR-206 conditional knockout (KO) mice were created by homologous recombination in embryonic stem cells followed by blastocyst injection. Resulting chimeras were bred with germline FLP-expressing mice to delete the neomycin resistance cassette, generating the miR-206 floxed allele, or with Cre-expressing mice to generate the miR-206 null allele. Constitutive miR-206 KO mice were obtained from miR-206 heterozygous matings. Proper targeting and deletion of the miR-206 locus was confirmed by Southern blot, PCR, and quantitative PCR for mature miR-206 in brain. miR-206 was conditionally deleted in various brain regions by injection of AAV-Cre-GFP virus into miR-206 floxed animals. Locomotor activity of adult mice was assessed by automated beam break detection in the open field, while cognitive ability was tested by training mice to perform a delayed non-match-to-place operant task. Pre-pulse inhibition of the acoustic startle response was quantified following exposure to 120 dB startle stimulus alone or with 4, 8, and 16 dB prepulesses preceding the startle stimulus. Other behaviors analyzed included latency to fall from an accelerating rotarod across three sessions and % freezing in response to 0.6 mV foot shock. Whole-cell patch clamp of medial prefrontal cortex pyramidal neurons was performed in acute slices prepared from adult male wildtype (WT) and miR-206 KO littermates. Miniature IPSCs (mIPSCs) were recorded over five minutes, and frequency and amplitude was quantified. For immunofluorescence studies, fixed brain sections from WT and miR-206 KO mice were stained with antibodies against GABAergic cell markers including GAD67 and PV. All data were analyzed by Student’s t-test or ANOVA, as appropriate.

Results: miR-206 constitutive KO mice are fertile, grossly normal, and display normal Mendelian inheritance. Baseline open field locomotor activity and performance on an accelerating rotarod are unaltered in miR-206 KO mice relative to WT littermates, suggesting that general motor function and coordination are unaffected. Compared to WT littermates, miR-206 null mice display no differences in amplitude of the acoustic startle response. However, KO mice demonstrate significantly impaired prepulse inhibition, likely due to deficits in sensorimotor gating. Impaired PPI is a hallmark of schizophrenia (SZ) in humans; other symptoms of SZ include heightened anxiety and cognitive dysfunction. miR-206 KO mice also display increased anxiety-related behaviors in response to stressful stimuli, including increased freezing following electrical shock and increased immobility following acute restraint stress. Moreover, female miR-206 KO mice are slower to learn a delayed non-match-to-place operant task, while male KOs have an increased percentage of omitted trials, cognitive deficits often associated with dysfunctional prefrontal cortical circuitry. Regional deletion of miR-206 by injection of AAV-Cre in PFC of miR-206 floxed mice induced PPI deficits. Consistent with a role for miR-206 in regulating cortical GABAergic function, whole-cell electrophysiological recordings revealed that mIPSC frequency was decreased in PFC pyramidal neurons of miR-206 KO mice.

Conclusions: miR-206 appears to be critical for proper inhibitory cortical transmission neurons and regulates the expression of schizophrenia-relevant behavioral deficits, including sensorimotor gating deficits, cognitive dysfunction and anxiety-like behaviors. Thus, miR-206 may be a candidate for the development of novel therapeutics relevant to schizophrenia.

Keywords: MicroRNA, schizophrenia, parvalbumin interneurons

Disclosures: Nothing to disclose.

T200. Heritability of Brain Structure and Glutamate Levels in the Anterior Cingulate and Left Thalamus Assessed with MR: A Twin Study

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Background: Changes in global and regional brain volumes in schizophrenia are known to be heritable and to
cosegregate with illness (McDonald et al., 2002; Peper et al., 2007). Changes in neurochemistry — and particularly changes in glutamate — are most likely linked to changes in brain volume (Kraguljac et al., 2013) but investigations on heritability of glutamate levels are sparse. Several genes associated with glutamate transmission were suggested to be involved in the pathophysiology of schizophrenia (Ripke et al., 2014). Moreover, altered glutamatergic neurochemistry was found in frontal and thalamic areas of both patients with schizophrenia and in prodromal subjects (Egerton et al., 2012; Stone et al., 2009; Théberge et al., 2002). Here we present our initial findings of a twin study in which we assess the heritability of regional cerebral glutamate levels as well as structural brain volumes.

**Methods:** Population: 18 monozygotic, 13 dizygotic twin pairs con- or discordant for schizophrenia (ICD-10, F. 20-29), 16 monozygotic healthy control pairs and 10 dizygotic healthy control pairs. Nine additional twins were included without their siblings. A 3D-T1W structural image and 1H nuclear magnetic resonance spectra (PRESS) was obtained from each subject using a 3 Tesla Philips MRI system. Total brain (TB), Gray matter (GM), white matter (WM), peripheral CSF (vCSF) volumes were calculated using the SIENAX tool provided with FSL. MRS data from the left thalamus and anterior cingulate cortex (ACC) (bilaterally) were processed using LCModel. Metabolite concentrations (Cramer-Rao Lower bound < 20; Full-width half maximum < 12 Hz) were referenced to internal water and corrected for CSF contamination. Outliers detected by Tukey's outlier labelling were discarded from further analyses.

**Results:** Brain volumes: ANOVA revealed a significant effect of group (probands, healthy co-twins, healthy controls) for normalized WM (F2,119 = 3.18; p = 0.0453) and TB (F2,119 = 3.49; p = 0.0338). No group effects were detected for pGM, vCSF, and GM. Post-hoc testing revealed significant reductions in the WM (t = 2.25; p = 0.025) and TB (t = 2.46; p = 0.016) of probands compared to healthy controls and trend-level significant reductions in healthy co-twins compared to healthy controls (p = 0.056 for WM and p = 0.061 for TB). Correlations within twin pairs were significant for both MZ and DZ pairs for all volume parameters (both normalized and absolute values), but only significantly higher in MZ compared to DZ pairs when considering absolute volumes.

MRS: No effect of group was identified by ANOVA. Significant correlations (positive) were found in monozygotic twin pairs in both the ACC (n = 56, r = 0.484, p = 0.009)) and the left thalamus (n = 56, r = 0.444, p = 0.018), but not in dizygotic twin pairs (ACC: n = 40, r = -0.123, p = 0.606; left thalamus: n = 40, r = 0.030, p = 0.902). No significant differences were detected between the correlations of monozygotic versus dizygotic twins.

**Conclusions:** These initial results confirm previous findings of reduced total brain and white matter volume in patients with schizophrenia compared to healthy controls. The stronger correlations in MZ compared to DZ pairs (absolute volumes) suggests a genetic contribution. Moreover, we found suggestive evidence that glutamate levels in the anterior cingulate cortex and thalamus are heritable. Future work includes a more sophisticated analysis of heritability using structural equation modeling (OpenMx) and investigating the potential of these measures to serve as an endophenotypic marker for schizophrenia.

**Keywords:** schizophrenia, neurochemistry, brain volumes, twins

**Disclosures:** Nothing to disclose.

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**T201. DNA Methylation of the RalA Binding Protein 1 Gene and Metabolic Syndrome Risk in Schizophrenia and Bipolar Disorder**

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**Background:** Metabolic syndrome is highly prevalent in schizophrenia and bipolar disorders due to various factors including lifestyle, genetics and medication use. The high rates of metabolic syndrome lead to cardiovascular disease and increased mortality. Previous work in animal models has identified the ralA binding protein (RALBP1) as playing a major role in the pathogenesis of metabolic syndrome. It is thought that this protein translates oxidative stress into the components of metabolic syndrome, namely hypertension, obesity, dyslipidemia and glucose dysregulation. To date, assessment of this gene’s regulation has not been translated to humans. This study aimed to translate preclinical work by analyzing whether the methylation of RALBP1 is associated with metabolic syndrome in schizophrenia and bipolar disorder.

**Methods:** Peripheral blood samples were utilized for epigenetic analysis from an ongoing cross-sectional study. DSM-IV diagnosed schizophrenia and bipolar subjects were included if they had been receiving an antipsychotic for at least 6 months with no changes. Subjects were excluded if a diagnosis of diabetes occurred prior to antipsychotic treatment or if they had an active substance abuse diagnosis. DNA methylation of 7 CpG sites was assessed in the RALBP1 gene using site-specific pyrosequencing in Chromosome 18 from position 9475607 to 9481258 and analyzed using multivariate regression in order to statistically control for known confounders.

**Results:** The average age of the included 186 schizophrenia and bipolar subjects was 46.9 ± 10.1 years, 55.0% were female, 75.0% were Caucasian, 48.9% had metabolic syndrome and 65.0% were on an antipsychotic known to cause weight gain (olanzapine, clozapine, quetiapine, risperidone and paliperidone). There were no significant demographic differences between the subjects with metabolic or without metabolic syndrome or between schizophrrenia and bipolar subjects with the exception that the bipolar subjects tended to have a higher percentage of females and the metabolic syndrome group was older. When analyzing the association between the presence of metabolic syndrome and RALBP1 CpG site while controlling for age, race, gender and antipsychotic use, only CpG site Chr18:9478570 was statistically significant (beta = 24.9, p = 0.011).

**Conclusions:** This is the first study to translate preclinical findings of a novel protein’s effect on the pathogenesis of metabolic syndrome in a human population at high risk for metabolic syndrome. Our results may suggest that regulation of this gene, through DNA methylation, could be an
important biomarker to prevent metabolic syndrome in the severely mentally ill. Future work will need to understand the lifestyle, environmental and medication factors that may influence this gene's methylation and thus risk for metabolic syndrome.

**Keywords:** Epigenetics, metabolic syndrome, Antipsychotic

**Disclosures:** Nothing to disclose.

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T202. Auditory Mismatch Negativity in Schizophrenia: A Neurodevelopmental Perspective

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**Background:** Abnormal auditory mismatch negativity (MMN) is a core feature of schizophrenia. In schizophrenia, auditory MMN has been associated with subtle disorganization in thought and language. Prior studies (Bishop DV et al., 2011) suggest a developmental trajectory for MMN, specifically to pitch or frequency deviants, from childhood through adolescence to adulthood. To gain insight into the potential time course over which MMN deficits might develop in schizophrenia, we obtained MMN to unattended, pitch, duration and intensity deviant stimuli (Friedman TJ et al., 2012) in both an adult schizophrenia and community-ascertained developmental cohort.

**Methods:** Forty-three healthy individuals, ages 6 to 25 (mean(SD) = 17.4 (5.5)), were ascertained from the community (NKI-Rockland Sample; Nooner et al., 2012). The 43 healthy individuals in this developmental cohort, and 30 adult schizophrenia patients, participated in a simultaneous visual and auditory ERPs experiment, in which attended visual ERPs were obtained to low- and high-spatial frequency stimuli, and simultaneously auditory MMN was obtained to unattended pitch, duration and intensity deviant stimuli (Friedman TJ et al., 2012).

**Results:** Mean amplitude of MMN to pitch deviant stimuli significantly increased with age, replicating the prior study by Bishop DV et al., 2011; MMN to pitch in children (ages 6-12) and adolescents (ages 13-17) significantly differed from that of adults (18-25). Mean amplitude of MMN to pitch deviant stimuli in schizophrenia patients was comparable to that of children and adolescents, suggesting early occurrence of deficit. In schizophrenia patients, MMN was correlated with PANSS ratings of thought disorder, particularly for duration and intensity.

**Conclusions:** In schizophrenia, cognitive impairments manifest as deficits in neurophysiological responses to simple auditory and visual stimuli, implicating glutamatergic pathways. Plotting these EEG-based deficits in respect to age-related normal trajectories can inform pathophysiological mechanisms of schizophrenia in a developmental cohort. Examining symptom correlates can inform mechanisms that may underlie different symptom domains in schizophrenia.

**Keywords:** mismatch negativity, schizophrenia, development

**Disclosures:** Nothing to disclose.

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T203. Maintenance ECT for Clozapine Resistant Schizophrenia

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**Background:** Clozapine is indicated for the treatment of medication-resistant schizophrenia. Nonetheless, up to 70% of patients who tolerate an adequate trial of clozapine fail to benefit from or partially respond to this drug. Historically, response to clozapine is defined as 20 or 30% reduction in the 4 psychosis items (delusions, hallucinations, disorganization and paranoia) in the Brief Psychiatric Rating Scale (BPRS-PS). In a randomized, controlled, single blind, NIMH-sponsored study we evaluated the efficacy of electroconvulsive therapy (ECT) as an augmentation strategy for the treatment of clozapine-resistant schizophrenia. Patients with schizophrenia receiving a stable dose of clozapine with serum levels > 250 mcg/ml for at least 8 weeks, with persistent psychotic symptoms (> 12 in the BPRS psychosis subscale) and no current 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 for 8 weeks. Patients in the pharmacotherapy arm, who did not respond after 8 weeks, crossed over to the ECT arm and received the combination treatment for another 8 weeks. Using as response criterion 40% reduction in the psychosis items of BPRS, we reported response rates of 50% in the single blinded phase of the study and 48% in the cross-over phase of the study.

We report here the results for the open-label follow-up study with maintenance ECT for up to 6 months.

**Methods:** Patients who completed either the blinded or open label phases of the above mentioned study and met the a priori set response criterion of 40% reduction in the BPRS psychosis subscale (BPRS-PS) were included in the study. The continuation phase lasted for up to 24 weeks during which patients received bilateral ECT with the same treatment parameters as in the acute phase. We followed a tapered schedule of 4 weekly ECT, followed by 4 ECT every 2 weeks and 2 monthly ECT for a total of 10 treatments in 6 months. Medication regimen including clozapine remained the same as in the acute phase and no additional psychotropic medications were allowed. Psychopathology ratings were performed at baseline (end of acute phase) before each ECT and at the end of the study.

**Results:** Nineteen patients who met the aforementioned response criteria were offered maintenance ECT for up to 6 months. Thirteen patients agreed to participate maintenance ECT for up to 6 months. Thirteen patients agreed to participate maintenance treatments for 1-2 months (4-7 maintenance treatments, but opted to discontinue before the completion of 6 months. None of the
13 patients had relapsed at the time they exited the study. For those who completed the 6-month period the mean BPRS-PS was 7.83 (±3.6) at baseline and 7.66 (±2.48) at the exit. For those who received 4-7 maintenance ECT BPRS-P was 8.0 (±3.83) at baseline and 8.5 (±3.96) at the exit. All 7 patients who received maintenance ECT for less than 6 months stated that they felt well and there was no further need for ECT, or could not continue for practical reasons (mostly lack of social support as outpatients). No patient discontinued the treatment because of side effects or worsening of psychotic symptoms.

The combination of ECT and clozapine was generally well tolerated and no unusual side effects were observed.

Conclusions: These data suggest that maintenance ECT seems to be protective against relapse for those patients who responded to ECT augmentation to clozapine, at least in the first six months after the acute phase. Further research is needed to confirm the efficacy and establish the optimal duration and frequency of maintenance ECT.

Keywords: electroconvulsive therapy, schizophrenia, clozapine, Treatment resistance

Disclosures: Nothing to disclose.

T204. Resolving “Deficit” and “Distress” Schizophrenia Subgroups from Positive and Negative Syndrome Scale Data

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Background: Years of research have produced considerable evidence that a form of schizophrenia characterized by enduring negative symptoms and low levels of emotionality or distress (“deficit”) is “a separate disease within the syndrome of schizophrenia” (Kirkpatrick, Buchanan et al., 2001). Other lines of work indicate that psychological and biological traits particularly associated with high emotionality and distress (“distress”) – neuroticism/negative affect, anxiety and depression, stress sensitivity, cortisol levels – are linked with positive and affective symptom configurations in schizophrenia but not with negative or deficit symptom configurations. Apart from discordant symptomatology, individuals in deficit and distress categories differ sharply in development, clinical course and behavior, and show certain distinct biological markers, perhaps signaling different etiologies. Interestingly, the deficit and distress constructs may correspond to core psychopathological processes (e.g., anhedonia and “anxious misery” [Krueger, 1999]) and to dispositional characteristics in the general population (e.g., negative and positive affect). The analyses described here tested whether subgroups showing these contrasting symptom configurations were present in a large schizophrenia sample and how subgroups differed on clinical, cognitive, and personality variables.

Methods: Data for the Positive and Negative Syndrome Scale data, a comprehensive cognitive test battery, and personality inventories, and other clinical and developmental data were available for 549 people with schizophrenia or schizoaffective disorder. Negative and distress symptom composite scores, only, were used as indicators in two-step cluster analyses (SPSS). ROC curve analyses tested whether the pattern of negative and distress composite scores underlying the cluster discrimination followed the expected pattern. GLM, logistic regression, and chi-square analyses, and bar graphs, were used to compare symptom subgroups on demographic, clinical, cognitive and personality variables that were external to the clustering, controlling for age, sex and race/ethnicity. Correlation analyses, before and after adjusting for cluster membership, examined the associations dimensionally.

Results: Cluster analyses divided the sample into low symptom (n = 301), distress (n = 121), and deficit (n = 127) subgroups. Those in deficit cluster were significantly younger than others in the sample. Sex and race were similar across clusters. ROC curve analyses showed that the clustering largely followed two dimensions suggested by previous research – deficit schizophrenia was separated from the rest of the sample according to a “negative minus distress” score and distress schizophrenia was distinguished on the basis of the distress score only. Relative to the low symptom group, the deficit and distress subgroups had comparably higher total PANSS symptoms (p’s < .001) and were similarly functionally and vocationally impaired (e.g., GAF p’s < .001 and currently working p’s < .01, respectively), but showed markedly different patterns on variables external to the clustering. In addition to severe negative symptoms and below average distress, the deficit group was characterized by greater disorganization (p’s < .001) and impaired cognition and educational attainment (e.g., IQ p’s < .001) relative the other subgroups. In addition to high distress symptom ratings, the distress group showed the highest positive symptoms (p’s < .001) and abnormally high scores on personality dimensions associated with anxiety and depression (e.g., harm avoidance p’s < .001).

Conclusions: The diagnosis of schizophrenia and psychiatric diagnostic systems in general have been strongly criticized for being polythetic – permitting the same diagnosis on the basis of very different, often non-overlapping, combinations of symptoms. The field seeks etiological and pathophysiological indicators of specific subtypes and facets of psychiatric illness, to refine diagnosis and treatment and to advance research and neurobiological understanding (Cuthbert & Insel, 2013), but progress has been slow. Using negative and distress symptom dimensions that lie at the intersection of well-developed lines of schizophrenia research we distinguished sizable subgroups from a larger sample. The schizophrenia subgroups identified here were strikingly different in patterns of symptoms and clinical markers, personality, and cognitive/academic performance, with some evidence of developmental distinctions. In short, while these groups showed similar levels of overall symptomatology and similarly poor everyday functioning, they arrived at these outcomes with very different experiences and characteristics, likely by very different routes. These differences may be equally important for etiological investigation and clinical planning.

Keywords: PANSS, Schizophrenia, Cognition, Cluster Analysis, Psychosis

Disclosures: Nothing to disclose.
T205. Understanding the Relationship Between Neuroinflammation and Cognitive Outcomes in First-Episode Psychosis

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**Background:** Recent years have brought renewed interest regarding the involvement and role of neuroinflammation in psychiatric diseases. Psychosis, especially during its first episode, is characterized by acute symptoms, and, according to some reports, increased levels of pro-inflammatory cytokines in CSF and blood. Limited evidence also suggests anti-inflammatory drugs might alter the course of psychosis during the first break. Yet, despite this renewed interest, the cause, location, or exact timing of the neuroinflammatory response in patients with psychosis is still unclear. Previous neuroimaging studies have reported increased extracellular free-water, a potential indicator of neuroinflammation, in recent-onset schizophrenia patients (Pasternak et al, 2012). Here, we extend this approach to a new cohort of first episode patients with psychosis to better understand the timing and functional significance of the increased neuroinflammatory response.

**Methods:** High-resolution diffusion weighted imaging (DWI) data was acquired on a 3-Tesla scanner in 63 patients experiencing a first-episode of psychosis and 70 healthy control subjects recruited from the Zucker Hillside Hospital, part of the North Shore-LIJ Health System in NY. We applied the free-water imaging analysis, which deconstructs the diffusion signal into two maps: free water (FW), a measure of the amount of extracellular water diffusion in each voxel, and the fractional anisotropy of the tissue (FA-t) in each voxel (Pasternak et al, 2009). In addition, a conventional fractional anisotropy (FA) map was calculated and the white matter skeleton was generated using a whole brain, automated analytic pipeline (ENIGMA TBSS). Group comparisons of FA, FW and FA-t projected onto the skeleton were calculated using nonparametric permutation-based tests with a threshold free cluster enhancement and family-wise error correction. The FW and FA-t values were also correlated with scores from the MATRICS Consensus Cognitive Battery that were collected at baseline and 12 weeks for patients.

**Results:** Our study revealed lower FA across the whole brain in first episode psychosis patients compared to healthy controls. Similar to previously published findings, FA changes were primarily mediated by significant increases in FW. These effects were relatively widespread, encompassing regions previously implicated in the neurobiology of psychotic disorders. In contrast, lower FA-t was also observed in patients, but only in small segments of the corpus callosum, left corona radiata, and left superior longitudinal fasciculus. Moreover, among patients, higher FW at the time of the scan was correlated with better neuropsychological functioning 12 weeks later. There were no significant differences between previously treated versus antipsychotic drug-naı¨ve patients suggesting that the observed effects were not influenced by prior treatment.

**Conclusions:** This is the first study to show that increased FW in white matter tracts during the initial presentation of psychosis may represent a potentially beneficial neuroinflammatory response to the underlying biological cause of psychosis. Moreover, our findings of neuroinflammation in white matter tracts at the first-episode of psychosis are highly consistent with prior work. Specifically, the FW effects observed in the current study were widespread throughout the brain and more pronounced compared to axonal degeneration, which was observed in relatively circumscribed regions. In summary, the use of FW imaging provides a first step towards a more complete understanding of the potential relationship between early inflammation and cognitive outcomes in psychosis.

**Keywords:** Diffusion Weighted Imaging, First-episode psychosis, Neuroinflammation

**Disclosures:** Nothing to disclose.


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**Background:** Positive allosteric modulators (PAMs) of the alpha7 nicotinic acetylcholine receptor (α7nAChR) better preserve the nature of endogenous cholinergic transmission than direct agonists because agonists stimulate receptors independent of presynaptic activity. PAMs’ effects depend upon cholinergic activity, with the extent of potentiation of transmitter release interacting with levels of ACh/choline in cellular models. Thus, nicotinic PAMs may have considerable potential as cognition-enhancing drugs. Since this effect on transmitter release has only been demonstrated in vitro, we determined if it would hold true in vivo by using an experimental protocol designed to a) dose-dependently evoke glutamate and choline release in PFC from awake rats and b) improve rodent performance on an attention task. Importantly, the glutamate release depends upon cholinergic activity at the α7nAChR.

**Methods:** Adult male Wistar rats received unilateral infusion cannulae into their nucleus accumbens shell (NaccSh) and a choline- or glutamate-sensitive biosensor in their ipsilateral medial prefrontal cortex (mPFC). On each of 3 consecutive test days, rats were infused with NMDA (aCSF, 0.05, or 0.30 μg/0.5 μL) 40 minutes after a systemic injection of either AVL3288 (type I PAM, 5% DMSO vehicle, 1, or 3 mg/kg) or PNU120596 (type II PAM, 5% DMSO vehicle, 3, or 9 mg/kg), and extracellular levels of choline and glutamate were measured in separate groups of rats.

**Results:** Infusion of aCSF into NaccSh did not evoke choline or glutamate release in mPFC, and neither PAM potentiated glutamate under these conditions. The low dose of AVL3288 (1 mg/kg) potentiated glutamate release after both the low (0.05 μg; 24.2% increase) and high (0.30 μg; 84.7% increase) doses of NMDA, relative to NMDA + vehicle. The high
T207. Accumbens Regulation of Prefrontal Glutamate and Dopamine Release Requires Stimulation of Cortical Alpha7 Nicotinic Receptors

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Background: Cognitive control is mediated by a distributed neural system containing the hippocampus, nucleus accumbens (NAC), basal forebrain (BF), medial dorsal thalamus, basal lateral amygdala, and prefrontal cortex (PFC). Dysregulations in this system contribute to the cognitive deficits seen in several neuropsychiatric disorders (e.g. schizophrenia, ADD, and drug addiction). Previously we demonstrated that stimulation of the NAC shell with NMDA evoked ACh release in the PFC and that this release was cognitively beneficial, increasing resistance to distractors in a sustained attention task. We also demonstrated, using a biosensor with sec-to-sec resolution, that NMDA activation elevates PFC choline and glutamate levels. Here we determined if a) NAC-evoked glutamate levels were also seen using more traditional microdialysis methods; b) NAC activation also increased DA release in PFC; and c) the relative contributions of nicotinic receptor subtypes to glutamate and DA release.

Methods: Rats were implanted with an infusion cannula into the NACshell and either a biosensor or microdialysis probe in the ipsilateral mPFC. NMDA was infused and extracellular levels of choline, glutamate, and DA were measured. In a separate group, the role of nicotinic (α7, α4β2) receptors in this stimulated release was determined following local perfusions (1.0 or 10.0 μM) of mecamylamine (MEC), α-bungarotoxin (α-BGT), or DHβE. The TTX (0.1 μM) dependency of basal and evoked glutamate and DA release was also determined.

Results: Infusions of NMDA (0.05, 0.15, or 0.30 μg/0.5 μL) produced dose dependent increases in choline (0.87 ± 0.15, 1.61 ± 0.17, and 1.73 ± 0.31 μM above baseline) and glutamate (2.01 ± 0.32, 3.34 ± 0.37, and 4.56 ± 0.42 μM above baseline) for each of the 3 NMDA doses as measured with the biosensor. The evoked choline and glutamate signals were rapidly cleared to basal levels in ~30 sec. Microdialysis-based measures also revealed an NMDA (0.30 μg) stimulation of glutamate efflux in PFC (75% increase). These levels were not cleared to baseline until 20 min later. Intra-NAC NMDA also increased prefrontal DA levels in PFC (100% increase). Evoked levels of glutamate and DA efflux (microdialysis) were dependent upon activation of cortical nicotinic receptors (α7) as local MEC or α-BGT blocked the increases. Blockade of α4β2 receptors (DHβE) resulted in partial reductions. Finally, TTX blocked stimulated release of glutamate and DA but only affected basal DA.

Conclusions: The results confirm, using two methods, the limbic stimulation of prefrontal acetylcholine (choline), glutamate, and DA release. Evoked release of glutamate and DA are secondary to the release of ACh/choline and, presumably, a subsequent activation of local nicotinic (α7) receptors on glutamate and DA terminals. The difference in clearance times suggest different pools of glutamate may be sampled by the MEA vs the dialysis probe. Research indicates that transient ACh/choline release is critical for cue detection and this release may also facilitate performance in tasks measuring working memory and cognitive flexibility.

Keywords: prefrontal cortex, glutamate, dopamine, microdialysis, alpha-7 nicotinic acetylcholine receptor

Disclosures: Nothing to disclose.

T208. Preclinical Abuse Potential Assessment of Flibanserin: Effects on Intracranial Self-Stimulation in Female and Male Rat

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Background: Flibanserin is a serotonin receptor subtype 1A (5HT1A) agonist and 2A (5HT2A) antagonist under consideration by the Food and Drug Administration for treating female sexual interest/arousal disorder. Preclinical abuse potential testing of flibanserin is warranted given its proposed clinical indication, its effects on central nervous system monoaminergic function, and its pharmacological similarity to the selective 5HT1A agonist 8-OH-DPAT, which produces abuse-related drug effects in some preclinical procedures. This study examined behavioral effects of flibanserin in an intracranial self-stimulation (ICSS) procedure that has been used previously to examine abuse-related effects of other drugs.

Methods: Adult female and male Sprague-Dawley rats with electrodes implanted in the medial forebrain bundle were trained to lever press for brain stimulation under a “frequency-rate” ICSS procedure. Initial studies determined the acute effect of flibanserin (1.0-10 mg/kg) and time course of effect at 10 mg/kg on ICSS. Subsequently, effects of flibanserin (3.2-18 mg/kg) were redetermined before and after five days administration of 5.6 mg/kg/day flibanserin. The effect of an acute dose of 1.0 mg/kg amphetamine was tested as a positive control.
Results: Both acute and repeated administration of flibanserin produced only rate-decreasing effects on ICSS, with female rats exhibiting greater decreases than male rats. Amphetamine produced significant ICSS facilitation that was equivalent between female and male rats.

Conclusions: These results suggest that flibanserin has low abuse potential. This study also provides evidence for sex differences in the rate-decreasing effects of flibanserin, with greater ICSS depression in females than males.

Keywords: Abuse Potential, flibanserin, intracranial self-stimulation

Disclosures: Nothing to disclose.

T209. EEG as an Information Transfer Device

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Background: Recently, many lines of investigation in neuroscience and statistical physics have converged to raise the hypothesis that the underlying pattern of neuronal activation which results in electroencephalography (EEG) signals is via power-law distributed neuronal avalanches, with self-affine dynamics, while scalp-recorded EEG signals themselves are nonstationary. Therefore, spectral analysis of EEG analysis may miss many properties inherent in such signals. A complete understanding of such dynamical systems implies knowledge of the thermodynamics. However, a complete thermodynamic assessment of non-equilibrium cortical dynamics is impossible with current technology. Principles of Information Equilibrium (IE) have been demonstrated to successfully characterize many systems far from equilibrium.

Methods: We apply principles of IE to model EEG as a system that transfers information over time.

Results: We find that human EEG is surprisingly well-modeled as an information transfer device, and that waking consciousness is readily distinguished from sleep stages 2 and 3 by differences in patterns of information transfer constants.

Conclusions: Application of IE to EEG may be useful in the study of cognitive neuroscience and states of brain pathology.

Keywords: Quantitative Electroencephalography (qEEG), sleep, Cognitive Neuroscience

Disclosures: Nothing to disclose.

T210. Effects of Chronic Adolescent Exposure to Cannabis Smoke on Adult Behavioral Outcomes

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Background: Cannabis (marijuana) is the most widely used illicit drug in the US and worldwide, and consumption among adolescents and young adults is rising. Animal studies have shown that adolescent exposure to delta 9-tetrahydrocannabinol (THC) or synthetic cannabinoid receptor type 1 (CB1) agonists causes alterations in cognition and measures of anxiety- and depression-like behavior upon maturation to adulthood. It is not known, however, whether similar behavioral alterations result from exposure to cannabis via smoke inhalation, which is the most common route of administration in humans. To begin to address these questions, we used a rat model to determine how exposure to cannabis smoke during adolescence affects behavioral and cognitive outcomes in adulthood.

Methods: Exposure sessions were conducted using an automated cigarette smoking machine. Male Long-Evans rats were placed into clean standard rat cages with wire lids, which were placed into exposure chambers connected to the cigarette smoking machine. Rats (n = 12/group) were exposed to smoke generated by burning either cannabis (5.3% THC) or placebo cannabis (<0.01% THC) cigarettes obtained from the NIDA Drug Supply program. In a previous study we showed that this exposure regimen leads to plasma THC levels of about 200 ng/ml. An additional group (air control, n = 12) was exposed to the same apparatus in the absence of smoke. Exposure sessions (approximately 1 h each) began on P29 and were conducted daily for 21 days. Following the final exposure session on P49, rats were left undisturbed in their home cages. Evaluation of the effects of cannabis smoke exposure on behavioral outcomes began with testing in an elevated plus maze (P76) followed by testing in a 120 x 120 cm open field (P84). Rats were then tested for 10 days in a serial probabilistic reversal learning task in operant test chambers, beginning on P98.

Results: The cannabis cigarettes produced smoke at a concentration of about 210 total suspended particulate (TSP)/m3 and CO levels of about 250 ppm. Placebo cigarettes produced smoke at a concentration of about 70 TSP/m3 and CO levels of about 240 ppm. Rats exposed to cannabis smoke showed significantly less of an increase in body weight compared to both placebo smoke and air control groups. This difference emerged during the exposure period, and was evident until at least P86. Despite these body weight differences, behavioral testing in adulthood revealed no differences between groups on either the elevated plus maze (percentage of open arm entries and percentage of time in open arms) or the open field (total distance traveled and entries into the center zone). On the probabilistic reversal learning task, there were no differences between groups on the number of reversals completed per session. Cannabis smoke-exposed rats had longer choice latencies than the other two groups, however, which could suggest a reduction in task motivation.

Conclusions: Previous studies in rats have found that adolescent exposure to THC or synthetic CB1 agonists can increase anxiety-like behavior and impair some aspects of cognition during adulthood. The current experiment was designed to determine whether exposure to cannabis smoke produces similar outcomes. The data collected to date revealed no effects of cannabis smoke exposure on several measures of anxiety-like behavior. There were similarly no effects on choice accuracy
in a reversal learning task, although cannabis-exposed rats showed some evidence of reduced motivation to perform the task. Ongoing experiments are testing additional measures of cognition and motivation to determine how they are affected by adolescent cannabis smoke exposure.

Keywords: cannabis, marijuana, Adolescence, addiction, Smoking

Disclosures: Nothing to disclose.

T211. Impairment of Neuroplasticity by Binge Drinking of Alcohol: A Paired Associative Stimulation Study

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Background: Binge drinking, resulting in acute alcohol intoxication, is considered an initial step in developing alcohol use disorders (AUDs). It has been suggested that alcohol intoxication may act on mechanisms of neuroplasticity to produce brain changes that contribute to the pathophysiology of AUDs. However, the effect of binge drinking on neuroplasticity has not been evaluated in humans. The current study was aimed at evaluating the effect of a binge drinking episode on LTP-like neuroplasticity.

Methods: In a within-subject randomized, cross-over design, fifteen otherwise healthy binge drinkers were administered paired associative stimulation (PAS) following consumption of alcohol or a placebo beverage. PAS is an experimental paradigm that allows for the induction of associative long-term potentiation (LTP)-like neuroplasticity. Subjects were administered alcohol at a dose of 1.5g/l of body water, producing a peak blood alcohol concentration (BAC) of 26.1mM (0.120% BAC). PAS induced neuroplasticity was measured at Post 0 (immediately following PAS), Post 15 (15 minutes following PAS), Post 30 (30 minutes following PAS), Post 60 (60 minutes following PAS) and Post Day 1 (the next day following PAS).

Results: The binge drinking episode inhibited LTP-like neuroplasticity, which was significantly different from placebo at 30 and 60 min following the PAS administration. Examination of longitudinal effects revealed no differences between alcohol and placebo beverages on LTP-like neuroplasticity the following day.

Conclusions: Findings suggest that binge drinking impairs neuroplasticity acutely. These effects are no longer evident the day after a single binge session. However, the long-lasting effects of repetitive binging on neuroplasticity are not yet clear. In individuals with AUDs, repetitive binging may contribute to long-lasting changes in neuroplasticity that contribute to the pathophysiology of the illness. Future studies examining PAS-induced neuroplasticity in abstinent individuals with an AUD will reveal whether baseline aberrancies in neuroplasticity exist in this population.

Keywords: binge drinking, Alcohol, transcranial magnetic stimulation, Neuroplasticity, alcohol use disorder

Disclosures: Nothing to disclose.

T212. Role of α5 Subunit Containing Nicotinic Receptors in Modulating Striatal Dopamine Release and Cue Evoked Learning

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Background: A loss of function single nucleotide polymorphism (SNP) in the gene coding for the α5 subunit of nicotinic receptors (nAChRs) is associated in chronic human smokers with an increase in cigarette consumption and enhanced pleasurable smoking experiences. Overall, this translates into a two fold increased likelihood for nicotine dependence. Alpha5 subunit expression is observed in layer 6 of the cortex, the hippocampus, the medial habenula, interpeduncular nucleus, the striatum and the ventral tegmental area. Interestingly, several studies report that developmental α5 KO animals self-administer more nicotine and will do so at higher doses. Given the expression pattern of α5 subunits and their implication in addiction we were interested in examining the role of these subunits in modulating striatal dopamine (DA) release as well as cognitive and motivational aspects of behavior.

Methods: To investigate the influence of these subunits on DA release in the ventral striatum we utilized a viral conditional knock-down (KD) strategy to selectively target expression in the VTA of Long-Evans rats. We then measured nicotine’s (.5mg/kg S.C.) modulation of DA release in the nucleus accumbens shell of urethane anesthetized animals using Fast Scan Cyclic Voltammetry (FSCV) while electrically stimulating the medial forebrain bundle. To examine for potential effects of nicotine on the dynamic range of DA release at the level of DA terminals we then performed a series of FSCV rat ex vivo slice experiments with varying concentrations of nicotine in the bath and varied local electrical stimulation pulse number and frequency parameters. To investigate the role of the α5 subunits in shaping motivation we directly infused virus in the ventral striatum of Sprague-Dawley rats and tested their performance on a Pavlovian Conditioned Approach (PCA) task in response to nicotine administration.

Results: In anesthetized rats VTA-α5 KD in the VTA lead to a significant increase in DA release in response to high dose nicotine. In contrast, control animals had reduced DA release in the presence of high dose nicotine. As reported elsewhere we observed a nAChR-desensitization related increase in the dynamic range of DA release in both controls and VTA-α5 KD animals. However, the increase in dynamic range from VTA-α5 KD animals was observed at bath nicotine concentrations several orders of magnitude lower than that for control animals. At a behavioral level, in the PCA task, control animals showed a nicotine mediated increase in the probability and speed of cue-evoked approach behaviors that was greatly diminished in ventral striatum-α5 KD animals.

Conclusions: Future experiments will investigate the influence of PFC α5 KD in a sustained attention task. Overall, through its influence on the kinetics of DA release, cue-evoked learning and attention, SNPs of the α5 nAChRs subunit are positioned to have a significant impact on...
reward and motivational processes that mediate not just the liability to nicotine addiction but potentially to other addictive substances as well.

**Keywords:** addiction, Dopamine, nicotinic acetylcholine receptors, alpha 5, motivation

**Disclosures:** All authors are Pfizer employees.

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**T213. Infralimbic PFC and Nucleus Accumbens Shell Connectivity in the Regulation of Habitual Reward Seeking**

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**Background:** The ability to flexibly regulate behavior is impaired in a number of neuropsychiatric disorders, including addiction. This loss of behavioral flexibility may involve a transition from goal-directed actions, where rewards are sought for their reinforcing properties, to habitual behaviors which are not performed in relationship to their outcome. Response strategy selection is mediated in part by corticostriatal circuitry. Previous research has implicated the infralimbic PFC (IL) in the expression of habitual reward seeking. For example, loss of IL function has been shown to restore goal-directed action in rodents that have acquired habitual reward seeking. While studies of extinction learning have implicated IL projections to the nucleus accumbens shell (NAcS) in the regulation of reward seeking, the downstream target of IL projections that mediate habitual behavior have not yet been assessed.

**Methods:** In order to investigate the role of NAcS glutamate signaling and the precise glutamatergic projections to this structure that mediate habitual reward seeking, we used pharmacological and pharmacogenetic strategies in tandem with a novel procedure in mice for testing habitual reward seeking. For mice used in pharmacological experiments, a bilateral cannula targeting the NAcS was implanted for the focal administration of drug. For mice used in the pharmacogenetic studies designed to dissect the precise circuits required for the regulation of response strategy, a microinjection of the inhibitory DREADD under control of the CAMKII promoter was infused into the IL. One half of these mice also received a bilateral cannula targeting the NAcS in order to selectively inhibit the terminals of IL projections. After recovery from surgery, mice were trained to respond for a 10% sucrose reinforcer, initially on a fixed ratio 1 (FR1) schedule in which each active lever press resulted in delivery of a single 20 ul reinforcer. After the mice exhibited stable performance on the FR1 schedule, they were moved to a random interval 30 (RI30) schedule for 3 sessions in which the first lever press after a randomly determined interval (averaging 30 seconds) was reinforced. After 3 RI30 sessions, the schedule was changed to RI60 for 6 sessions. To test the expression of habitual behavior, mice experienced a single test session in which the relationship between the action (lever press) and the outcome (sucrose delivery) was degraded by provision of non-contingent sucrose at a schedule determined by each individual’s performance. The role of NAcS glutamate signaling in contingency-mediated behavior was assessed by administering an mGlur2/3 agonist into the NAcS (0.2 ul/side) 5 min prior to testing. The effect of IL inhibition on response strategy selection was assessed by systemically administering the DREADD agonist CNO (2mg/kg) 30 min prior to testing. Lastly, to determine the role of IL projections to the NAcS, CNO was microinjected (0.2ul/side; 10 min prior to test). For each condition, the control group received a matched saline vehicle injection or cannula infusion, and mice were assigned to conditions based on response rates on the last test session. Data were analyzed using repeated measures ANOVA in SPSS.

**Results:** Our findings revealed that NAcS glutamate signaling mediates the expression of habitual reward seeking such that mGlur2/3 agonism can restore goal-directed actions (p < 0.05). In addition, IL inhibition can restore goal-directed behavior in a contingency degradation test session (p<.05). Excitingly, we further demonstrate that inhibition of IL projections to the NAcS is sufficient to mimic this effect, indicating that IL-NAcS projections are critical for the expression of habitual sucrose seeking behavior.

**Conclusions:** Together, these studies demonstrate that IL and its projections to the NAcS are critical for the expression of habitual reward seeking. In addition, regulation of NAcS glutamate signaling can restore goal-directed actions. These exciting new data suggest that glutamatergic projections from IL to NAcS are necessary for the regulation of response strategy, and indeed are critical for the expression of habitual sucrose seeking behavior. Future research is planned to investigate the contributions of additional glutamatergic projections to the NAcS, including those from the ventral hippocampus and the basolateral amygdala.

**Keywords:** infralimbic cortex, Nucleus Accumbens Shell, habit, glutamate

**Disclosures:** Nothing to disclose.

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**T214. Activation of Accumbal Nitric Interneurons and Subsequent Nitric Oxide Release Underlies Relapse to Cocaine Seeking**

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**Background:** The gaseous transmitter nitric oxide (NO) is produced in the nucleus accumbens core (NAcore) by a subpopulation of interneurons that express neuronal nitric oxide synthase (nNOS). Apart from its role as the ligand for soluble guanylyl cyclase, NO also mediates the nitrosylation and activation of matrix metalloproteinases (MMPs). Interestingly, MMP activity is required for the dendritic spine head (dh) expansion on medium spiny neurons (MSNs) in the nucleus accumbens core (NAcore), responsible for cue-induced cocaine seeking. Further, we have previously shown that cocaine exposure enhances activity of the nNOS enzyme in the NAcore leading to the nitrosylation of MMPs, while inhibition of nNOS inhibits cue-induced activation of MMPs and cocaine seeking. These data suggest
that activation of nNOS neurons and the subsequent release of NO is a crucial mediator of reinstated drug seeking.

**Methods:** In order to test the hypothesis that activation of mGluR5 in the NAcore precipitates reinstatement of cocaine seeking that is mediated by activation of nNOS interneurons, rats underwent 10 days of daily cocaine self-administration followed by 14 days of extinction training. Rats were then tested for reinstatement of drug seeking precipitated by microinfusion of the mGluR5 agonist RS-2-Chloro-5-hydroxyphenylglycine (CHPG) or by the microinfusion of CHPG + N-propyl-L-arginine (NPLA), a selective inhibitor of nNOS. In order to validate that activation of NAcore nitrergic interneurons translates to NO release in real time, evoked NO levels were measured in anesthetized animals using Nafion + o-PD coated S2 multi electrode arrays (MEA) with the Quanteon FAST16mkII system. Using a picospritzer glutamate, CHPG or CNO was puff applied directly in front of MEAs while NO levels were recorded in real-time.

**Results:** In our rodent cocaine relapse model we show here that infusion of CHPG into the NAcore reinstated drug seeking, which was blocked by the co-infusion of nNOS inhibitor NPLA. During anesthetized NO recordings in the NAcore, we show that puff application of glutamate or CHPG produced a reproducible dose-dependent increase in NO release, which was inhibited by the mGluR5 antagonist MTEP or NPLA, respectively. Moreover, NO efflux was dose-dependently evoked by stimulation of Gq-coupled designer receptors exclusively activated by designer drugs (DREADDs), selectively expressed in NAcore nitrergic interneurons.

**Conclusions:** Our results demonstrate that activation of glutamate receptors (including mGluR5) in the NAcore produced NO release. Further, we show that activation of Gq-signaling specifically in NAcore nitrergic interneurons also induced NO release. Combined, these data indicate that activation of nitrergic interneurons, a remarkably small population of cells in the NAcore, and the subsequent release of NO is a crucial step in the signal transduction cascade between cue-induced glutamate release, activation of MMPs and increased dh associated with cued cocaine seeking. These data provide insight towards new and exciting possibilities for therapeutic intervention in the prevention of chronic relapse disorder.

**Keywords:** Nucleus Accumbens, nitric oxide, DREADD Receptor

**Disclosures:** Nothing to disclose.

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**Background:** Non-invasive brain imaging has contributed important insight into the structural and functional alterations linked with drug (substance) and behavioral (non-substance) addictions. While these neuroimaging studies have typically assessed brain alterations among individuals addicted to specific drugs/behaviors, neurobiological theories of addiction often emphasize common alterations across conditions [1-5]. This gap between practice and theory highlights the need for novel strategies facilitating cross-drug/condition comparisons. Neuroimaging meta-analytic tools are available to systematically curate, quantitatively synthesize, and more fully interpret neuroimaging results; they also allow for exploration of novel research questions not generally feasible within a single neuroimaging study. Structural magnetic resonance imaging (sMRI) has been widely employed to estimate grey matter alterations among addicted versus comparison individuals. As such, we adopted a meta-analytic analysis strategy to quantitatively synthesize this body of literature in the service of identifying common grey matter alterations across drug (e.g., alcohol, nicotine, marijuana, stimulant, opiates) and behavioral addictions (e.g., gambling, internet gaming). We hypothesized that structural alterations in the insula, ventromedial prefrontal cortex (vmPFC), thalamus, and striatum represent a potential addiction-general neural signature.

**Methods:** We performed an iterative literature search to compile structural neuroimaging studies interrogating grey matter alterations among individuals with addictive disorders compared to healthy controls. We included studies in this meta-analysis that assessed grey matter using sMRI, reported a set of coordinates (i.e., foci) in a defined stereotoxic space from a whole-brain analysis, and provided sufficient information regarding participant characteristics and data analysis strategies. The studies included reported foci obtained by between-groups comparisons and distinguished alterations in grey matter by the control > addict (grey matter decrease) and addict > control (grey matter increase) directions. Given the limited number of foci, grey matter increases were not considered further. To identify common structural alterations, we performed coordinate-based meta-analysis using the revised version [6] of the Activation/Anatomical Likelihood Estimation (ALE) algorithm [7, 8]. Specifically, voxel-wise ALE scores, which quantify spatial convergence of grey matter alterations across included studies, were calculated and then compared with those from a null distribution resulting in p-value maps. To identify clusters of significant convergence, the resultant maps were thresholded at a cluster corrected-level of p < 0.05 (voxel-level: p < 0.005).

**Results:** When considering drug (e.g., alcohol, nicotine, marijuana, stimulant, opiates) and behavioral addictions (e.g., gambling, internet gaming), we identified 79 peer-reviewed articles for inclusion involving 3,086 participants with addictive disorders and 3,287 controls. The meta-analysis included 520 distinct foci from 81 experimental contrasts representing grey matter decreases among addicted individuals. Across these studies, convergent grey matter decreases were observed in the left insula, vmPFC, and mediodorsal thalamus as well as in left and right lateral prefrontal cortex, right parahippocampus, and left orbitofrontal cortex.

**Conclusions:** To elucidate structural alterations associated with addictive disorders, we conducted an anatomical meta-analysis and observed convergent grey matter reductions...
across drug and behavioral addictions notably in the insula, vmPFC, and mediodorsal thalamus. These outcomes provide meta-analytic support for addiction-general structural alterations, which may represent consequences or antecedents (or both) of addiction and contribute to dysregulated cognitive, emotional, and/or reward processing. Improved insight into the common as well as distinct brain alterations across addictive disorders may expedite the evolution of heuristic frameworks guiding future research, identification of neurobiological intervention targets, and development of strategies to fractionate the addiction phenotype by brain-based markers.

**Keywords:** addiction, neuroimaging, Insula, prefrontal cortex

**Disclosures:** The authors have no conflicts to declare and NIMH (ARL: R01-MH074457, R56-MH097870) and the NIDA Intramural Research Program (EAS).

**References:**


T216. **Cognitive Functioning as a Marker of Resting-State Connectivity in Cocaine Addiction**

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**Background:** Resting-state functional connectivity has recently emerged as a reliable marker of abnormal brain functioning in addiction, whereby compared to healthy controls drug users show deficits in prefrontal connectivity. Drug addicted individuals also show impairments on cognitive tasks that assess functions mediated by the prefrontal cortex. However, although resting-state functional connectivity has been linked to task-related functioning of discrete brain regions and to corresponding behavior in health, resting-state connectivity has rarely been investigated using a whole-brain approach and such brain-behavior studies are yet to be performed in individuals with cocaine use disorder (iCUD). Cognitive function was assessed here with the Cambridge Neuropsychological Test Automated Battery (CANTAB), which was developed to reliably measure select brain functions across cognitive domains and specific neural systems as grounded in animal neuroscience. By tapping into individual differences in both brain connectivity at rest and baseline cognitive functioning, and at the same time combining them with information about drug use, we aim to advance the study of the neurocognitive deficits underlying loss of cognitive control, in order to ultimately develop targeted trainings for preventing relapse in addiction.

**Methods:** Ten minute resting-state functional magnetic resonance imaging (fMRI) scans were acquired in iCUD (N = 44) [age 47 ± 8 yrs; iCUD+ (urine positive) N = 25, iCUD- (urine negative) N = 19] and healthy controls (N = 31; age 41 ± 8 yrs) matched on race and gender. Controls differed from iCUD in depression scores (Beck Depression Inventory) and smoking status; both were used as covariates in all between-group comparisons. Participants’ drug use histories and baseline craving were assessed by a structured interview and the Cocaine Craving Questionnaire. Participants’ cognitive profile was assessed using five CANTAB tasks of memory and executive function (listed below). For data reduction, the scores were submitted to factor analysis with oblique rotation in SPSS 22. To assess relation with brain functioning, emerging factor loadings were used as regressors in the resting-state analysis. The imaging data was preprocessed following standard procedures and analyzed with CONN toolbox (MIT, Cambridge). One connectivity matrix per subject was derived using a 638 region anatomical template, computing local and global efficiency of each brain region. While local connectivity captures within region processing, global connectivity indexes processing between different brain regions across whole brain networks. All group level analyses were performed controlling for motion during the scan and age.

**Results:** A three factor neurocognitive model, explained 59% of the variance in CANTAB scores. The Delayed Matching to Sample and Spatial Span tasks loaded on Factor 1 (“Memory”); the Intra-Extra Dimensional Set Shift loaded on Factor 2 (“Executive Functioning”); and the Stop Signal Task loaded on Factor 3 (“Inhibitory Control”). None of the loadings for the Verbal Recognition Memory task were significant (loadings < 0.40). There was a trend for a group difference on Inhibitory Control (p = 0.08). Further, lower inhibition scores were correlated with earlier age of onset of cocaine use in the iCUD- subgroup (0.42, p = 0.03).

The resting-state data analysis revealed that compared to healthy controls iCUD showed increased local efficiency (p = 0.05) in select frontal (inferior frontal gyrus), parietal (inferior/superior), cuneus and visual regions, and a trend for decreased global efficiency (p = 0.07) in other frontal [dorsal anterior cingulate (dACC), supplementary motor area], posterior cingulate and subcortical regions (e.g. putamen).

Importantly, Inhibitory Control modulated brain function in a network of regions, including the posterior cingulate, cuneus, insula and visual regions for local connectivity (p = 0.02) and the dACC, inferior and superior parietal cortex for global connectivity (p = 0.04). Specifically, in the dACC reduced global connectivity was associated with worse Inhibitory Control within iCUD. In the iCUD-subgroup, reduced dACC global connectivity was also associated with shorter lifetime cocaine use (controlling for age, r = 0.47, p = 0.02). In contrast, in the parietal cortex increased global connectivity was related to worse Inhibitory Control and higher craving in iCUD (r = 0.37, p < 0.01) and shorter duration since last drug use in the iCUD+ subgroup (r = -0.48, p = 0.04).
Conclusions: In summary, in iCUD global connectivity was reduced in the dACC and local connectivity was increased in the parietal cortex, linked to worse inhibitory control in both regions (this effect was significant for global connectivity in the dACC). Results in a subgroup of abstinent users revealed an unexpected direction of a correlation whereby decreased global connectivity in the dACC was associated with shorter lifetime cocaine use. As higher global connectivity in this region was linked to better inhibitory control, these results could support prior evidence that cocaine use may partially serve a self-medication purpose due to preexisting deficits; this speculation remains to be validated in future studies. The link between global connectivity in the parietal cortex, higher craving and shorter duration since last drug use further suggests relevance of connectivity in large-scale brain networks to drug use behaviors, as craving has been shown to predict drug taking in current users and relapse in abstinent users. The present results thus extend previous reports about altered connectivity in prefrontal regions to show that whole-brain connectivity states involved in inhibitory control are altered. Targeting these whole-brain states by cognitive training (e.g., to enhance inhibitory control) may lead to new development of relapse interventions grounded in neuroscience.

Keywords: Addiction, Cocaine, Resting state, Functional connectivity, Inhibitory control

Disclosures: Nothing to disclose.

T217. Do We Need to Treat Risk? Attitudes Toward Risk and Ambiguity in Opioid Addiction
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Background: Drug addicted individuals are thought to be excessively reckless and risky, but the relevant factors that drive these behaviors are poorly understood. Economics provides a set of tools to quantify at least three factors that distinctly contribute to an individual's propensity for risk taking: their tolerance of known risk (technical risk attitude), their tolerance of ambiguous or unknown risk (ambiguity attitude), and the randomness in their decision process (stochasticity). Therefore, to more fully understand the behavior of opioid users in risky situations, in the present study we focused on these three factors, which have not been the subject of previous experimental decomposition in addiction – although such decomposition may have important implications for understanding and potentially treating this disorder. Our specific goals were to determine the relationship of these factors to (1) a diagnosis of an opioid use disorder (OUD) and (2) relevant clinical outcomes in a longitudinal study of treatment-seekers with OUD and matched community controls (CC).

Methods: 23 individuals seeking outpatient maintenance treatment for an OUD and 21 CC participated in weekly experimental sessions (1-11 per subject, mean: 3.2) during which subjects completed a decision making task and provided information about their psychological state(s) and drug use over the previous week (i.e., since the last session). Drug use (both groups) and treatment adherence (OUD only) was objectively monitored via routine urine drug tests and chart review. The decision making task consisted of 124 trials each of which presented a choice between a certain gain of $5 and a lottery offering a chance to win more than $5 or nothing. Across trials, we varied the magnitude of the potential win ($5-$66), the probability of winning (25%, 50%, or 75%), and the level of ambiguity (how much was known about the probability of winning; 3 levels). The task was incentive compatible meaning that subjects were paid according to their actual choices. The data were analyzed with a modified power utility model that treats ambiguity as a subtrahend term to probability. From this model, we derived subject-specific parameters for technical risk attitude, ambiguity attitude, and stochasticity. We additionally computed two model-free measures that capture the quality of subjects' decisions: the percentage of trials in which the objectively better option was chosen (e.g., $5 for sure versus a 50% chance of $5) and the proportion of choices in which the option that would maximize expected task earnings was chosen.

Results: OUD subjects were more ambiguity tolerant than CC, with no other group differences reaching significance. All subjects made more choices that maximized expected earnings with experience in the study (likely due to adopting a slightly more ambiguity tolerant attitude). Despite this learning effect, weekly fluctuations in task variables predicted (and were predicted by) clinical variables. The strongest contributing factor to task behavior was heroin use in the preceding week. A positive use event predicted more risk tolerant attitudes, more stochasticity, and poorer decision making as indexed by the two model-free measures. The latter reduction in choices that maximize task earnings may be explained by an increase in reported heroin craving with use. In contrast, the only observed predictor of heroin use in the following week was increased risk tolerance (with a similar trend observed for increased ambiguity tolerance).

Conclusions: These preliminary results suggest that there is a ranking in the relative contribution of these individual factors to the behavior seen in addicts. While ambiguity attitudes most clearly differentiated OUD and CC subjects, technical risk attitudes more closely tracked recent and prospective heroin use. Perhaps surprisingly, stochasticity and overall quality of decision making fell intermediate, emerging as reliable markers of recent use. Together, these data strongly support the decomposition of risk taking behavior into its constituent driving factors for improving our understanding, and potentially treatment, of this complex disorder.

Keywords: Opioid dependence, Risk, Ambiguity, Decision-making, Treatment

Disclosures: Nothing to disclose.

T218. A Role for the Dynorphin/Kappa Opioid Receptor System in Binge-Like Alcohol Consumption in Mice
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Background: Repeated episodes of binge-like drinking may facilitate transition to a state of alcohol dependence and,
thus, increase risk for numerous long-term negative consequences associated with alcohol use disorders. Dynorphins, endogenous opioid neuropeptides, along with their principal target, kappa opioid receptors (KOR), have been implicated in excessive drinking and negative affect associated with dependence. For example, previous studies have shown that pharmacological blockade of KOR in the central nucleus of the amygdala (CeA) reduced elevated drinking associated with alcohol dependence. However, a role for KOR has not been established in the modulation of binge-like drinking. The present set of studies was designed to address this question by first examining the effects of systemic administration of a KOR agonist and a KOR antagonist on binge-like alcohol consumption. A second study employed a chemogenetic approach, examining whether selective inhibition of prodynorphin-containing neurons in the CeA influences binge-like drinking.

Methods: Binge-like alcohol consumption was assessed using the ‘drinking-in-the-dark’ (DID) model, a procedure that results in high intake and significant elevation of blood alcohol levels. The 4-day DID procedure involved providing mice a single bottle containing 20% ethanol starting 3 hours into the dark cycle for 2 hr on Days 1-3, with the drinking session extended to 4 hr on Day 4. In Study 1, adult male C57BL/6J mice received vehicle injections prior to the 2-hr drinking sessions (Days 1-3), and systemic injections (ip) of the KOR agonist U50, 488 (0 or 5 mg/kg) or the short-acting KOR antagonist LY2444296 (0, 1, 5, or 10 mg/kg) 30 min prior to the 4-hr session on Day 4 (N = 8-11/group). In Study 2, Pdyn-ires-Cre mice (N = 9) received bilateral infusions of a cre-dependent virus containing an inhibitory DREADD (hM4Di) into the CeA. After at least three weeks to ensure viral infection, mice were tested in the DID model, receiving vehicle injections (ip) on Days 1-3, and then either vehicle (saline) or clozapine-N-oxide (CNO; 3 mg/kg) 30 min prior to the 4-hr alcohol drinking test on Day 4. Histological verification of extent and location of viral expression is currently in progress.

Results: Systemic administration of the KOR agonist KOR U50, 488 (5 mg/kg) significantly elevated alcohol consumption (5.6 ± 0.2 g/kg) relative to vehicle (4.0 ± 0.3 g/kg) in the DID model. Conversely, KOR blockade via systemic administration of LY2444296 decreased alcohol intake in a dose-related manner, with significant reduction produced by 5 mg/kg (2.01 ± 0.5 g/kg) and 10 mg/kg (2.33 ± 0.3 g/kg) doses compared to 1 mg/kg (3.75 ± 0.2 g/kg) and vehicle (3.31 ± 0.3 g/kg) dose conditions. Additionally, administration of CNO (i.e., to selectively activate the inhibitory DREADD expressed in neurons containing prodynorphin in the CeA) resulted in a significant reduction binge-like alcohol consumption (2.8 ± 0.4 g/kg) compared to intake in the vehicle condition (4.1 ± 0.4 g/kg).

Conclusions: Collectively, results from these studies indicate a role for dynorphin/KOR activity in binge-like alcohol consumption. Further, results from the chemogenetic study indicate that prodynorphin-containing neurons in the CeA play a role in mediating binge-like drinking since selective inhibition of these neurons significantly reduced alcohol consumption in the DID model. Ongoing studies involve use of both excitatory and inhibitory DREADDs (along with GFP viral controls) to examine whether modulation of prodynorphin neurons in CeA produces bidirectional effects on alcohol consumption in this binge-drinking model. Taken together, results from the present studies suggest that the kappa opioid receptor system represents a promising therapeutic target for pharmacological intervention of alcohol binge-like drinking and dependence.

Keywords: Dynorphin, binge drinking, mouse model, Behavioral Pharmacology, DREADD

Disclosures: Supported by NIH grants P50 AA010761, U01 AA014095, U01 AA020929 (HCB) and F32 AA023700 (RIA), and VA Medical Research (HCB).

T219. White Matter Abnormalities in Individuals with Cocaine Use Disorder

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Background: Using diffusion-weighted MRI the general integrity of white matter can be assessed by fractional anisotropy (FA), myelination can be assessed by radial diffusivity (RD), and axonal damage can be assessed by axial diffusivity (AD). In previous MRI studies, reduced FA and increased RD were found to be associated with cocaine use disorder (CUD) implying that the differences are related to myelin. Differences were localized to large fiber tracts including the anterior corpus callosum (CC), anterior frontal white matter, body and splenium of the CC, internal capsule, superior longitudinal fasciculus, corticospinal tract, and the superior corona radiata. It has been postulated that these decreases are the result of myelin reduction caused by hypo-perfusion related to cocaine exposure. Nevertheless, conflicting results, where FA increases were observed in the anterior cingulate in individuals with CUD, were also reported. In general, in these previous studies, there was no clear consensus about the regions affected. The aim of this study is to examine the effect of CUD on white matter integrity using more powerful modern diffusion-weighted MRI acquisition.

Methods: Subjects: Diffusion-weighted MRI was performed in 46 subjects with CUD and 36 healthy controls. Groups were matched on sex, non-verbal intelligence (matrix reasoning scale of the Wechsler Abbreviate Scale of Intelligence) and ethnicity but not on age (controls: 41 ± 8 yrs, CUD: 47 ± 8 yrs, p < 0.01) which was used as covariate in all between-group comparisons. MRI: The acquisition was performed with a high-angular-resolved single shot spin echo EPI sequence with monopolar diffusion encoding [TR/TE = 407200/81.462 ms, 1.8 mm isotropic resolution over the whole brain and multi-band factor of 3 (R = 3) without in-plane acceleration]. Paired acquisitions with reversed phase encoding in the LR/RL direction were acquired, each with 64 matched diffusion-encoding directions (b = 1200 s/mm2) and 5 un-weighted (b = 0) scans. The total scan time for each acquisition was ~ 5 min. Addiction Severity Measure: Disease severity was assessed
with three metrics: the cocaine selective severity assessment scale (a measure of withdrawal signs and symptoms in the 24 hours preceding the study), cocaine craving questionnaire, and the severity of dependence scale. These metrics were combined into a composite standardized Z score (by z-transforming each metric independently and then taking the mean). Analysis: Pre-processing of the diffusion-weighted data was performed using FSL. The corrected images were fit to a tensor model to obtain FA, RD, and AD maps with MITK. Voxelwise statistical analysis of the FA data from all subjects was carried out using tract-based spatial statistics in FSL with age as a covariate.

A corrected t-statistic was obtained for the test that the FA, RD, and AD of the CUD subjects was different (both directions) than those of the healthy controls. Voxelwise linear fitting of the skeletonized FA versus the addiction severity measure was performed in MATLAB. Maps of the p-value for the correlation were computed for each voxel.

**Results:** Subjects with CUD had lower FA than controls over a wide range of white matter tracts including the corpus callosum, cingulum, internal capsule, frontal white matter, posterior corona radiata, and the anterior commissure (there were no significant differences in FA between the groups in the opposite direction, i.e., CUD > controls). The mean effect size for the reduction in FA (skeletonized FA voxels reaching significance, 0.025 < p < 0.05) was 0.45. Most areas where subjects with CUD had lower FA than controls also showed a trend for significantly higher RD in the CUD than control group (0.03 < p < 0.06). This result suggests that the FA differences are driven by increases in the RD, which are commonly interpreted to be driven by myelin damage or loss of myelin. Tests for differences in the AD did not reach significance. The composite addiction severity score was significantly and negatively correlated with FA in the anterior CC (R2 = 0.08, p = 0.02), anterior commissure (R2 = 0.1, p = 0.01), and inferior portion of the internal capsule (R2 = 0.09, p = 0.01).

**Conclusions:** In this study we report significantly lower FA across numerous white matter areas in subjects with CUD as compared to demographically matched healthy controls (and after statistically controlling for age). In contrast to previous studies that showed focal regions of decreased FA, these current results indicate a more widespread effect of CUD on white matter integrity. These results further suggest that the FA reductions are driven exclusively by increases in RD (and not AD, a measure of axonal damage), directly implicating myelin involvement. Furthermore, the regional correlation between disease severity and FA is a novel finding in the study of CUD, suggesting a cumulative or a predisposing effect of drug use on these white matter impairments. To further follow-up on these correlations, future work will be focused on contributions to results of the different severity measures (e.g., separating effects of withdrawal from those of craving) as well as potential differences between subgroups with CUD that could be attributed to abstinence length vs. an active cocaine use status.

**Keywords:** Cocaine, Diffusion Weighted Imaging, addiction, Fractional-anisotropy

**Disclosures:** Nothing to disclose.

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**T220. Nicotine Enhances Initial Sensitivity and Acute Functional Tolerance to Alcohol**

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**Background:** Alcohol drinking and tobacco smoking are strongly linked; smoking promotes alcohol drinking, particularly of hazardous levels, and individuals who use nicotine are more likely to meet criteria for alcohol dependence. In this study we aimed to examine possible behavioral mechanisms underlying the co-use of these substances. Specifically, we sought to assess the influence of a low dose of nicotine upon the subjective experience produced by alcohol with respect to changes in breath alcohol concentration i.e., increasing vs. decreasing.

**Methods:** Healthy moderate drinkers who were also light non-dependent smokers (N = 15) participated in the experiment. They completed two experimental sessions with administration of 0.6 mg/kg alcohol (ALC), one after pretreatment with 7 mg nicotine (NIC) and the other after placebo (PL, in randomized order). Before and at repeated times after drug administration, subjects completed questionnaires to rate subjective drug effects, and breath alcohol concentration was measured.

**Results:** Overall, NIC enhanced ALC-induced euphoria (p < 0.05) without influencing breath alcohol concentration. Analysis of effects with respect to limb of the breath alcohol curve showed that, in comparison to PL, pretreatment with NIC enhanced reports of “desire to drink alcohol” on the ascending limb of the breath alcohol concentration curve (p < 0.05). Later, when breath alcohol concentrations were falling, NIC attenuated reports of feeling high in comparison to PL.

**Conclusions:** These findings support those of previous studies which show that nicotine enhances the rewarding experience of alcohol without altering alcohol pharmacokinetics. The results also show that nicotine alters the level of response to alcohol by enhancing both initial sensitivity and acute functional tolerance to alcohol subjective effects. Level of response to alcohol has been linked to alcohol consumption, thus our findings suggest that this may be a mechanism by which nicotine promotes alcohol consumption and dependence. Future studies should focus on specific receptor mechanisms and systems that mediate these interactions.

**Keywords:** alcohol use disorder, nicotine, subjective effects

**Disclosures:** Nothing to disclose.

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**T221. Changes in mGluR5 Glutamate Receptor Availability Following Sensitization to D-Amphetamine: A [11C]ABP688 PET Study**

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**Background:** Sensitization to psychostimulants is thought to be a critical process indexing the neuroplastic events implicated in a variety of neuropsychiatric disorders, including schizophrenia, addiction, and some anxiety disorders. The metabotropic glutamate receptor subtype 5 (mGluR5) modulates signaling through NMDA receptors...
and regulates some forms of synaptic plasticity. In animal experiments, repeated stimulant exposure induces changes in the expression of mGluR5. The positron emission tomography (PET) ligand [11C]ABP688 is a selective allosteric antagonist at mGluR5. Recently, it has been used in several samples of experimental animals and humans to identify alterations in brain mGluR5 binding availability. The present study uses PET with [11C]ABP688 to assess the effects of psychostimulant-induced sensitization on mGluR5 availability in healthy volunteers.

**Methods:** Healthy adults were randomly assigned to receive 0.3mg/kg d-amphetamine (Dexedrine) or placebo. Participants were administered three consecutive doses approximately 2 days apart, followed by an identical challenge dose 16 days later. Behavioural drug responses were assessed through measures of motor activation including speech and eye-blink rates, as well as subjective ratings of activation and alertness using visual analog scales (VAS) and the Profile of Mood States (POMS). Behavioural sensitisation was operationally defined as a larger drug-induced change from baseline in these measures following the challenge dose compared to the first dose. All participants underwent a one-hour high-resolution PET scan with 370mBq [11C]ABP688 on day 1 and again on follow-up on day 21. Scans were performed under drug-free conditions, prior to the administration of amphetamine or placebo. Binding potential (BPND) values for mGluR5 receptors were calculated across the whole brain as well as in striatal, limbic, and cortical subregions using a simplified reference tissue model. Changes in BPND from day 1 to day 21 were calculated within each treatment group.

**Results:** To date, seven participants have completed this study. Preliminary analyses suggest that behavioural sensitisation is observed for subjective ratings of alertness and vigour. Larger changes from baseline were seen in the amphetamine group compared to placebo group, and response tended to increase with subsequent doses (mean change from baseline in VAS-Alertness score: placebo group day 1, 0.80 ± 1.3, day 21, 0.60 ± 0.89; amphetamine group day 1, 1.5 ± 2.1, day 21, 4.5 ± 0.71). [11C]ABP688 BPND values did not change systematically from scan 1 to scan 2 in the placebo group, but showed a tendency to increase following the drug regimen in the amphetamine group (mean change in whole brain BPND at follow-up: placebo group, 0.71 ± 4.8%, amphetamine group, 50 ± 16%).

**Conclusions:** These preliminary results indicate that mGluR5 expression may be altered following sensitization to d-amphetamine.

**Keywords:** Positron emission tomography, metabotropic glutamate receptor, sensitization, Psychostimulant

**Disclosures:** Nothing to disclose.

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**T222. Differences between Treatment and Non-Treatment Seeking Alcoholics: Do They Matter?**

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**Background:** While the prevalence of lifetime alcohol dependence (AD) is estimated to be 12.5%, only about a quarter of people with AD receive treatment in their lifetime. The distinction between treatment seekers and non-treatment seekers is highly relevant when considering medication development efforts. Given the prominent role of behavioral pharmacology in medication development for alcoholism, understanding the degree to which clinical and behavioral pharmacology samples are representative of one another is highly significant for informing treatment development.

**Methods:** Combining non-treatment seeking participants with AD who completed behavioral pharmacology studies in our laboratory (n = 211) with treatment seekers who participated in the COMBINE study (n = 1383), the present study addresses the following aims: (a) compare treatment seekers to non-treatment seekers on demographic and clinical variables related to AD presentation, and (b) test whether the variables found to differ across samples (if any), are indeed predictive of clinical outcomes in the COMBINE Study. Measures of interest include both demographic and clinical variables, such as Alcohol Dependence Scale (ADS), Drinker Inventory of Consequences (DrInC) and Obsessive Compulsive Drinking Scale (OCDS) scores, number of DSM-IV symptoms of AD, total drinks, and drinks per drinking day.

**Results:** Group comparisons revealed that treatment seeking participants were older (t(1592) = -15.90, p < 0.001, d = 1.18), had more years of education (t(1558) = -2.36, p = 0.018, d = 0.18), greater ADS scores (t(253) = -6.71, p < 0.001, d = 0.57), DrInC scores (t(1391) = -9.44, p < 0.001, d = 0.73), and OCDS scores (t(250) = -8.43, p < 0.001, d = 0.70), met more DSM-IV symptoms of alcohol dependence (t(253) = -11.24, p < 0.001, d = 0.95), and consumed more standard drinks in the 30 days prior to randomization (t(301) = -2.62, p = 0.009, d = 0.18) and more drinks per drinking day (t(323) = -4.34, p < 0.001, d = 0.27) compared to non-treatment seeking participants. Furthermore, relationship status (X2(2,1591) = 178.57, p < 0.001, Cramer’s V = 0.335), ethnicity (X2(3,1590) = 157.89, p < 0.001, Cramer’s V = 0.315) and sex (X2(1,1592) = 4.58, p = 0.032, Cramer’s V = 0.054) were not equally represented between treatment seeking and non-treatment seeking participants. All patient characteristics that differed between treatment seeking and non-treatment seeking participants predicted at least one clinical outcome in the COMBINE Study (i.e., time to relapse, percent days abstinent, and probability of good clinical outcome; ps < 0.05).

**Conclusions:** These data provide indirect evidence for the clinical significance of differences between treatment seekers and non-treatment seekers with AD on those measures. The results indicate that the behavioral pharmacology and clinical trial samples in this study differed significantly on various clinical and demographic variables such that differences across samples should be carefully considered and addressed in order to promote greater consilience across stages of medication development.

**Keywords:** human laboratory, CNS Clinical Trials, alcohol use disorder, Medication Development

**Disclosures:** This work was supported by the following grants: grants from ABMRF (L.A.R.), UCLA CTSI UL1TR000124 (L.A.R.), M01-RR00865 (L.A.R.), R21AA02214 (L.A.R.), F32 AA023449 (M.M.Y.), TRDRP 23FT-0102
These data support a model of aberrant NRG3-induced general impulsivity through modulation of long-term synaptic plasticity in the orbitofrontal cortex (OFC). A high-frequency tetanic stimulation paradigm induced long-term depression (LTD). Since NRG3 signals through activation of the ErbB4 receptor, we next applied Afatinib, a selective ErbB4 inhibitor, to test whether ErbB4 activation is involved in this phenomenon. We observed that the NRG3-induced LTD was attenuated by Afatinib. Furthermore, similar to NRG3, bath application of nicotine also induced LTD. This nicotine-induced LTD was also attenuated by the ErbB4 inhibitor, Afatinib, suggesting that nicotine is eliciting this response via ErbB4 activation.

Conclusions: These data suggest that nicotine may influence general impulsivity through modulation of long-term synaptic plasticity via NRG3-ErbB4 signaling in the OFC. These data support a model of aberrant NRG3-induced plasticity that may potentially underlie nicotine dependence, suggesting a new therapeutic target for smoking cessation. Current studies are evaluating the molecular mechanism of chronic nicotine treatment on NRG3-mediated synaptic plasticity in the OFC, as well as the effects of NRG3-ErbB4 singaling on impulsive behaviors.

Keywords: neuregulin-3, nicotine dependence, Synaptic Plasticity

Disclosures: Nothing to disclose.

T223. NRG3-ErbB4 Signaling Mediates Nicotine-Induced Synaptic Plasticity in Orbital Frontal Cortex

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Background: The neuregulin signaling pathway, which has been widely implicated in schizophrenia, has also been recently linked to nicotine dependence. For example, work from our lab (Turner et al, 2014) and that of our colleagues (Loukola et al, 2014) has shown that polymorphisms within two genes in the neuregulin signaling pathway, NRG3 and ErbB4, have been linked to failed smoking cessation. Cigarette smokers have difficulty quitting, which is thought to be related to deficient impulse control. Recently, neuregulin 3 (NRG3) expression in the frontal cortex was shown to regulate certain aspects of impulsivity (Loos et al, 2014). However, the mechanism by which neuregulin signaling may regulate neuronal circuitry in this area, and consequently influence impulsivity, is currently unknown. Therefore, this work aims to evaluate how NRG3 signaling impacts long-term synaptic plasticity in the orbitofrontal cortex (OFC), a brain region highly involved with impulse control, and what potential consequences this may have for nicotine dependence.

Methods: To evaluate these questions, we used electrophysiological field potential recordings in brain slices to assess whether NRG3 and nicotine alters long-term potentiation (LTP) in the OFC.

Results: We found that a high-frequency tetanic stimulation protocol could successfully induce LTP in the OFC. However, in the presence of NRG3, the same stimulus paradigm induced long-term depression (LTD). Since NRG3 signals through activation of the ErbB4 receptor, we next applied Afatinib, a selective ErbB4 inhibitor, to test whether ErbB4 activation is involved in this phenomenon. We observed that the NRG3-induced LTD was attenuated by Afatinib. Furthermore, similar to NRG3, bath application of nicotine also induced LTD. This nicotine-induced LTD was also attenuated by the ErbB4 inhibitor, Afatinib, suggesting that nicotine is eliciting this response via ErbB4 activation.

Conclusions: These data suggest that nicotine may influence general impulsivity through modulation of long-term synaptic plasticity via NRG3-ErbB4 signaling in the OFC. These data support a model of aberrant NRG3-induced plasticity that may potentially underlie nicotine dependence, suggesting a new therapeutic target for smoking cessation. Current studies are evaluating the molecular mechanism of chronic nicotine treatment on NRG3-mediated synaptic plasticity in the OFC, as well as the effects of NRG3-ErbB4 signaling on impulsive behaviors. Keywords: neuregulin-3, nicotine dependence, Synaptic Plasticity

Disclosures: Nothing to disclose.

T224. The Role of μ-Opioid Receptor (OPRM1) Gene A118G Polymorphism on Cortisol and β-Endorphin Response to Alcohol

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Background: Individuals with lower cortisol and higher beta-endorphin release after alcohol consumption have been shown to be at increased risk for alcohol use disorders. One potential moderator of alcohol response is the μ-opioid receptor (OPRM1) gene polymorphism A118G at rs1799971. The functional consequence of this polymorphism remains unclear, but pre-clinical and clinical studies indicate that the OPRM1 A118G allele is associated with increased striatal dopamine release in response to alcohol administration (Ramchandani et al., 2011), as well as moderating the effects of opioid antagonists on alcohol reward and consumption (Bilbao et al., 2015). Here we tested the hypothesis that this polymorphism may alter hypothalamic-pituitary-adrenal (HPA) axis and β-endorphin response to alcohol, potentially indicating risk for alcohol use disorders.

Methods: Twenty-eight healthy male social drinkers were classified into two groups: (1) subjects homozygous for the major 118A allele (N = 16) and (2) subjects heterozygous or homozygous for the variant 118G allele (N = 12). All participants completed the timeline follow back to assess drinking patterns over the 90 days prior to the study. Participants underwent two sessions where they received, in counter-balanced order: (1) an intravenous alcohol infusion that brought blood alcohol level (BAL) up to 80 mg % and clamped at that level for 30 min, or (2) a placebo (saline infusion) for the same duration. Plasma levels of adrenocorticotropic hormone (ACTH), cortisol, and β-endorphin were measured at baseline, 15, 30, and 45 minutes after start of the infusion. Repeated measures ANOVA was conducted to examine the effect of treatment, time, genotype, and baseline levels on change in hormone levels.

Results: ACTH, cortisol, and β-endorphin levels were similar at baseline across sessions. ACTH and cortisol levels were not significantly altered by alcohol relative to placebo infusion. β-endorphin levels were significantly lower following alcohol relative to placebo infusion [F(1, 24) = 7.98, p = 0.009]. AA homozygotes did not differ from G carriers on ACTH, cortisol, or β-endorphin response to alcohol (all ps > 0.05). Participants who drank more frequently during the previous 90 days had a trend for lower release of ACTH and β-endorphin, but these relation-
sections are used to collect high resolution images of CA1 weeks prior to preference test after which coronal brain injections of AAV-CamKII-eYFP were performed three NMDA receptor subunits and Rho family signaling proteins. Subcellular fractionation and western blot analysis of ROCK inhibitor, prior to morphine pairing. Mice were given intra-hippocampal injections of 100nM H1152, received the same doses of morphine and were placed horizontal chambers on alternating days. Home cage mice with either the horizontal or vertical striped chamber. Consecutive days. Paired mice received morphine paired with particular context induces an impairment in synaptic plasticity while the same doses of morphine paired with alternating contexts does not produce this impairment. Dendritic spines are the structural units of synaptic function and plasticity, and examining changes in spine morphology can elucidate essential signaling pathways that are involved in the remodeling of synapses. Spine morphology is controlled by actin polymerization which is regulated by NMDA receptor-mediated Ca2+ influx and activation of the Rho signaling cascade. The goal of these studies is to determine the role of contextual cues in morphine induced dendritic spine morphology and elucidate which signaling pathways are involved in morphine induced structural plasticity. Methods: For these studies three treatment groups were used; paired, unpaired and home cage. All mice received subcutaneous (s.c.) morphine (5, 8, 10 and 15 mg/kg) over 4 consecutive days. Paired mice received morphine paired with either the horizontal or vertical striped chamber. Unpaired mice received morphine in both vertical and horizontal chambers on alternating days. Home cage mice received the same doses of morphine and were placed immediately back into their home cage. Some paired mice were given intra-hippocampal injections of 100nM H1152, ROCK inhibitor, prior to morphine pairing. Mice were sacrificed for either confocal imaging or western blotting. Subcellular fractionation and western blot analysis of NMDA receptor subunits and Rho family signaling proteins were performed. For confocal imaging intra-hippocampal injections of AAV-CamKII-eYFP were performed three weeks prior to preference test after which coronal brain sections are used to collect high resolution images of CA1 pyramidal neuron dendritic segments. Additionally, the Barnes maze was used to determine if morphine CPP affects hippocampal dependent spatial learning. All experimental protocols in animal studies were approved by the Institutional Animal Care and Use Committee at Columbia University. Results: We found a decrease in thin spines independent of whether morphine was given in a paired or unpaired design. This decrease was not associated with impairment in spatial memory examined using the Barnes maze. Additionally, we found an increase in synaptosomal RhoA expression in both paired and unpaired mice. However, there was no change in either RhoA or NMDA receptor expression when mice were given morphine in their home cage. Finally, preliminary findings show that intra-hippocampal administration of H1152 prior to each morphine pairing may attenuate morphine preference in the paired design. Conclusions: Decreases in thin spines occurred in both paired and unpaired mice both of which received morphine in the context of the vertical and horizontal striped chambers. This indicates that morphine-induced decreases in thin spines may be triggered by administering morphine in a context outside their home cage environment. In support of this idea, we observed increases in synaptosomal RhoA in both paired and unpaired mice but not in mice which received morphine in their home cage. Previous studies demonstrated that increases in RhoA can mediate a loss of thin spines. This evidence suggests that the decrease in thin spines we observe may be due to an increase in synaptosomal RhoA in the paired and unpaired mice. Additionally, we have evidence that RhoA signaling may be involved in morphine preference as intra-hippocampal ROCK inhibitor, a kinase downstream of RhoA, may attenuate morphine CPP. Finally, we observed that the morphine CPP induced decrease in thin spines we observed does not affect spatial learning suggesting that although synaptic plasticity is impaired and there are less spines these mice are still capable of learning spatial information. Future studies will determine 1) if mice given morphine in their home cages have the same decreases in thin spines as the paired and unpaired mice and 2) if intra-hippocampal ROCK inhibitors administered during the acquisition of morphine CPP can reverse the decrease in thin spines. Keywords: Hippocampus, morphine, Context, spines, RhoA

Disclosures: Nothing to disclose.

T225. Morphine-Associated Contextual Cues Induce Structural Plasticity on CA1 Pyramidal Neurons in the Hippocampus
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Background: Opiate abuse is an emerging problem in the US with 2.1 million people abusing opiate prescription drugs. Relapse occurs in 80% of abstinent abusers indicating a need to develop methods to prevent cravings that drive relapse. Our previous studies demonstrate that the NMDA-NR2B subunit in the hippocampus is necessary for opiate induced behaviors and that pairing morphine with a particular context induces an impairment in synaptic plasticity while the same doses of morphine paired with alternating contexts does not produce this impairment. Dendritic spines are the structural units of synaptic function and plasticity, and examining changes in spine morphology can elucidate essential signaling pathways that are involved in the remodeling of synapses. Spine morphology is controlled by actin polymerization which is regulated by NMDA receptor-mediated Ca2+ influx and activation of the Rho signaling cascade. The goal of these studies is to determine the role of contextual cues in morphine induced dendritic spine morphology and elucidate which signaling pathways are involved in morphine induced structural plasticity.

Methods: For these studies three treatment groups were used; paired, unpaired and home cage. All mice received subcutaneous (s.c.) morphine (5, 8, 10 and 15 mg/kg) over 4 consecutive days. Paired mice received morphine paired with either the horizontal or vertical striped chamber. Unpaired mice received morphine in both vertical and horizontal chambers on alternating days. Home cage mice received the same doses of morphine and were placed immediately back into their home cage. Some paired mice were given intra-hippocampal injections of 100nM H1152, ROCK inhibitor, prior to morphine pairing. Mice were sacrificed for either confocal imaging or western blotting. Subcellular fractionation and western blot analysis of NMDA receptor subunits and Rho family signaling proteins were performed. For confocal imaging intra-hippocampal injections of AAV-CamKII-eYFP were performed three weeks prior to preference test after which coronal brain sections are used to collect high resolution images of CA1 pyramidal neuron dendritic segments. Additionally, the Barnes maze was used to determine if morphine CPP affects hippocampal dependent spatial learning. All experimental protocols in animal studies were approved by the Institutional Animal Care and Use Committee at Columbia University.

Results: We found a decrease in thin spines independent of whether morphine was given in a paired or unpaired design. This decrease was not associated with impairment in spatial memory examined using the Barnes maze. Additionally, we found an increase in synaptosomal RhoA expression in both paired and unpaired mice. However, there was no change in either RhoA or NMDA receptor expression when mice were given morphine in their home cage. Finally, preliminary findings show that intra-hippocampal administration of H1152 prior to each morphine pairing may attenuate morphine preference in the paired design.

Conclusions: Decreases in thin spines occurred in both paired and unpaired mice both of which received morphine in the context of the vertical and horizontal striped chambers. This indicates that morphine-induced decreases in thin spines may be triggered by administering morphine in a context outside their home cage environment. In support of this idea, we observed increases in synaptosomal RhoA in both paired and unpaired mice but not in mice which received morphine in their home cage. Previous studies demonstrated that increases in RhoA can mediate a loss of thin spines. This evidence suggests that the decrease in thin spines we observe may be due to an increase in synaptosomal RhoA in the paired and unpaired mice. Additionally, we have evidence that RhoA signaling may be involved in morphine preference as intra-hippocampal ROCK inhibitor, a kinase downstream of RhoA, may attenuate morphine CPP. Finally, we observed that the morphine CPP induced decrease in thin spines we observed does not affect spatial learning suggesting that although synaptic plasticity is impaired and there are less spines these mice are still capable of learning spatial information. Future studies will determine 1) if mice given morphine in their home cages have the same decreases in thin spines as the paired and unpaired mice and 2) if intra-hippocampal ROCK inhibitors administered during the acquisition of morphine CPP can reverse the decrease in thin spines.

Keywords: Hippocampus, morphine, Context, spines, RhoA

Disclosures: Nothing to disclose.

T226. Intranasal Oxytocin Reduces Cue-Induced Craving in Cigarette Smokers
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Background: Cigarette smoking is a major health concern worldwide and a leading cause of preventable death. Despite moderate success with both pharmacological and behavioral treatments, smoking relapse rates are high. Many smokers report being exposed to smoking cues or triggers in their environment, which can lead to relapse. Therefore, treatments that target cue reactivity are needed to help smokers...
successfully abstain. One potential candidate for reducing smoking craving is the neuropeptide oxytocin (OT). OT reportedly has calming effects and reduces drug self-administration in animals. However, there have been no studies examining the effect of OT on cue-induced craving in smokers. Here, we investigated the effects of intranasal OT on two types of craving for cigarettes: spontaneous craving after overnight abstinence, and craving induced by smoking-related cues, in an interim analysis of 17 daily smokers.

**Methods:** In this within-subject, placebo-controlled study, daily smokers (N = 17) abstained from smoking for 12 hours before attending two separate laboratory sessions. Participants were randomized to receive two doses of intranasal OT (20 IU per dose) nasal spray on one testing session and placebo nasal spray on the other. They rated their smoking craving before and after the first spray. After the second spray one hour later, they were exposed to smoking cues (smoking-related images followed by lighting and holding a lit cigarette) and again rated their craving.

**Results:** OT did not reduce levels of spontaneous craving after the first spray. However, OT significantly dampened increases in cigarette craving and negative mood states induced by the smoking cues.

**Conclusions:** These results provide preliminary evidence that OT can reduce cue-induced smoking craving and negative mood in smokers. These findings provide an important link between preclinical and clinical studies aimed at examining the effectiveness of OT as a novel treatment for drug craving.

**Keywords:** Smoking urge, Oxytocin, Cue-Exposure

**Disclosures:** Research supported by the University of Chicago Comprehensive Cancer Center Pilot Grant and NIMH T32MH020065.

**T227. Varenicline-Induced Elevation of Dopamine in Smokers: A Preliminary [11C]-(+)PHNO Pet Study**

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**Background:** Varenicline, a nicotinic partial agonist, may produce its therapeutic efficacy in smoking cessation by elevating dopamine (DA) in the human brain during periods when smokers are abstinent and may crave cigarettes due to withdrawal-induced negative affect. However, no study has explored this in human participants and the preclinical literature is unclear as to the ability of varenicline to elevate DA levels. [11C]-[(+)-PHNO ([11]C- ( +)-4-propyl-3,4,4a,5,6,10b-hexahydro-2H-naphtho[1,2-b][1,4]oxazin-9-ol) is a positron emission tomography (PET) radiotracer that allows for the detection of changes in DA levels in the human brain with good sensitivity, and changes in binding of [11C]-(+)PHNO have been used to detect elevations of DA induced by smoking.

**Methods:** Here, we used PET with [11C]-(+)PHNO to explore the impact of varenicline on binding, and DA levels, in the D2-rich striatum and D3-rich extra-striatal regions and its relationship with craving, withdrawal and smoking behavior. Eleven treatment-seeking smokers underwent two PET scans with [11C]-(+)PHNO each following 12 hr overnight smoking abstinence (i.e. under abstinent conditions) both prior to receiving varenicline and following 10-11 days of varenicline treatment (i.e. at steady state drug levels). Subjective measures of craving and urges to smoke were also assessed on the days of the PET scans.

**Results:** Varenicline treatment significantly reduced [11C]-(+)PHNO binding in the dorsal caudate and some craving measures.

**Conclusions:** These findings provide evidence that varenicline is able to increase DA levels in the human brain, a factor that may contribute to its therapeutic efficacy.

**Keywords:** smoking cessation, dopamine, imaging

**Disclosures:** Nothing to disclose.

**T228. Buprenorphine Reduces Perception of Negative Social Stimuli in Healthy Young Adults**

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**Background:** Buprenorphine, a μ-opioid partial agonist and κ-antagonist, has been has been used successfully to treat opioid use disorders for more than 30 years. Although its therapeutic effects are mainly through opioid replacement, recent preclinical evidence suggests that it may also modulate reactivity to emotional stimuli. The endogenous opioid system is known to mediate critical social and affective processes, and we have recently shown that buprenorphine dampens responses to social stress in a laboratory setting. However, the effects of the drug on responses to other types of social and affective stimuli remain largely unexplored in human subjects. Here we examined the effects of buprenorphine on three dimensions of social processing: i) responses to simulated social rejection, ii) attention to emotional facial expressions, and iii) subjective ratings of images with and without social content.

**Methods:** Healthy young adults (N = 36) attended two laboratory sessions during which they received either placebo or 0.2mg sublingual buprenorphine in randomized order, under double-blind conditions. Ninety minutes after drug administration, they completed the three behavioral tasks: i) a virtual ball-toss game in which they were first included and then excluded by the other players; ii) an attention task in which they were shown pairs of faces (posed by the same actor), one emotional and one neutral, while the direction of their gazes was recorded using electrooculography, and iii) a picture-viewing task, in which they rated standardized images with and without social content.

**Results:** Buprenorphine significantly reduced participants’ perception of the degree to which they were excluded during the virtual ball tossing game. It significantly reduced initial attention to fearful facial expressions, without influencing attention to angry, happy, and sad faces during the attention bias task. Finally, buprenorphine significantly increased ratings of positivity of images with social content,
without affecting ratings of nonsocial images during the picture-viewing task.

Conclusions: These results suggest that even at low doses, buprenorphine dampens responses to some types of negative social stimuli, which may contribute to its efficacy in treating opioid use disorders. This provides further support for the role of the opioid system in mediating responses to social rejection and other aversive social states.

Keywords: Buprenorphine, Social stimuli, emotion processing, Cyberball, opioid

Disclosures: This research was supported by NIDA DA02812. The study drug was supplied by Reckitt Benckiser Pharmaceuticals Inc. (RBP) as an unrestricted, unsolicited grant of non-financial support. RBP had no role in study design; collection or analysis and interpretation of the data; in the writing of the manuscript; or in the decision to submit the manuscript for publication, but did review the report for scientific accuracy.

T229. SERT Inhibition Modulates Molecular and Behavioral Effects Arising from Non-Serotonergic Cocaine Targets

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Background: Cocaine, like many abused psychostimulants, has a complex mode of action. Understanding the contributions of specific targets is crucial to comprehend the complex circuit adaptations that result in addiction, and therefore to design improved intervention strategies. Cocaine inhibits the serotonin- (5-HT), dopamine- (DA), and norepinephrine (NE) transporters with relatively equal potencies, and accordingly, cocaine-evoked neuroadaptations in any of the three neurotransmitter systems may contribute to behavioral alterations. To identify molecular and behavioral effects arising from cocaine inhibition of the 5-HT transporter (SERT), we implemented a novel knock-in (KI) mouse model wherein the Slc6a4 gene has been mutated to express the Met172 variant that eliminates SERT-dependent 5-HT elevations that are thought to be involved in adaptive changes leading to addiction and ultimately to addiction and here we demonstrate that several of these changes are suppressed as a consequence of SERT inhibition. Our differential findings identified networks associated with NFkB, ERK, and Akt as differentially regulated in the PrL of acutely cocaine-treated WT and KI. qPCR analyses showed no contribution of SERT inhibition to cocaine-induced up-regulation of the IEGs Egr2 and Arc, whereas a genotype effect leading to greater Junb and c-Fos mRNA expression in the KI vs. WT was evident (p<0.05, Sidak post-hoc in 2-way ANOVA, N = 6-9). Although no genotype differences were observed with respect to locomotor sensitization in open field testing, KI mice showed a remarkably blunted locomotor sensitization during the CPP paradigm (p<0.01, Sidak post-hoc in RM-ANOVA, N = 50-53). The KI mice also exhibited a significantly higher preference for the cocaine-paired chamber in the CPP paradigm (p<0.05, Sidak post-hoc in RM-ANOVA, N = 48-51), whereas no differences were found for extinction and cocaine reinstatement. However, after a mild swim stress KI mice spent less time in the cocaine-paired chamber than the WT (p<0.05, unpaired t-test, N = 26-29).

Conclusions: Our studies reveal multiple molecular, cellular, and behavioral contributions of SERT-inhibition to the acute and chronic actions of cocaine. Whereas extracellular elevations of DA likely dominate in establishing cocaine’s reinforcing effects, SERT-dependent 5-HT elevations that modulate neuronal activation and transcriptional programs in the PrL and the piriform cortex appear to drive discrete cellular and molecular changes that ultimately are integrated into the wholistic experience of cocaine action. IEGs are thought to be involved in adaptive changes leading to neuroplasticity and ultimately to addiction and here we demonstrate that several of these changes are suppressed as a consequence of SERT inhibition. Our differential findings with locomotor sensitization in the open field and CPP paradigms revealed a context-dependence to the impact of cocaine-induced elevated 5-HT, which may derive from differences in behavioral contingencies or differences in stress imposed by these environments. Possibly, the mildly stressful environment of non-contingent drug administration and open field testing normalized the locomotor sensitization differences that emerged when animals were tested in the well-habituated CPP context. The genotype-dependent changes exposed by acute swim stress point to key roles of elevated 5-HT signaling in reinstatement.
Together, these studies provide a novel perspective on the role of SERT-inhibition in cocaine action and indicate the SERT Met172 mouse to be a powerful platform for the deconstruction of the action of cocaine in distinct monoaminergic circuits.

Keywords: serotonin transporter, cocaine, genetic mouse model, gene expression, conditioned place preference

Disclosures: Nothing to disclose.

T230. Evidence of Incubation of Cue-Induced Craving in Human Individuals with Cocaine Use Disorder: Objective Bottom-Up Measures Diverge from Self-Reports in Support of Preclinical Studies

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Background: Cue-induced craving or craving precipitated by the re-exposure to cues previously associated with drug use is thought to be a major contributor to relapse in treatment-seeking individuals with substance use disorders, yet its trajectory during abstinence has mostly been studied using animal models. Results of parallel human studies have not presented as clear a pattern, which could be attributed to divergence from the preclinical studies on several measures. A major difference is in the use of subjective (self-report) measures that are inherently influenced by demand characteristics, inaccurate self-knowledge and may also reflect the possible conflation of certain baseline states (i.e., unprompted) with more reactive influences. Here, we hypothesized that the late positive potential (LPP) component of the electroencephalogram (EEG), a non-invasive and temporarily precise measure of stimulus salience that circumvents the recognized pitfalls of self-reports, can be used to objectively track the trajectory during abstinence of cue-induced craving in human individuals with cocaine use disorders (iCUD).

Methods: Sixty-two iCUD participated in this cross-sectional study. Prior to LPP analyses, iCUD were stratified into four groups based on their abstinence duration. The selected abstinence time windows were meant to parallel the preclinical time windows [Group 1: abstinence = 2.85 ± 1.04 days (2-5 days), N = 20; Group 2: abstinence = 25.38 ± 8.59 days (11-37 days), N = 16; Group 3: abstinence = 126.43 ± 50.1 days (45-180 days), N = 14; and Group 4: abstinence = 427.58 ± 328.99 days (200-1380 days), N = 12]. The four abstinence groups did not differ on age, gender, duration of cocaine use or severity of dependence (all p > 0.1). All participants completed the Cocaine Craving Questionnaire to report their baseline unprovoked (state) craving. EEG data from 64 electrodes were recorded as participants passively viewed 30 cocaine pictures as used in our prior work and 30 neutral pictures selected from the International Affective Pictures System. All participants were subsequently asked to rate the ‘liking’ (hedonic properties) and ‘wanting’ (craving) for cocaine in response to each picture on a SAM scale (range: 1-9), such that a higher score reflected more cocaine liking and wanting. The LPP was scored in response to the cocaine-related picture type relative to the neutral pictures (cocaine minus neutral) as an averaged activity over 400–2000 ms time window post stimulus onset.

Results: As expected, baseline craving decreased progressively with abstinence [linear: F = 13.66, p < 0.001]. Cue-induced liking and wanting self-reported ratings showed a similar progressive decline [linear: Liking: F = 1.88, p = 0.006; Wanting: F = 2.03, p = 0.003]. In contrast, the LPP amplitudes (cocaine minus neutral contrast) followed an inverted U-shaped trajectory [quadratic: F = 87, p = 0.022], such that the LPP amplitude at the third time window (abstinence: 45-180 days) increased significantly as compared to the first time window (during early abstinence, range 2-5 days) (p = 0.006), showing a similar trend when compared with the second time window (abstinence range 11-37 days) (p = 0.07), before significantly declining with longer-term abstinence duration (abstinence > 200 days) (p = 0.019).

Conclusions: To our knowledge, using an objective marker of brain function, these findings provide the first confirmation of incubated cue-induced craving in human cocaine addiction. Using abstinence periods that parallel preclinical reports, we show that cue-induced craving peaks at about four months of abstinence before declining at 14 months. Importance of these results further derives from the divergence between self-reported and objectively assessed measures of cue-induced craving, suggesting that objectively ascertained periods of high vulnerability to relapse may not be accessible to traditional self-reported craving. Hence, because EEG is more affordable and portable than other imaging technologies (e.g., MRI, PET), results suggest that its deployment in treatment centers for online assessment of cue-induced craving could help guide the implementation of individually-tailored detection followed by intervention, prevention, and treatment efforts. Specifically, the utilization of such objective bottom-up measures that do not rely on higher-order cognitive processing or introspection can help bypass obstacles of impaired cognitive function and insight/self-awareness, which may impede the effective, reliable and valid assessment of drug-cue reactivity and craving in a substantial subgroup of iCUD. Taken together, our results promise exciting comparisons between species to enhance understanding of the basic neurobiological mechanisms underlying incubated cue-induced craving in humans.

Keywords: craving, Cue-Exposure, EEG biomarkers, ERP

Disclosures: Nothing to disclose.

T231. Estimated Probability of Becoming Alcohol Dependent: Extending a Multiparametric Approach

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Background: United States (US) epidemiological studies suggest that for every 5-8 who start drinking alcoholic beverages, at least one drinker will develop an alcohol dependence (AD) syndrome within the first 10 years after onset of drinking (Lopez-Quintero et al., 2011; Wagner & Anthony, 2002). Recently, we described a multiparametric functional analysis approach for new research to estimate these transition probabilities with a one-dimensional function (1D; Vsevolozhskaya & Anthony, 2015). Here, we demonstrate extension of this analysis to two-dimensional (2D) functions that combine information about number of recent drinking days and number of drinks on the typical drinking day.

Disclosures: Nothing to disclose.
Methods: Data are from the United States National Survey on Drug Use and Health (NSDUH) Restricted-use Data Analysis System, 2002-2011, with nationally representative samples of newly incident drinkers and rapid-onset AD syndromes ascertained via standardized audio computer self interviews, completed for surveys of non-institutionalized civilian US citizens, age 12 years and older. Drinking history, including DSM-IV AD status, were assessed via the standardized computer-assisted interview assessments. The 2D functional estimates are based on a non-linear parametric Hill equation evaluated for (1) number of drinking days in 30 days just before NSDUH assessment, and (2) typical number of drinks on recent drinking days. Results: Among newly incident drinkers with just one drink per drinking day, the estimated AD risk ranges from more or less 1% among infrequent drinkers with a single drinking day per month (95% bootstrap confidence interval, CI: 0.7, 1.0), upward to about 3% among daily drinkers (95% CI: 1.4, 3.7). Among newly incident drinkers with ~2 drinks per drinking day, estimated AD risk is much larger among daily drinkers (21.4%; 95% CI = 5, 21). Across subgroups defined by 3, 4, and 5 or more drinks per day, the estimated AD risk is larger, as can be seen clearly for those who have progressed to daily drinking: 31% for 3 drinks, 84% for 4 drinks, 90% for 5+ drinks, respectively, with some degree of CI overlap. However, among infrequent drinkers, with no more than one drinking day per month, the estimated AD risk does not appreciably differ from 1% irrespective of the number of drinks consumed per typical drinking day. Conclusions: Via the multiparametric functional analysis approach extended beyond the number of drinks per typical drinking day, this evidence helps clarify that AD risk apparently is relatively constant and quite limited when newly incident drinking is limited to no more than one drinking day per month. When newly incident drinkers are observed within 12 months after drinking onset, there is substantial increase in AD risk among daily drinkers, provided the typical number of drinks per day increases from 1 to 5+ drinks. This study is novel in its focus on newly incident drinkers and variations in risk of developing alcohol dependence soon after drinking onset. A new agenda for research AD risk among newly incident drinkers can be built upon this initial platform of new evidence, particularly if family history and individual-level genomic characteristics can be assessed and brought into play in future national surveys of this type. Keywords: Alcohol dependence, statistical methods, epidemiology

Disclosures: Nothing to disclose.

T232. Buprenorphine during Pregnancy: Clearance, Fetal Exposure and Neonatal Outcomes

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Background: Opiate exposure during pregnancy is a growing concern with recent data indicating that over 11% of pregnant women receive an opiate (Kellogg et al 2011). Opiate abuse during pregnancy is associated with a significant increase in obstetrical complications. Exact numbers of pregnant women abusing opiates is unknown; however, ~1/3 of all patients entering treatment for opioid dependence are women of childbearing age (Johnson 2003). The landmark study by Jones and colleagues (2010), solidified a role for buprenorphine (BUP) over methadone for opiate maintenance in pregnancy based on infant outcomes. Previous studies have shown that BUP crosses the placenta, though pharmacokinetic data in pregnancy is limited. BUP is metabolized by N-dealkylation (CYP3A4) to norbuprenorphine and subsequently both the parent and metabolite undergo glucuronidation (Cone 1984) with low bioavailability in healthy populations. These metabolic systems undergo alterations in pregnancy and guidelines for optimizing buprenorphine maintenance during pregnancy have not been forthcoming. The current study examines the clearance of BUP during pregnancy to quantify the impact, if any, of gestational physiology on serum [BUP]. In addition, preliminary assessment of the impact of cumulative exposure as measured by area under the curve for maternal daily dose, maternal serum concentrations of both BUP and norbuprenorphine, and placental passage on infant outcome. Methods: Pregnant women in a BUP maintenance program at a tertiary referral center were enrolled in the study. Study participation did not influence treatment in anyway. Study visits during pregnancy and the postpartum period were conducted at 4-8 weeks with collection of current BUP dose, psychometric scales for depression/stress/withdrawal, completion of medication/exposure tracking for additional exposures, urine and blood samples. At delivery, maternal blood and umbilical cord blood samples were collected. Medical records for mother and baby were obtained. Biological samples were labelled with a HIPPA compliant code and stored at -80 C. Assays for maternal plasma and cord blood concentrations of BUP and norbuprenorphine were conducted in a single batch blind to maternal daily dose, gestational/postpartum timing of the sample, and time post dose using SPE and Absciex 5500 LC/MS/MS method with a reportable limit of 0.2 ng/ml for both BUP and norbuprenorphine (Castle Medical – SLN, TD). The relative clearance (CL) was calculated using CL = daily dose (mg) / body weight (kg) / concentration (ng/ml) as described previously with anti-epileptic drugs in pregnancy by our group (Pennell et al 2008, Polepally et al 2014). Placental passage was defined as [umbilical cord] / [maternal plasma]. Results: At time of submission, 13 subjects (mean age 26.7 years) completed pregnancy with a total of 45 maternal plasma samples collected in pregnancy and 5 umbilical cord samples obtained at delivery that were available for assay. Additional subjects are enrolled, and sample collection is ongoing. All but one of the 13 subjects were Caucasian. Seven women increased their daily dose of buprenorphine over the course of the pregnancy with average buprenorphine dose rising from 7.5 mg per day in first trimester, to 11.2 mg in the second trimester and 15.25 mg per day in third trimester. Initial inspection of the individual clearance plots indicated a pattern of increasing clearance from early gestation – peaking between 20-26 weeks, and then noticeably trending back towards baseline. Per clinical
program recommendations, BUP is discontinued 48-72 hours prior to a planned cesarean section, as such two cord sample were below the limit of detection. For those maternal / cord pairs taking BUP through delivery (n = 3), the placental passage of BUP was 37.5% and norbuprenorphine was 76.9%. Additional analyses of concomitant medication effects, postpartum clearance, and infant outcome are underway.

Conclusions: In our analysis, albeit a small cohort, the preliminary analyses indicate that BUP clearance changes across pregnancy with a high degree of individual variability in a pattern similar to that seen for other medications such as lamotrigine. Adding to existing literature, these findings suggest likelihood of required titration of BUP dose during this window to prevent additional cravings, potential for relapse and withdrawal symptoms (Conceiheiro 2011). The impact of gestational changes in metabolic capacity may underlie the clinical decision to increase maternal daily dose in mid pregnancy. In contrast, the relative decrease in clearance in the third trimester suggests dose reductions may be feasible and potentially improve neonatal outcomes. Improving our understanding of the gestational timing of such changes has direct clinical import in the management of opiate dependent pregnant women. Quantification of fetal exposure and/or estimates of fetal/neonatal metabolic capacity via measures of parent and metabolite may serve to identify neonates at greater risk for neonatal abstinence syndrome.

Keywords: Buprenorphine, pregnancy, Pharmacokinetics

Disclosures: Nothing to disclose.

T233. A Novel CRF-VTA Microcircuit in the Mouse Midbrain as Critical Site for Social Stress to Escalate Cocaine Self-Administration

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Background: Corticotropin releasing factor (CRF) signaling in the posterior ventral tegmental area (pVTA) regulates stress-induced psychostimulant self-administration. However, the source of pVTA-CRF and the molecular mechanisms underlying this drug self-administration remain unclear.

Methods: We used viral-vector-based tract-tracing by stereotaxically infusing an AAV-Flex-ChR2 virus bilaterally in the ventral-posterior medial (VPM) region of the pVTA in CRF-Cre mice, optogenetic or Gq-DREADD stimulation, c-FOS-immunoreactivity, 10-days of repeated social defeat stress, intravenous self-administration of cocaine.

Results: CRF neurons in the lateral hypothalamus (LH) and dorsal raphe nucleus (DRN) make reciprocal connections with the paranigral (PN) and parainters fascicular (PIF) subnuclei of the pVTA. DRN-VPM CRF processes form putative synapses on subsets of dopaminergic (DA-ergic) neurons co-express CRF1/2 receptors in the VPM, while LH-VPM CRF processes do not. Both of these neuronal circuits were activated, evident by c-FOS-immunoreactivity (-ir), following a single 30-minute restraint stress. The DRN-VPM, and not LH-VPM, CRF microcircuit was recruited after 10 days of repeated social defeat stress but not after a single social defeat or handling experience. Furthermore, there are more synapses triple-labeled with post-synaptic density marker PSD95, presynaptic VGLUT1, and DRN-VPM CRF dendrites expressed on DA-ergic neurons in the pVTA of mice that experienced repeated social defeat. Thirty-minutes of restraint stress increased CRF-ir fibers in the PN/PIF, consistent with microdialysis studies demonstrating CRF release in the VPM of intruder rats and mice experiencing social defeat stress. Specifically, activating the PN/PIF using either optogenetics or Gq-DREADD in CRF-Cre mice was sufficient to mimic chronic social defeat stress-induced drug-seeking behavior. Daily 30-minute activation of CRF signaling in the PN/PIF, for 10 consecutive days followed by 10-days of rest, induced behavioral sensitization to a single intra-peritoneal injection of d-amphetamine (d-AMPH, 1.5mg/kg) and a low dose (0.3 mg/kg/infusion) of intravenous self-administered cocaine.

Conclusions: These data suggest that CRF neurons in the DRN are specifically activated by social stress, releasing CRF in the PN/PIF of the pVTA, which modulates behavioral sensitization to and active drug-taking of abused psychostimulants.

Keywords: Social defeat stress, cocaine, CRF, optogenetics, DREADD

Disclosures: Nothing to disclose.

T234. Adverse Effects of Cannabis on Adolescent Brain Development: A Longitudinal Study

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Background: Cannabis is widely perceived as a safe recreational drug and its use is increasing in youth. It is important to understand the implications of cannabis use during childhood and adolescence on brain development.

Methods: This is the first longitudinal study that compared resting functional connectivity (FC) of frontally mediated networks between 43 healthy controls (HC; 20 females; age M = 16.5 ± 2.7) and 22 treatment-seeking adolescents with cannabis use disorder (CUD; 8 females; age M = 17.6 ± 2.4).

Results: Increases in resting FC between caudal anterior cingulate cortex (ACC) and superior frontal gyrus across time were found in HC, but not in CUD. Also, while there were no significant longitudinal changes in resting FC between caudal ACC and dorsolateral and orbitofrontal cortices across time in HC, CUD showed a decrease. Lower FC between caudal ACC cortex and orbitofrontal cortex at baseline predicted severity of cannabis use during the following 18 months. Finally, the amount of cannabis use during the 18-month interval predicted IQ and cognitive function measured at follow-up.

Conclusions: These data provide compelling longitudinal evidence suggesting that repeated exposure to cannabis during adolescence may have detrimental effects on brain resting functional connectivity (FC), intelligence and cognitive function.

Keywords: cannabis, Cognition, development, Resting State Functional Connectivity, longitudinal

Disclosures: Nothing to disclose.
T235. Distinct Dorsal and Ventral Hippocampal Inputs to Lateral Septum Drive Context vs. Cue-Induced Cocaine Seeking

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Background: Stimuli associated with drug experiences can trigger relapse in human addicts. Drug-associated contexts and discrete drug cues initiate relapse by activating distinct brain regions. However, neural circuits involved in distinct relapse modalities have not been fully characterized. Previous results from our lab revealed a functional dorsal CA3 hippocampus-lateral septum (LS) ventral tegmental area circuit (Luo et al., 2011, Science). We further investigated the hippocampus to LS circuit during both contextual and discrete cue reinstatement. Based on the involvement of the hippocampus in contextual processing and its dense projections to LS, we hypothesized that this circuit is important specifically for context-, but not cue-induced cocaine seeking.

Methods: Using a modified self-administration model, all rats self-administered cocaine with light/tone cues in one context, extinguished this behavior in an alternative context (without light/tone cues), and reinstated in either the training context without light/tone cues (context reinstatement, ABA) or in the extinction context with light/tone cues (cued reinstatement, ABB) to dissociate context vs. cued reinstatement of cocaine seeking. We measured expression of the neural activity marker Fos to identify activated brain regions, and combined Fos signaling with a retrograde tracer to determine activated circuits during these reinstatement modalities. We then examined the functional role of hippocampal inputs to lateral septum in these reinstatement modalities using pharmacologic (GABAA/B agonists) or chemogenetic (Designer Receptors Exclusively Activated by Designer Drugs; DREADDs) approaches. Specifically, we inhibited lateral septum with microinjections of a baclofen-muscimol mixture or we transiently inhibited hippocampal (CA3) terminals in LS by virally transducing inhibitory (hM4Di) DREADDs into dorsal CA3, which transported to terminals in LS and were activated by local microinjections of the ligand clozapine-N-oxide (CNO).

Results: Both dorsal hippocampus (CA1, CA3, and dentate gyrus) and LS (caudal and rostral LS) expressed more Fos-positive cells during context compared to cued reinstatement. Furthermore, a greater percentage of dorsal CA3 neurons that project to LS expressed Fos during context compared to cued reinstatement or extinction. DREADD hM4Di-induced inhibition of CA3 terminals in LS attenuated context-, but not cue-induced reinstatement, indicating the dorsal CA3-LS circuit is particularly important for relapse induced by drug-associated contexts. Interestingly, pharmacological inhibition of LS attenuated both context- and cue-induced reinstatement. Therefore we investigated if cue-induced reinstatement involved ventral hippocampus inputs to LS. Results revealed that a greater percentage of ventral CA1 hippocampal neurons that project to LS expressed Fos during both context- and cue-induced reinstatement compared to extinction.

Conclusions: Together these findings confirm the importance of LS in cocaine-seeking behavior, and that dorsal CA3 hippocampal inputs to LS drive context-induced reinstatement, whereas ventral CA1 hippocampal inputs to LS activate during both cue-induced and context-induced reinstatement. Further experiments will include functional inactivation of the ventral CA1 hippocampal inputs to LS during context- and cue-induced reinstatement. Elucidating the circuitry involved in different relapse modalities will identify therapeutic targets for specific relapse triggers in recovering drug addicts.

Keywords: cocaine, Hippocampus, Lateral Septum, reinstatement, DREADDs

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T236. Methamphetamine Preference in Female Rats in the Conditioned Place Preference Test Increases with Altitude

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Background: In demographic studies, methamphetamine (Meth) and cocaine abuse have both been shown to increase with altitude of residence. This implies that living at altitude could potentially alter brain biochemistry to increase the rewarding effects of Meth and cocaine abuse. Several other groups have documented both acute and chronic increases in dopamine and/or dopaminergic effects in the rat brain with exposure to hypobaric hypoxia (the low partial pressure of oxygen at altitude). We therefore examined whether housing rats at altitude alone could increase Meth conditioned place preference.

Methods: Initial studies were conducted on female rats. Female SD rats were housed for a week at altitudes of sea level (SL), 4,500ft (4.5K, local conditions) or 10,000ft (10K). Animals were then tested for Meth preference in the conditioned place preference (CPP) test. The 10 day CPP protocol consists of a pretest session on day 1, 8 days of conditioning, and the test CPP on day 10. Chamber preference was assessed in the pretest for each animal. Meth Group: For conditioning, rats were treated with either Meth (0.5mg/kg, subcutaneous injection) and placed in the non-preferred chamber on odd days, or with saline and placed in the preferred chamber on even days. Control Group: Control rats received saline injections each day for the conditioning period, and all else remained similar. All animals were returned to their home altitude daily after the 30min CPP sessions. In the test CPP, rats were given unrestricted access to all chambers to determine the effects of Meth conditioning on place preference.

Behavioral data was presented as percent time spent in each chamber. Reward benefits of Meth or saline were calculated as test preference (PREF = time spent in Meth-paired chamber- time spent in saline-paired chamber in the test session) or test difference (DIFF = time spent in Meth-paired chamber in the test - time spent in Meth-paired chamber in the pretest). Statistical analyses were performed...
T237. Behavioral Effects of Tobacco Smoke Constituents in Squirrel Monkeys

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Background: Recent preclinical studies in rodents suggest that tobacco constituents other than nicotine also exhibit pharmacological properties that may play a role in maintaining tobacco consumption. The present studies were conducted to evaluate nicotine-like behavioral (schedule-controlled responding for food reinforcement, discriminative-stimulus, and reinforcing) effects of minor tobacco alkaloids (e.g., nornicotine, anabasine, anatabine, myosmine, and cotinine) in nonhuman primates.

Methods: In schedule-controlled behavior studies, the ability of minor tobacco alkaloids to produce nicotine-like decreases in rates of responding maintained by food was examined in squirrel monkeys (n = 4). In drug discrimination studies, the ability of minor tobacco alkaloids to engender nicotine-like discriminative-stimulus effects was determined in squirrel monkeys (n = 4) trained to discriminate a highly potent nicotine-like agonist (+-)epibatidine from vehicle. In intravenous (IV) self-administration studies, second-order fixed-interval schedule procedures in nonhuman primates (n = 3) were utilized to determine whether selected minor tobacco alkaloids (e.g., nornicotine, anabasine, and myosmine) exhibit nicotine-like reinforcing effects.

Results: Results from scheduled-controlled performance show that nicotine and minor tobacco alkaloids produced dose-dependent decreases in rates of responding maintained by food reinforcement. Results from DD studies show that: a) NIC and minor tobacco alkaloids engendered full (nornicotine, anabasine, myosmine, anatabine), or no (cotinine) substitution for (+)-epibatidine. Results from our self-administration studies show that nicotine (0.032–0.032 mg/kg/injection) reliably produced dose-related IV self-administration behavior under the second-order fixed-interval schedule, with peak rates of responding during availability of the unit dose of 0.01 mg/kg/injection. In contrast, nornicotine (0.032–0.18 mg/kg/injection) and anatabine (0.01–0.18 mg/kg/injection) produced response rates that are between those engendered by nicotine and those during saline availability. In contrast, myosmine (0.32–5.6 mg/kg/injection) failed to maintain IV self-administration under the second-order fixed-interval schedule; response rates were no greater than for vehicle.

Conclusions: Taken together these findings suggest that non-nicotine tobacco constituents may differentially produce nicotine-like addiction-related effects that contribute towards maintaining long-term tobacco consumption.

Keywords: nicotine addiction, behavioral pharmacology, Nonhuman Primates, Minor Tobacco Alkaloids, Self-Administration

Disclosures: Nothing to disclose.

T238. Orbitofrontal Cortex Neurons Are Activated during Alcohol and Sucrose Seeking

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Background: The orbitofrontal cortex (OFC) is involved in reward valuation, preference, and motivation, and OFC disruption has been associated with addiction to drugs of abuse. Research from our group has shown that OFC neurons are activated during seeking of natural rewards such as sucrose, as well as drug rewards such as cocaine. Recent work has also shown that alcohol exposure has pronounced effects on OFC neuron excitability and plasticity in vitro. However, it is completely unknown how OFC neurons signal alcohol reward and motivation in behaving animals. Furthermore, there are significant differences between lateral and medial OFC with respect to behavioral regulation, and some studies have reported opposite functions for these subregions. Characterizing the contributions of different OFC networks to alcohol reward and seeking has the potential to provide a mechanistic understanding of critical neural systems that drive motivation for alcohol in both addicted and non-addicted states.

Methods: Here we investigated the activity of multiple single neurons in lateral and medial OFC during self-administration and extinction of alcohol- and sucrose-seeking. Long-Evans rats received intermittent access to 20% ethanol in their home-cage for four weeks before being...
trained to self-administer ethanol and/or sucrose. In self-administration sessions, nosepoke-initiated trials triggered one of two auditory cues. Tone 1 (1 kHz) predicted delivery of 20% ethanol. Tone 2 (5 kHz) predicted delivery of 15% sucrose solution. Reward was delivered if the animal left the nosepoke in less than 500 ms of the cue and initiated licking the reward-delivery tube within 500 ms of leaving the nosepoke. Ethanol and sucrose cues/rewards were randomly interleaved in some sessions and blocked separately in others. Rats were initially trained on the sucrose-only variant of the task. Upon stable performance, we implanted unilateral 32 microwire arrays in medial and lateral OFC (16 electrodes per region). After recovery, rats were retrained on sucrose and ethanol self-administration and recordings commenced upon stable performance. Following self-administration recordings, animals underwent extinction sessions in which nosepokes produced tones cues but no reward. Recordings were performed on four consecutive extinction sessions. After final recording sessions, lesions were made at electrode tips, animals were perfused, and brains were sectioned to identify electrode placement. All experimental protocols were approved by the University of Massachusetts 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:** Rats increased 20% ethanol drinking over intermittent access sessions, reaching an average of 12.44 ml on the final day of homecage testing. Individual rats, however, exhibited variable degrees of ethanol preference during homecage intermittent access testing. Rats increased 20% ethanol drinking over intermittent access testing. 

**Conclusions:** Here we demonstrated for the first time that OFC neurons are activated during ethanol self-administration. We are currently in the process of further data collection and analysis, but these data show that OFC neurons encode both ethanol and sucrose cues and outcomes during self-administration. These results indicate that OFC neuronal activity is not specifically tied to the preferred outcome, as all rats exhibited greater motivation for sucrose than alcohol. Rather, the data indicate that OFC neuronal activity reflects both preferred and less-preferred outcomes, though signaling of different outcomes occurs through differential activation across neurons. Additional analysis and further recordings will assess whether differential encoding of preferred vs. non-preferred outcomes segregate anatomically (into lateral and medial OFC neurons respectively) and whether encoding differs across rats with differing levels of alcohol preference. The results analyzed thus far, however, indicate that OFC neurons encode information related to alcohol seeking and that the OFC is therefore an important structure for study in the field of alcohol abuse and addiction.

**Keywords:** Alcohol-seeking behavior, Orbitofrontal cortex, Cortical neurons, Rat, Reward

**Disclosures:** Nothing to disclose.

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**T239. Midbrain Functional Connectivity and Ventral Striatal Dopamine D2-type Receptors: Link to Impulsivity in Methamphetamine Users**

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**Background:** Stimulant Use Disorders are associated with deficits in striatal dopamine receptor availability (BPND), abnormalities in mesocorticolimbic resting-state functional connectivity (RSFC), and impulsivity. The extent to which these correlates of methamphetamine (MA) use are inter-related, however, is unknown. This question was addressed in two studies. In the first study, MA users and controls were compared on the association of ventral striatal D2-type BPND with midbrain RSFC. In the second study, involving an independent sample of MA users and controls, the relationship of midbrain RSFC to self-reported impulsivity was evaluated. Two hypotheses were tested. The first, that ventral striatal D2-type BPND would be negatively related to RSFC between midbrain and the ventral and dorsal striatum, was based on findings that MA users exhibit lower D2-type BPND throughout the striatum (Lee et al, 2009) and greater RSFC between midbrain and striatum than controls (Kohno et al, 2014). Extending the first hypothesis, it was expected that midbrain-to-ventral striatum RSFC, which was expected to depend on ventral striatal D2-type BPND (first hypothesis), would be positively related to impulsivity in MA users. This second hypothesis was based on observations that measures of impulsivity are negatively related to ventral striatal D2-type BPND in MA users, and that amphetamine-induced striatal dopamine release in control subjects is positively correlated with impulsivity (Buckholtz et al, 2010).

**Methods:** The study included 44 healthy control subjects and 39 MA users. In Study 1, 26 control subjects and 19 MA users took part in positron emission tomography (PET) as well as resting-state fMRI. In Study 2, an independent sample of 18 controls and 20 MA users took part in resting-state fMRI and provided self-report measures of impulsivity. Any current Axis-I diagnoses other than nicotine...
dependence (any group) and MA dependence (MA user groups) were exclusionary.

Barratt Impulsiveness Scale - Self-report data were collected using the Barratt Impulsiveness Scale (BIS-11) (Patton et al., 1995). A 2-factor model was implemented to determine scores for cognitive and behavioral impulsivity, respectively (Reise et al., 2013).

fMRI and PET Acquisition - Resting-state fMRI was performed on a 3-T Siemens Tim Trio tomograph. Images were acquired for 5 min while participants viewed a black screen. Dopamine D2-type BPND was assessed using [18F]fallypride and PET. Images of 90 slices were obtained in two scanning blocks of 80-min each.

Resting-state fMRI Image Processing - Standard image pre-processing was performed using FSL. Images were further pre-processed to include cerebrospinal fluid and white-matter signal, and two metrics of motion-related artifacts (Power et al., 2011). A midbrain ROI was created using coordinates from a study that demonstrated differences in midbrain RSFC between MA users and controls (Kohno et al., 2014). The mean time series across all voxels within the midbrain seed from pre-processed images were used as covariates in separate whole-brain voxelwise correlation analyses.

PET Image Processing - Reconstructed PET data were averaged into 16 frames. VOI-based time-activity data were extracted from PET images and imported into PMOD 3.1 for kinetic modeling. Time-activity curves were fit using the simplified reference tissue model (SRTM). A volume-weighted average of k2, estimated from high-activity regions was computed. Time-activity curves were refit using SRTM2 applying the computed k2 values to the VOIs. Midbrain RSFC and Dopamine D2-type Receptor BPND - Ventral striatal BPND was used as a regressor in whole-brain voxelwise analysis of midbrain RSFC. A group x ventral striatal BPND interaction on midbrain RSFC was followed by post hoc, within group analyses. All whole-brain analyses were corrected for multiple comparisons with age and sex as covariates.

Midbrain to Ventral Striatum RSFC and Impulsivity - Following the observation of a significant group x ventral striatal BPND interaction with midbrain RSFC to left (but not right) ventral striatum, a VOI of the left ventral striatum was used to extract average connectivity values from the midbrain RSFC contrast maps. These estimates were entered as an independent variable in ANCOVA with outcome measures being BIS cognitive and behavioral impulsivity scores, separately.

Results: Midbrain RSFC and Ventral Striatal BPND - There was a significant group by ventral striatal BPND interaction on RSFC between midbrain and orbital frontal cortex, ventral and dorsal striatum, and insula, which was driven by a significant negative in the MA Group and no significant relationship in the Control Group. Midbrain RSFC and Impulsivity - MA users exhibited greater cognitive (p = 0.012) and behavioral (p = 0.04) impulsivity than controls. There was a significant group by RSFC interaction on cognitive impulsivity (p = 0.016), with the MA Group showing a positive relationship and the Control Group showing a negative relationship. There was no main effect of midbrain and ventral striatum RSFC on behavioral impulsivity.

Conclusions: These data suggest that chronic methamphetamine use leads to cognitive impulsivity, at least in part, by augmenting connectivity of mesocorticollimbic structures, presumably due to stimulant-induced loss of striatal D2-type receptors. The results, indicating that ventral striatal D2-type receptor signaling affects system-level activity within the mesocorticollimbic system, may provide a functional link that may help explain high impulsivity in methamphetamine users.

Keywords: Resting State Functional Connectivity, Dopamine (D2, D3) receptors, Methamphetamine, impulsivity

Disclosures: Nothing to disclose.

T240. Coupling Between Corticostriatal Structural and Functional Connectivity is Disrupted in Methamphetamine Dependence

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Background: Chronic methamphetamine use is associated with functional and structural abnormalities in corticostriatal circuitry. For example, methamphetamine-dependent individuals have lower fractional anisotropy (FA) than healthy individuals in prefrontal white matter (Tobias et al., 2010). Furthermore, studies of brain function show that in healthy control subjects sensitivity of the right dorsolateral prefrontal cortex (rDLPFC) during risk-taking is related to resting-state functional connectivity (RSFC) between the rDLPFC and striatum, but this relationship is not found in methamphetamine-dependent individuals (Kohno et al., 2014). This study sought to extend previous findings by examining the relationship corticostriatal structural and functional connectivity in controls and methamphetamine-dependent individuals.

Methods: Seventeen healthy control and 21 methamphetamine-dependent participants underwent diffusion tensor imaging (DTI) and resting-state functional magnetic resonance imaging (fMRI) on a 3T Siemens Magnetom Trio system. The DTI pulse sequence included 64 diffusion gradient directions and one image with no diffusion weighting. Briefly, DTI images were aligned across shots (i.e., diffusion directions) and native space maps of DTI derived indices were generated. BedpostX was used to calculate probability distributions on fiber direction at each voxel and probabilistic tractography between the rDLPFC and the striatum was conducted. Connectivity distributions were transformed to standard space and only voxels where at least 80 percent of subjects had connectivity between the right DLPFC and striatum were retained for extraction of average FA from each participant.

Analysis of fMRI data included realignment to compensate for small head movements (Jenkinson et al., 2002), spatial smoothing, and registration to Montreal Neurological Institute space (Jenkinson and Smith, 2001). To generate voxel-wise estimates of RSFC with the striatum, the time-series extracted from a striatal mask was regressed voxel-wise across the whole brain along with average signal of the cerebrosplinal fluid and white matter and indices of motion related artifacts. Finally, a general linear model with age, sex...
and frequency of marijuana use as covariates was conducted to examine the relationship between striatum-rDLPFC FA and striatum-rDLPFC RSFC.

**Results:** There was a significant group-by-FA interaction on RSFC between the striatum and the rDLPFC. The interaction was driven by the positive association between striatum-rDLPFC FA and striatum-rDLPFC RSFC in control participants; no such association was detected in the methamphetamine-dependent participants. Although there were no differences between the groups in structural or functional connectivity (p’s > 0.05), striatum-rDLPFC FA was negatively associated with frequency of methamphetamine use (days of methamphetamine use in the 30 days preceding study entry) and striatum-rDLPFC RSFC was negatively associated with years of methamphetamine use.

**Conclusions:** These results of this study suggest that normal coupling between brain structure and function (Damoiseaux and Greicius, 2009) is disrupted in methamphetamine dependence. Similar disruptions have been observed other neuropsychiatric disorders such as schizophrenia (Cocchi et al., 2014). As corticostratial RSFC and FA was correlated with indices of methamphetamine use/exposure, future studies could test the hypothesis that corticostratial function or structure is constrained by other methamphetamine-induced changes in brain such as reductions in dopamine receptor availability in the striatum (Groman et al, 2012; Lee et al., 2009).**

**Keywords:** Methamphetamine, Fractional-anisotropy, Resting State Functional Connectivity

**Disclosures:** Nothing to disclose.

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**T241. Contribution of Withdrawal-Induced Neurogenesis to Drug Context-Induced Reinstatement of Methamphetamine-Seeking Behavior in Rats**

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**Background:** The hippocampus is required for drug-cues or in drug-context paired with drug-cues to trigger drug-seeking behavior. The dentate gyrus in the hippocampus is an important region for adult neurogenesis, and drugs of abuse, including methamphetamine reduce adult neurogenesis during drug experience. Withdrawal from methamphetamine experience enhances proliferation and differentiation of neural progenitors and increases adult neurogenesis and we hypothesize that neurogenesis during withdrawal contributes to drug context-induced drug-seeking behaviors.

**Methods:** A novel transgenic rat model for genetically ablating neurogenesis in the dentate gyrus was used to inhibit neuronal proliferation, differentiation and neurogenesis in animals intravenously self-administering methamphetamine (0.05 m/kg) in a 6h extended access paradigm. GFAP-TK rats were trained to self-administer methamphetamine for 17 sessions after which animals received valcyte (to reduce neurogenesis) or chow (to maintain control conditions) for 3 weeks. Following abstinence, animals experienced 6 days of extinction sessions in a novel context and following extinction animals were exposed to single sessions in drug-context without drug-cues or in drug-context paired with drug-cues to determine reinstatement of drug-seeking behaviors. One hour after cued reinstatement, animals were euthanized and brain tissue was processed for immunohistochemical analysis of proliferation and cell differentiation markers in the hippocampus.

**Results:** GFAP-TK rats self-administered methamphetamine and demonstrated escalation in methamphetamine intake over 17 sessions. Valcyte treatment impaired extinction and impaired relapse to methamphetamine seeking triggered by methamphetamine context. Notably, valcyte treatment did not affect relapse to methamphetamine seeking triggered by methamphetamine cues. Valcyte treatment reduced proliferation of hippocampal progenitors by 60% and ablated the number of immature neurons.

**Conclusions:** These findings demonstrate that withdrawal and protracted abstinence-induced neurogenesis is required for methamphetamine seeking during extinction and relapse to methamphetamine seeking triggered by methamphetamine context. Thus neurogenesis during drug withdrawal is aberrant and promotes drug context-induced motivated behavior.

**Keywords:** Hippocampus, adult neurogenesis, Methamphetamine

**Disclosures:** Nothing to disclose.

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**T242. Systemic Oxytocin Acts Within the Nucleus Accumbens Core to Attenuate Methamphetamine Seeking**

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**Background:** Evidence indicates that oxytocin, an endogenous peptide well known for its role in social behaviors, childbirth, and lactation, may be a promising addiction pharmacotherapy. Here we employed a within-session behavioral-economic (BE) model for methamphetamine (meth) self-administration to examine the potential of oxytocin as a pharmacotherapy. Our rat BE paradigm was modeled after BE procedures commonly used to assess motivation for reward in humans and non-human primates. Importantly, the same BE variables (x, Q0) are assessed across species, and these variables have been shown to predict later relapse behavior. Therefore, the translational potential of preclinical BE studies is particularly strong. This approach also allowed us to assess individual variability in meth demand in relation to relapse-like behaviors, and in response to oxytocin administration.

**Methods:** Male and female Sprague Dawley rats were trained to self-administer meth for multiple days at each fixed ratio (FR) value used in the BE paradigm (1, 3, 10, 32, 100), and subsequently switched to daily BE sessions. During the BE sessions, rats self-administer at each FR value in 5 min bins
in descending FR order, with 20-min timeouts between each bin. Separate groups of rats were tested with systemic oxytocin (or vehicle) during BE and cue-induced reinstatement or with microinfusions of oxytocin or an oxytocin antagonist (after systemic oxytocin injection) into the nucleus accumbens core (NAc core).

**Results:** The BE model predicted cue-induced relapse behavior. Systemic oxytocin in both males and females decreased demand (i.e., motivation) for meth and attenuated reinstatement to meth seeking. Oxytocin showed the greatest efficacy at decreasing meth seeking in rats with the highest meth demand (i.e., strongest addiction phenotype). Finally, we found that microinfusion of an oxytocin antagonist into the NAc core blocked the effects of systemic or microinfused oxytocin.

**Conclusions:** These results demonstrate that oxytocin modifies multiple meth-seeking behaviors. They also show that the NAc core is both necessary and sufficient in mediating the attenuation of meth seeking by oxytocin, and they identify a unique role of oxytocin in the mesolimbic addiction circuitry. Overall, these data indicate that oxytocin-based therapies are a promising treatment approach for meth addiction in humans.

**Keywords:** oxytocin, Self-Administration, addiction, behavioral economics, Nucleus Accumbens

**Disclosures:** Nothing to disclose.

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**T243. Resting State Functional Connectivity of the Basal Nucleus of Meynert in Cigarette Smokers: In Comparison with the Ventral Striatum and Gender Differences**

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**Background:** Analysis of resting state functional connectivity (rsFC) using low frequency BOLD signals has provided a volume of information on cerebral functional organization and how the network circuits are compromised in neuropsychiatric conditions including nicotine addiction. Previous studies have examined how rsFC of the default mode network (DMN) is altered and the effects of comorbid marijuana misuse in cigarette smokers. Smokers have reduced rsFC between DMN and executive control network. Functional coupling between the DMN, saliency and executive control network distinguished states of craving and satiety in smokers. In a longitudinal study, decreased rsFC between bilateral posterior insula and somatomotor cortices predicted relapse in cigarette smokers receiving nicotine replacement therapy following a target quit date. Both nicotine and varenicline down regulated insula-amygdala and insula-DMN rsFC. Other analyses described the effects of nicotine replacement on cerebral network efficiency in relation to sustained attention during a go/no-go task in smokers. Together, these studies indicate changes in cerebral functional connectivity in smokers that relate to craving, cognitive functions and treatment outcomes. Nicotine influences cerebral functions by way of its action on nicotinic receptors. Cholinergic innervations of the brain arise from a few distinct nuclear groups each in the basal forebrain, brain stem and the diencephalon (medial habenula). In the basal forebrain, the basal nucleus of Meynert (BNM) projects to the amygdala and cerebral cortex. Specifically, cholinergic projections from the BNM are widespread in the cerebral cortex. Using a template derived from a study of post-mortem human brains, we recently characterized whole-brain functional connectivity of the BNM in a large sample of adult humans (Li et al., 2014). Here, building on this work, we aimed to examine differences in rsFC of the BNM between smokers and non-smokers.

**Methods:** Resting-state fMRI scans of current smokers were selected from Nathan Kline Institute (NKI)/Rockland sample (Nooner et al., 2012) of the 1,000 functional connectomes project (http://www.nitrc.org/projects/fcon_1000/). Scans were collected using the latest version of the Multiband EPI sequence (Xu et al., 2012). A total of 20 smokers (19-57 years of age; 20 men) and 173 non-smokers (18-59 years of age; 72 men) were included in the study.

Brain imaging data were preprocessed using Statistical Parametric Mapping, following our published work (Li et al., 2014). We applied a temporal band-pass filter (0.009Hz < f < 0.08Hz) to the time course in order to obtain low-frequency fluctuations, as in previous studies (Lowe et al. 1998). We used the same seed regions of the basal nucleus of Meynert (BNM) and ventral striatum (VS) as in Li et al., 2014.

The BOLD time courses were averaged spatially over each of the two seeds. For individual subjects, we computed the correlation coefficient between the averaged time course of each seed region and the time courses of all other brain voxels. To assess and compare the rsFC, we examined and compared the normalized Z maps in group random effect analyses. We performed one-sample t test each on the Z maps each for smokers and non-smokers groups and 2 by 2 ANOVA (group x gender) models on Z maps each for BNM and VS seed.

**Results:** Overall, the BNM and VS shared but also showed a distinct pattern of cortical and subcortical connectivity, replicating our previous work of a large cohort of young adults (Li et al., 2014). Compared to non-smokers, smokers demonstrated decreased BNM connectivity to precuneus, cuneus and posterior cingulate cortex (PCC) as well as increased VS connectivity to the supplementary motor cortex (SMA), dorsal anterior cingulate cortex (dACC), and bilateral somatomotor cortex (SMC) and superior temporal gyrus (STG). When men and women were examined separately, the differences in connectivity were significant only in women; compared to non-smokers, women smokers showed decreased BNM connectivity to the precuneus and PCC and increased BNM connectivity to the SMA, STG/supramarginal gyrus and bilateral anterior insula (AI). With respect to the VS, compared to non-smokers, women smokers showed increased connectivity to the SMA/dACC and bilateral SMC/STG as well as decreased connectivity to the inferior and middle occipital cortices. Post-hoc analysis of variance confirmed significant gender interactions in the majority of these regions of interest.

We also performed a linear regressions of these functional connectivities against the FTND score. The results showed that the effect size of the SMA connectivity to the BNM...
showed negative correlation with the FTND score for men (p = 0.0027, r = -0.63) but not for women (p = 0.14, r = 0.34). Thus, while women smokers showed increased BNM connectivity to the SMA, men smokers demonstrated a negative relationship between addiction severity and BNM connectivity to the SMA.

Conclusions: To our knowledge, this is the first study to explore rsFC of the BNM in smokers as contrasted to non-smokers. Specifically, women smokers showed increased BNM connectivity to a network of cerebral structures, including the anterior insula, involved in processing salient stimuli, compared to non-smokers. These results are consistent with many previous studies indicating the importance of stress and cue-related stimuli in eliciting smoking in women smokers. Compared to non-smokers, women smokers also showed increased VS connectivity to the medial prefrontal cortex and bilateral somatomotor cortices, suggesting an enhanced circuit relating reward related and action processes. On the other hand, because of the relatively small sample size of smokers investigated in the current work, more studies are needed to confirm these gender differences and to investigate the functional correlates of these findings and their relationship to clinical characteristics and treatment outcome for smoking cessation.

Keywords: nicotine, Resting State Functional Connectivity, gender, Insula

Disclosures: Nothing to disclose.

T244. Adverse Effects of Cannabis on Adolescent Brain Development: A Longitudinal Study

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Background: Cannabis is widely perceived as a safe recreational drug and its use is increasing in youth. It is important to understand the implications of cannabis use during childhood and adolescence on brain development.

Methods: This is the first longitudinal study that compared resting functional connectivity (FC) of frontally mediated networks between 43 healthy controls (HC; 20 females; age M = 16.5 ± 2.7) and 22 treatment-seeking adolescents with cannabis use disorder (CUD; 8 females; age M = 17.6 ± 2.4).

Results: Increases in resting FC between caudal anterior cingulate cortex (ACC) and superior frontal gyrus across time were found in HC, but not in CUD. Also, while there were no significant longitudinal changes in resting FC between caudal ACC and dorsolateral and orbitofrontal cortices across time in HC, CUD showed a decrease. Lower FC between caudal ACC cortex and orbitofrontal cortex at baseline predicted severity of cannabis use during the following 18 months. Finally, the amount of cannabis use during the 18-month interval predicted IQ and cognitive function measured at follow-up.

Conclusions: These data provide compelling longitudinal evidence suggesting that repeated exposure to cannabis during adolescence may have detrimental effects on brain resting functional connectivity (FC), intelligence and cognitive function.

Keywords: marijuana, Adolescence, Human Neuroimaging, Cognition

Disclosures: Nothing to disclose.

T245. WITHDRAWN

T246. Glucocorticoid Regulation of Food Reward in Humans: Evidence from Cushing’s Disease

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Background: Glucocorticoids (GC) regulate food intake and resulting body mass, but the mechanisms in humans are not fully known. One potential mechanism could involve modulation of reward processing. Indeed, stress influences behavioral and neural responses to rewarding stimuli, with transient stress (e.g., experimental administration of cortisol or laboratory stressors) increasing responsiveness and chronic stress (e.g., childhood maltreatment) decreasing responsiveness, respectively. However, neither model can capture the unconfounded effects of long-term GC exposure on humans (i.e., experimental stress is short-lived, and childhood stress may have other downstream consequences that can cloud interpretations). A disease characterized by excessive cortisol, which provides a unique model of chronic GC exposure in humans, is Cushing’s disease (CD). CD is a rare endocrine disorder (1.2-2.4/million/year) characterized by chronic excess endogenous GCs due to an adrenocorticotropic hormone-secreting pituitary adenoma; left untreated, CD results in increased mortality and multiple morbidities including obesity, diabetes, hypertension, and cardiovascular disease. A potential contribution of GCs to reward processing in CD could provide insight into the role of GCs in the pathogenesis of excess food intake and resulting obesity. Here, we examined the effects of CD on a laboratory test of food reward in which participants chose to view high-caloric food images versus viewing standardized pleasant (e.g., smiling babies), unpleasant (e.g., disfigurement), or neutral (e.g., household objects) images; members of our team originally developed this task for use in cocaine addiction (i.e., evaluating choice for cocaine images). Given data suggesting that chronic GC exposure is associated with reduced reward processing, and in the context of our focus on obesity, we hypothesized that CD patients would choose fewer food-related images for viewing than healthy controls (HC). Moreover, given data suggesting that prior GC exposure has lasting effects, we also examined food-image choice in successfully treated CD patients in remission.

Methods: Twenty-three patients with active CD (N = 13) or remitted CD (N = 10) (age: 40.5 ± 14.3 yr., BMI: 31.2 ± 4.5 kg/m2), and 12 BMI-matched HC (age: 36.7 ± 12.9 yr., BMI: 29.4 ± 4.5 kg/m2) completed an “explicit” and a “probabilistic” picture viewing task. On the “explicit” task,
participants chose, via continued button pressing, which of two fully visible side-by-side images they preferred to view from four picture categories (pleasant, unpleasant, neutral, food). On the “probabilistic” task, participants sampled from flipped-over decks of playing cards containing the same image categories, such that their deck preference needed to be learned (and re-learned, once deck identities changed) through task experience. Participants also completed a self-report measure of state food craving (e.g., “I am hungry”) and provided a urine sample for assessment of cortisol (currently processed in a third of participants).

**Results:** On both choice tasks, mixed ANOVAs revealed image category (pleasant, unpleasant, neutral, food) x study group (CD, HC) interactions [explicit: F(3,30) = 5.55, p = 0.004; probabilistic: F(3,30) = 3.16, p = 0.039]. In both cases, and consistent with hypotheses, CD participants (both active and remitted) made fewer food-related choices than HC, reaching significance for the explicit task [t(22) = 2.31, p = 0.031] though not for the probabilistic task (p > 0.25). Nevertheless, within CD but not HC, probabilistic food choice positively correlated with state food craving (CD: r = 0.65, p = 0.001; HC: r = -0.24, p = 0.45; difference: z = 2.54, p = 0.011), validating these tasks as a model of food reward for this population. Further analyses linked these effects to GC levels. In CD but not HC, 24-hour urine free cortisol correlated with probabilistic food choice (CD: r = 0.94, p = 0.002; controls: r = 0.04, p = 0.93; difference: z = 2.29, p = 0.022). Moreover, in analyses that split CD into active disease versus remission, active CD participants made fewer food choices than remitted CD participants, who made fewer food choices than HC [linear contrast: F(1,32) = 7.79, p = 0.009]. No effects emerged for pleasant choice, indicating specificity to food.

**Conclusions:** Chronic GC exposure from CD is associated with reduced food (but not pleasant) reward. This blunted sensitivity to food may increase eating in CD (paralleling effects in addiction literature, whereby reduced sensitivity to drugs is associated with increased use). Alternatively, results could reflect the motivation of these patients to reduce their food intake and lose weight. However, if results were driven by demand characteristics or participant motivation alone, controls (also overweight) ostensibly would be similarly unmotivated to choose food images. Our results also revealed that CD remission improves but does not normalize food reward processing, suggesting that prior GC exposure has lasting effects on brain reward systems. Continued alterations in motivation to eat could play a role in the cardiovascular and metabolic risk reported in CD patients (and chronically stressed individuals), even after remission. Overall, our findings can inform mechanisms for development of obesity in people exposed to GCs from CD, with relevance for potentially elucidating underpinnings of oral GC therapy and/or chronic stress.

**Keywords:** glucocorticoid, reward processing, Obesity, Neuroendocrine system

**Disclosures:** Nothing to disclose.

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**T247. Synapse-Specific Persistent Activation of VTA Kappa Opioid Receptors Following Acute Stress**

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**Background:** Stressful experiences drive many adaptive and maladaptive behaviors, and even acute stressors can have lasting behavioral consequences. Emerging evidence shows that dopaminergic neurons in the ventral tegmental area (VTA) are an important locus in stress. We previously identified a long-term potentiation of GABAergic synapses on these neurons (LTPGABA) that is blocked by acute stress (Graziane et al, Neuron, 2013). Administration of a kappa opioid receptor (KOR) antagonist (norBNI) in vivo prevents the block of LTPGABA. Intra-VTA injection of the KOR antagonist also prevents reinstatement of cocaine seeking by acute stress, suggesting that KOR-mediated regulation of VTA inhibitory plasticity may play a role in stress-induced drug seeking. Our recent work shows that a single five minute cold water swim stress blocks LTPGABA for at least five days. Surprisingly, blocking KORs with norBNI even well after stress restores LTPGABA, and cocaine self-administration is prevented even when norBNI is administered after stress (Polter et al, Biological Psychiatry, 2014). In this study we examine the mechanism by which KORs are persistently activated by acute stress and the circuitry in which this stress-induced neuroadaptation occurs.

**Methods:** Sprague-Dawley rats were subjected to cold-water swim stress or to administration of the KOR agonist U50488 (5 mg/kg). Midbrain slices were prepared following stress, and whole-cell patch clamp recordings of inhibitory and excitatory postsynaptic currents were performed from ventral tegmental area dopaminergic and GABAergic neurons. LTPGABA was induced by bath application of the nitric oxide donor SNAP. In some experiments, projection-specific dopaminergic neurons were labelled by intracranial injection of retrobeads into target regions.

**Results:** Here we show that the long-lasting block of LTP–GABA by stress is due to persistent KOR signaling. While bath application of an inverse agonist (norBNI, 100 nM) rescues LTPGABA in slices from stressed animals, a neutral antagonist (6-□-naltrexol, 10 µM) does not (LTP magnitude: norBNI after stress = 144 ± 18% of baseline, 6-□-naltrexol after stress = 99 ± 8% of baseline; p < 0.05). These results suggest that LTPGABA is blocked by constitutive activation of KORs rather than by persistently elevated dynorphin, the effects of which would be blocked by both drugs. The ability of norBNI to recover LTPGABA was blocked by pre-treatment of slices with the JNK inhibitor SP600125 (20 µM), suggesting that JNK signaling can prevent persistent KOR signaling after stress.

**Transient activation of KORS was sufficient to induce a lasting blockade of LTPGABA, as (U50488, blocked LTPGABA for 5 days (LTP: saline = 140 ± 10% of baseline, 1 day post U50488 = 108 ± 5% of baseline, 5 days post U50488 = 99 ± 9% of baseline). The activation of KORS by stress does not affect all synapses, as bath application of norBNI did not potentiate excitatory synapses on either dopaminergic (IPSC amplitude after norBNI: control = 92 ± 6% of baseline, FSS = 94 ± 2% of baseline) or GABAergic VTA neurons (IPSC amplitude after norBNI: control = 109 ± 4% of baseline, FSS = 112 ± 3% of baseline). Expression of LTP–GABA in dopamine neurons projecting to the nucleus accumbens and prefrontal cortex was also examined.

**Conclusions:** Our results show that a single exposure to acute stress or to a KOR agonist both cause long-lasting changes in plasticity of GABAergic synapses in the VTA.
T248. Upregulation of Nicotinic Acetylcholine Receptors in Cigarette Smokers: Effect of Concomitant Heavy Caffeine or Marijuana Use

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**Background:** Upregulation of alpha4beta2* nicotinic acetylcholine receptors (nAChRs) is one of the most well-established effects of chronic cigarette smoking on the brain. Prior research by our group gave a preliminary indication that concomitant caffeine or marijuana use affects the extent of nAChR upregulation. We sought to determine if heavy caffeine or marijuana use affects the extent of nAChR upregulation in smokers.

**Methods:** Positron emission tomography (PET) scans using the radiotracer 2-FA (a ligand for beta2-containing nAChRs) and the bolus-plus-infusion method were obtained in four groups: smokers with heavy caffeinated use (mean of 4.0 coffee cup equivalents per day; n = 22), smokers with heavy marijuana use (mean of 22.3 days of use per month; n = 17), smokers without heavy caffeine or marijuana use (n = 27). Specific binding volume of distribution (designated as Vs/fp) was determined for the mean of left and right prefrontal cortex (PFC) and brainstem, along with the thalamus (a region previously found not to have upregulation in smokers) as a control region.

**Results:** An overall MANCOVA revealed a significant between-group effect on Vs/fp values (F = 24.4, df = 3.97, p < 0.0005). Smokers with heavy caffeine or marijuana use had the highest Vs/fp values (for PFC, mean values of 6.2 and 5.6, respectively; for brainstem, mean values of 12.7 and 12.5, respectively), followed by Vs/fp values for smokers without heavy caffeine or marijuana use (mean for PFC of 4.0 and brainstem of 7.5), followed by Vs/fp values for non-smokers (mean for PFC of 3.0 and brainstem of 5.5). Differences for Vs/fp values between the smoker groups with heavy caffeine or marijuana use and smokers without this additional drug use were significant (Student t tests, range of p values 0.02 to 0.0001). Similarly, differences in Vs/fp values between all smoker groups and the non-smoker group were significant (Student t tests, p values 0.003 to < 0.0001). The smokers with concomitant heavy caffeine or marijuana use did not have a higher mean number of cigarettes per day. Vs/fp values for the control region (thalamus) were not significantly higher for smokers than non-smokers.

**Conclusions:** Smokers with heavy caffeine or marijuana use have greater upregulation of alpha4beta2* nAChRs than smokers without these concomitant drug usages. Based on prior research, the present findings may represent a pharmacokinetic effect, an interaction on a molecular level, or some other mechanism. Study findings also support much prior research demonstrating overall up-regulation of nAChRs in smokers compared to non-smokers in brain regions other than the thalamus.

**Keywords:** nicotine dependence, Positron emission tomography, caffeine, marijuana, nicotinic acetylcholine receptors

**Disclosures:** Nothing to disclose.

T249. Medicine and the Law in the Courtroom: Marijuana as Medicine

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**Background:** Twenty-three states and the District of Columbia have legalized the use of botanical marijuana for “medical indications”, circumventing the FDA drug approval process and violating the Controlled Substances Act (CSA). Recent literature reviews and meta-analysis of existing data conclude that the evidence is inadequate to support the use of whole plant marijuana for these indications. These conflicting positions were recently argued and adjudicated in an evidentiary hearing in Federal Court in California, in which the defense moved to have marijuana removed from Schedule 1. In late October 2014, Federal Court Judge Kimberly Mueller postponed the trial to hold an evidentiary hearing on whether marijuana placement in schedule; I was consistent with the best current scientific evidence. Bertha Madras was the sole expert witness for the government.

**Methods:** The defense called on three medical experts to testify in court, Gregory T. Carter, MD, Philip A. Denny, MD, and Carl Hart, PhD. Additionally, they produced testimony of Marine Sgt. Ryan D. Begin, an Iraq war veteran with PTSD, Jennie Stormes, mother of a child with a rare form of pediatric epilepsy, James J. Nolan III, PhD an associate professor of sociology and anthropology at West Virginia University, and Christopher Conrad, an expert on cultivation of marijuana.

To sustain the view that marijuana should remain in Schedule I, the prosecution team had to show that marijuana has, a high potential for abuse, no currently accepted medical use in treatment in the United States, and unacceptable safety standards for its use under medical supervision. To prevail on the motion, the prosecutors also had to show that marijuana failed to meet even one of five legal standards as a medicine that would permit removal from Schedule I. Conversely, the legal defense team had to prove that marijuana was misclassified in Schedule 1 of the CSA, that it is a safe and effective medicine and is a relatively harmless drug.

**Results:** Defense expert witnesses dismissed DSM-V diagnostic criteria of Cannabis Use Disorder or CUD, claiming that marijuana’s risk of addiction is disputed within the...
medical community and that “marijuana was less addictive than nicotine, alcohol, cocaine and caffeine”. They also testified that marijuana associated problems were less prevalent than legal drugs, including over-the-counter medicines. Testifying initially that marijuana had “high potential for abuse, one defense witness advocated for reclassifying marijuana as a Schedule II substance, even though the first criterion under both Schedule I and II is the same, “a high potential for abuse.” Dr. Madras noted the scientific foundation of DSM-V criteria for CUD and the prevalence of CUD as 4.3 million, higher than for any other illicit drug and higher among adolescents. She claimed that frequent marijuana use for chronic medical conditions could increase the risks of CUD. The defense claimed that numerous randomized controlled trials (RCT) documented marijuana efficacy for more medical conditions than typically are required for FDA approval. Dr. Madras countered that rigorous RCT using botanical marijuana were scant and compromised by an array of weaknesses. She cited recent meta-analyses concluding that evidence for whole plant marijuana was insufficient for use in various medical conditions. While reasonable experts could conclude that whole plant marijuana has medicinal value, she opined that they would be ignoring some of the evidence and FDA standards to reach that conclusion. Claiming that the benefits of marijuana outweighed the risks, the defense questioned the validity or significance of research revealing relationships between marijuana use and adverse consequences. One witness maintained that marijuana-induced brain changes were meaningless because brain changes occur with conversation or playing Tetris. Another witness had recommended marijuana to 12,000 patients in California and testified that none of his patients admitted marijuana use”. Defense witnesses believed that the majority of physicians think marijuana has medical benefits. Dr. Madras countered by citing contrary position, policy statements of major medical associations dedicated to the medical community and that “marijuana was less addictive than nicotine, alcohol, cocaine and caffeine”. They also testified that marijuana associated problems were less prevalent than legal drugs, including over-the-counter medicines. Testifying initially that marijuana had “high potential for abuse, one defense witness advocated for reclassifying marijuana as a Schedule II substance, even though the first criterion under both Schedule I and II is the same, “a high potential for abuse.” Dr. Madras noted the scientific foundation of DSM-V criteria for CUD and the prevalence of CUD as 4.3 million, higher than for any other illicit drug and higher among adolescents. She claimed that frequent marijuana use for chronic medical conditions could increase the risks of CUD. The defense claimed that numerous randomized controlled trials (RCT) documented marijuana efficacy for more medical conditions than typically are required for FDA approval. Dr. Madras countered that rigorous RCT using botanical marijuana were scant and compromised by an array of weaknesses. She cited recent meta-analyses concluding that evidence for whole plant marijuana was insufficient for use in various medical conditions. While reasonable experts could conclude that whole plant marijuana has medicinal value, she opined that they would be ignoring some of the evidence and FDA standards to reach that conclusion. Claiming that the benefits of marijuana outweighed the risks, the defense questioned the validity or significance of research revealing relationships between marijuana use and adverse consequences. One witness maintained that marijuana-induced brain changes were meaningless because brain changes occur with conversation or playing Tetris. Another witness had recommended marijuana to 12,000 patients in California and testified that none of his patients admitted marijuana use”. Defense witnesses believed that the majority of physicians think marijuana has medical benefits. Dr. Madras countered by citing contrary position, policy statements of major medical associations dedicated to the relevant medical conditions; scientists deeply familiar with the research would attest that, presently marijuana has no high quality research support. She conceded that non-psychoactive cannabinoids in the marijuana plant should be evaluated because there is preclinical evidence of their therapeutic potential. 

Conclusions: Judge Kimberly Mueller issued her pivotal ruling on the case April 17, 2015.7 The Judge’s ruling recognized that “defendants have not submitted any evidence that Congress classified marijuana as a Schedule I controlled substance because of animus or some discriminatory legislative purpose. The record here does not demonstrate there is only one supportable point of view about marijuana’s safe, medical value or abuse potential. The ongoing vigorous dispute as to the physical and psychological effects of marijuana, its potential for abuse, and whether it has any medical value, supports the rationality of the continued Schedule I classification. Congress could rationally find marijuana has a high potential for abuse and Congress could rationally conclude that marijuana, the undifferentiated plant that appears on Schedule I, has no established medical value. Having carefully considered the facts and the law as relevant to this case, the court concludes that on the record in this case, this is not the court and this is not the time. The court DENIES the motion”. As this judgment came from a federal court, it stands as the law of the land.

Keywords: Medicinal Marijuana, addiction, Abuse Potential

Disclosures: Dr. Madras was the sole expert witness for the Department of Justice
null
Background: Cocaine addiction represents a significant public health problem for which there are no Food and Drug Administration-approved pharmacotherapies. Previous preclinical research has implicated a role for serotonin (5HT) in modulating cocaine abuse-related effects by interactions with mesolimbic dopamine (DA) neurotransmission. In particular, activation of 5HT2A receptors has been shown to facilitate mesolimbic activity and subsequent dopamine release within the nucleus accumbens. Conversely, acute pretreatment with a 5HT2A antagonist has been shown to attenuate cocaine self-administration in monkeys. Thus, 5HT2A receptors represent one target for serotonin-mediated modulation both of mesolimbic DA signaling and of abuse-related cocaine effects dependent on mesolimbic DA signaling. Pimavanserin is a 5HT2A inverse antagonist that mechanistically should be more efficacious than a 5HT2A antagonist in attenuating cocaine reinforcing effects. This study evaluated effects of repeated 7-day treatment with pimavanserin on cocaine self-administration under a concurrent cocaine vs. food choice procedure. A choice procedure was utilized because a major goal of treating cocaine addiction is not only to decrease cocaine-taking behavior, but also to increase behavior maintained by alternative, non-drug reinforcers. The study tested the hypothesis that pimavanserin would attenuate cocaine choice and produce a reciprocal increase in food choice.

Methods: Adult, male rhesus monkeys (n=3) were surgically implanted with a chronic indwelling venous catheter and trained to self-administer cocaine under a concurrent schedule of food delivery (1-gram pellets, fixed-ratio 100 schedule) and cocaine injections (0.0032 – 0.1 mg/kg/injection, fixed-ratio 10 schedule). Daily choice sessions were implemented from 0900 – 1100 and consisted of five 20-min components, with a different unit cocaine dose available during each successive component (0, 0.0032, 0.032, and 0.1 mg/kg/injection during components 1-5, respectively). Once cocaine vs. food choice was stable, pimavanserin (0.32-3.2 mg/kg, intramuscular) was administered 30 min before the daily choice session for seven consecutive days. Pimavanserin doses were studied in a mixed order across monkeys, and treatment blocks with pimavanserin were followed by baseline conditions for at least 5 days and until the cocaine choice dose-effect function returned to pre-test levels.

Results: Under saline treatment conditions, food was primarily chosen during availability of small cocaine doses (0, 0.0032, and 0.01 mg/kg/injection), and cocaine was primarily chosen during availability of larger cocaine doses (0.032 and 0.1 mg/kg/injection). Repeated pimavanserin treatment failed to reduce cocaine choice. Rather, pimavanserin decreased food choice and partially reallocated responding to increased cocaine choice during early components of daily sessions when food was primarily chosen over cocaine under baseline conditions.

Conclusions: The present results do not support pimavanserin as a candidate medication or 5HT2A inverse agonism as a viable strategy to treat cocaine addiction. Furthermore, pimavanserin treatment effects on cocaine vs. food choice observed in this study were qualitatively similar to effects observed previously during treatment with dopamine receptor antagonists.

Disclosures: Nothing to disclose.

T253. Glutamatergic Mechanisms Mediate Enduring Vulnerability to Drug Use Following an Acute Stressor


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Background: There is substantial comorbidity between stress disorders and substance use disorders (SUDs). Using rodent models of stress and substance use, most studies reveal that previous exposure to stress predisposes animals to the behavioral effects of psychostimulants and opioids, including the development of behavioral sensitization and drug self-administration. While the face validity of these animal models relative to stress disorders can be argued, stress exposure potentiates both the rewarding and psychomotor stimulant effects of addictive drugs. Here we endeavor to understand the neural underpinnings of comorbid stress disorders and drug use by determining if the glutamatergic neuroadaptations (glutamate transport and glutamate mediated synaptic currents) that characterize cocaine self-administration are induced by acute stress, and if restoring glutamate transport in the accumbens core (NAcore) with Ceftriaxone (CEF) the stress-induced potentiation in cocaine-induced locomotor activity and increase in cocaine self-administration is prevented.

Methods: Adult male Sprague-Dawley rats were double housed with a 12:12 hr dark/light cycle. Acute stress group was restrained for 2 hours, while sham animals were left undisturbed in their home cage. The animals appeared healthy and no difference in body weight was measured between groups. Three weeks after acute stress or sham: 1) Animals were trained to self-administer cocaine during
seven days on an FR1 schedule (2 h per day), where responses on an active lever resulted in a drug infusion (0.2 mg) paired with discrete light and tone cues. Criterion defined as the first day animals obtain >10 infusions. 2-4) Animals were treated with CEF (200 mg/kg IP) or vehicle (saline) for 5 days and were sacrificed to measure AMPA and NMDA currents, H3-Glutamate uptake, GLT-1 expression in NAcore, or locomotor activity in response to cocaine challenge (15 mg/kg) or saline. 5) Animals were treated with CEF (200 mg/kg IP after each operant session) or vehicle (saline) for 3 days prior and 7 days during the acquisition of cocaine self-administration. All procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Assessment and Accreditation of Laboratory Animal Care.

Results: 1) Stress pre-exposure potentiated the acquisition of cocaine self-administration (Log-rank Mantel-Cox test Chi2 = 4.33, p = 0.038). 2) Acute stress-induced increase in the AMPA/NMDA ratio in the NAcore (one-way ANOVA F(2,43) = 8.14, p < 0.001), which was not reversed by CEF. 3) CEF pretreatment restored stress-induced decrease in glutamate uptake in Na+ -dependent, but not Na+ -independent uptake of 3H-glutamate into slices of the NAcore (2-way ANOVA stress vs sham F(1,19) = 8.98, p < 0.01; VEH vs CEF F(1,19) = 11.12, p < 0.001; interaction F(1,19) = 10.20, p < 0.001), and restored expression of the glial glutamate transporter, GLT-1 (2-way ANOVA VEH vs CEF F(1,20) = 12.98, p < 0.01; interaction F(1,20) = 19.78, p < 0.001). 4) CEF pretreatment reversed stress-induced potentiation acute cocaine-induced locomotor activity (2-way ANOVA stress vs sham F(1,80) = 4.83, p < 0.05; saline vs cocaine F(3,80) = 27.81, p < 0.001; interaction F(3,80) = 5.74, p < 0.01) and 5) reversed stress-induced augmented acquisition of cocaine self-administration (Chi2(3) = 5.51, p = 0.138).

Conclusions: These results probed aspects of glutamate transmission in the NAcore known to be altered by addictive drugs, and found that akin to cocaine, at three weeks following a single exposure to stress the AMPA/ NMDA ratio was increased, while glutamate uptake and GLT-1 content were reduced. In contrast to elevated AMPA/ NMDA, which occurs after withdrawal from cocaine but not heroin, reduced GLT-1 in the NAcore is observed following withdrawal from all drugs of abuse examined to date, and pharmacological restoration of GLT-1 with CEF inhibits drug seeking. Accordingly, when we restored GLT-1 function in NAcore with CEF, and we prevented acute stress-induced increases in cocaine-induced locomotion and acquisition of cocaine self-administration. These data provide a mechanistic link between acute stress-induced down-regulation of glutamate transport in NAcore and stress-induced vulnerability to use cocaine, and pose common points of pharmacological intervention that may be particularly useful in treating stress disorder and SUDs comorbidity.

Keywords: Acute Stress, cocaine, GLT-1, Nucleus Accumbens

Disclosures: Nothing to disclose.
unmeasured differences in key variables (e.g. genetic differences in DA metabolism). These results suggest that interventions targeting anhedonia in CM may need to take a different approach, perhaps intervening in inflammatory processes, which are active in CUD and can produce anhedonia, or adding behavioral interventions to target anhedonia, such as behavioral activation or exercise, to CM for CUD.

Keywords: cocaine addiction, anhedonia, Dopamine, treatment outcome prediction

Disclosures: Nothing to disclose.

T255. Nicotine Causes Parallel Increases in Medial Prefrontal Cortex Gamma Oscillations and Visual Attention

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Background: Nicotine improves attentional performance, particularly in subjects with previous nicotine exposure. Here, we measured single unit activity and local field potential (LFP) signaling in medial prefrontal cortex (mPFC) during repeated nicotine exposure, and assessed how these neural measures are associated with visual attention and behavioral sensitization.

Methods: We recorded LFP oscillations and single unit activity in mPFC of awake, behaving rats during five consecutive sessions of experimenter-administered nicotine exposure (.2 mg/kg freebase concentration). Then, after a nine day washout period, we again recorded mPFC activity during a nicotine challenge. In a separate cohort, we used a well-established attentional paradigm (McGaughey and Sarter, 1995) to assess visual attention performance during a comparable nicotine schedule.

Results: Modulations of mPFC low gamma LFP power (40-60 Hz) manifested on day three of nicotine exposure, whereas a consistent reduction of theta and beta power (5-25 Hz) was evident across all days. Nicotine induced a net inhibition of mPFC single unit activity after the first injection, but this effect was not observed in subsequent sessions. In the behavioral experiment, we observed that nicotine did not affect visual attention on day one, then improved attentional performance beginning on day three. This time scale was remarkably similar to the progression of effects of nicotine on low gamma oscillations, with effects on both attention and gamma beginning on day three and persisting across subsequent treatment sessions. Finally, rats were given an additional injection of nicotine after a nine-day washout period. This caused locomotor sensitization, improved attentional performance, and increased both low and high gamma oscillations in mPFC.

Conclusions: These parallel findings demonstrate that nicotine may improve attention via modulation of low gamma LFP in medial prefrontal cortex, and that nicotine sensitization is reflected by increased high gamma LFP. These data provide a possible mechanism for nicotine’s acute effects on behavior and cognition, and suggest that different aspects of LFP in mPFC may have utility for assessing nicotine history and attentional state.

Keywords: nicotine, Medial Prefrontal Cortex, Attention, electrophysiology, sensitization

Disclosures: Nothing to disclose.

T256. FAAH Inhibitor Treatment for Cannabis Dependence

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Background: Cannabis is the most widely used illicit drug worldwide. Cannabis dependence is associated with tolerance and withdrawal. There are no FDA approved treatments for cannabis dependence, and while many medications have been tested, few have been found to be consistently effective. Substitution treatment with THC may reduce cannabis withdrawal syndrome (CWS) is limited by its psychoactive effects, abuse liability, and by its limited relapse prevention effects. An alternative to substitution treatment may be to potentiate the signaling through the endogenous cannabinoid system.

Anandamide a principal endocannabinoid is broken down by the enzyme fatty acid amide hydrolase (FAAH). Recently, a FAAH inhibitor which increases anandamide levels was shown to reduce CWS in THC-dependent animals. Compared to THC or cannabis, FAAH-inhibitors 1) do not have psychoactive effects, 2) are not rewarding, 3) do not increase the abuse liability of other addictive drugs, 4) are not associated with tolerance and 5) produce fewer changes in CB1-R function. PF-04457845 is an orally active, long-acting, potent and selective FAAH inhibitor that does not have psychoactive or cognitive effects and is well-tolerated at the proposed dose, does not have effects suggestive of abuse liability or discontinuation-related withdrawal symptoms.

Hypothesis: The FAAH-Inhibitor PF-04457845 will attenuate cannabis withdrawal syndrome, reduce cravings and reduce relapse rates in cannabis dependent individuals.

Methods: Cannabis-dependent subjects (n = 60) with a history of CWS will be randomized to receive PF-04457845 (4mg) or placebo a 2:1 ratio in a double-blind, placebo-controlled, parallel group study. After a screening period, subjects will enter a 4 week treatment phase. Subjects will be hospitalized on an inpatient research unit for up to 1 week to achieve abstinence and precipitate CWS. Subjects will continue the remaining 3 weeks of treatment as outpatients. The treatment phase will be followed by an 8 week follow up phase to assess the durability of any treatment effects. L be randomized to receive placebo or (4 mg) provided through an agreement with Pfizer. The treatment phase consists of a 1-week inpatient stay to achieve abstinence and precipitate withdrawal, followed by a 3-week outpatient phase to assess relapse prevention. Urine toxicology for THC-COOH, cannabis use, withdrawal symptoms, craving for cannabis, self-report of sleep and appetite, sleep architecture, mood, cognition, serum endocannabinoid levels will be measured.
Adherence to study medication was assessed almost daily by video confirmation using Cellphone Assisted Remote Observation of Medication Compliance (CAROMA).

**Results:** The study has enrolled 52 of 60 subjects. Enrollment is projected to be completed shortly. The study medication has been well tolerated. There have been no serious adverse events. Adherence to study medication was very high. Results of the study will be available to be presented.

**Conclusions:** Results of the study will be available to be presented.

**Keywords:** Cannabis Dependence, FAAH, FAAH inhibitor, Withdrawal, craving

**Disclosures:** Nothing to disclose.

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**T257. Nicotinic Receptor Stimulation Affects Reversal Learning in Smokers**

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**Background:** The ability to learn which behaviors lead to rewarding outcomes and to flexibly adapt behavior to environmental changes is important for human functioning. This ability is captured by reversal learning (RL) tasks, where participants must learn reward contingencies and adjust their behavior as these contingencies change. Reversal learning deficits, specifically the inability to change behavior when a response is no longer rewarded, are an indicator of cognitive inflexibility, which is a hallmark of compulsive drug use in addiction. RL relies on prefrontal and striatal circuitry, and is dependent on serotonergic and dopaminergic processing (Clark, Cools, & Robbins, 2004; Cools, Clark, Owen, & Robbins, 2002). Preclinical evidence indicates that manipulation of dopaminergic pathways impairs RL (Lee, Groman, London, & Jentsch, 2007). Although it is well known that both dopaminergic and serotonergic systems are modulated by cholinergic afferents, the effects of nicotinic receptor stimulation and nicotine dependence on RL in humans are unknown. Here, we investigate the effects of nicotinic receptor stimulation on RL in smokers and non-smokers through the administration of nicotine and varenicline. Varenicline, an efficacious smoking cessation aid, acts as a partial agonist/partial antagonist to nicotine at nACh receptors, and may therefore mimic nicotine's effects on reversal learning in its absence, and blunt these effects when nicotine is present. We hypothesize that 1) nicotine will improve performance compared to placebo, especially in smokers; 2) the effects of nicotine will be partially blocked by varenicline, while varenicline on its own will serve as a partial agonist; and 3) the neural response in striatal and prefrontal brain areas will reflect these effects.

**Methods:** 24 smokers participated in 6 fMRI sessions (4 reported herein) during a two-drug, placebo-controlled, double-blind crossover study. Subjects underwent ~17 days of varenicline and placebo pill administration (counterbalanced between subjects) and were scanned under each condition wearing a transdermal nicotine and placebo patch. Subjects performed a probabilistic RL task in the scanner. Two visual stimuli were presented, one of which was associated with a $1 reward, while the other resulted in a $1 loss. Participants learned which stimulus was associated with the reward through feedback-guided learning, and were required to detect and adjust to reversing reward contingencies. Rewards were probabilistic, such that a correct response received a reward 75% of the time. A proportion of the final earned sum was awarded to the participants. The dependent measure of interest was the number of lose-stay events: events where the participant did not receive a reward but persisted in selecting the same response on the next trial. Repeated-measures ANOVA with factors group (smokers or nonsmokers), PATCH (nicotine or placebo) and PILL (varenicline or placebo) were carried out on the behavioral performance (number of win-stay choices and number of lose-stay choices), and the BOLD response during lose-stay events. Regions of interest were a priori defined in orbitofrontal cortex, dorsal and ventral striatum, amygdala, anterior insula and ACC, and results were FWE corrected to p<0.05 within region of interest masks.

**Results:** Behavioral results demonstrate that acutely abstinent smokers (i.e. in the absence of either drug) show a decrease in lose-stay choices (Nicotine vs. placebo: t(42) = 9.75, p<0.001; Varenicline vs. placebo: t(42) = 10.87, p<0.001). Non-smokers and smokers who are taking varenicline or nicotine do not differ in the number of lose-stay events. This effect was specific to the lose-stay events, as the number of win-stay choices and the number of correct responses did not vary by group or conditions. Imaging results during lose-stay events showed group-x-nicotine interactions, such that there was decreased activity in bilateral caudate and putamen and left amygdala in abstinent but not sated smokers; this difference was not present in non-smoking controls. No significant nicotine-x-varenicline interactions were observed in the lose-stay events in the smoker group, but non-significant trends were observed in anterior insula and anterior cingulate.

**Conclusions:** These data show that RL performance is affected by nicotinic receptor stimulation in smokers. The tendency to persevere in a no longer rewarded response was reduced in smokers in the absence of nicotinic stimulation. With either nicotine or varenicline present, smokers' behavior was not different from the healthy non-smoking group. This pattern was reflected in brain areas on which RL depends: dorsal striatum and areas in the salience network (i.e. dorsal anterior cingulate and insula) showed reduced neural activity during lose-stay events in smokers, whereas this difference was absent in nonsmokers. These results indicate that cognitive flexibility may be exaggerated in abstinent smokers, perhaps indicating an exploratory drive state induced by acute withdrawal.

**Keywords:** nicotine, Varenicline, fMRI, Reward-based decision-making, reversal learning

**Disclosures:** Supported by the NIDA-IRP

**References:**


T258. The Circadian Transcription Factor Clock Represses the Expression of the Dopamine Rate-Limiting Enzyme Tyrosine Hydroxylase via Recruitment of the Metabolic Sensor SIRT1

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Background: Both basic and clinical studies strongly implicate alterations or disruptions to circadian rhythms as putative contributors to the pathophysiology of mood and addiction disorders. However, the molecular mechanisms underlying these associations are poorly understood. At the cellular level, the molecular clock is comprised of several transcriptional-translational feedback loops, whereby CLOCK and BMAL1 drive the rhythmic transcription of many genes that control tissue and cell type specific metabolic programs and functions. CLOCK, in particular, is highly expressed in the ventral tegmental area (VTA), which is a brain region that sends the primary dopaminergic projections throughout the mesocorticolimbic system and is highly implicated in mood, reward, and motivation behaviors. We have shown previously that Clock mutant mice (Clock19) display a behavioral repertoire similar to human bipolar mania with a particular sensitivity to rewarding stimuli, including cocaine and other drugs of abuse. Clock19 displayed enhanced cocaine conditioned place preference (CPP), along with increased dopamine cell firing and dopamine levels in the VTA. Interestingly, mRNA levels of tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis, was also increased in the VTA of Clock19 mice, suggesting TH is a direct target of CLOCK. We investigated how CLOCK negatively regulates TH expression in the VTA, and whether these mechanisms are involved in the hyperhedonic phenotype. We focused on two particular proteins that dynamically interact with CLOCK across the light-dark cycle, phosphoactive CRE-element binding protein (P-CREB) and the histone deacetylase sirtuin 1 (SIRT1), a sensor of intracellular changes in metabolism.

Methods: Male wild-type (Wt) and Clock mutant mice (n = 6-15) were used for gene, protein, co-immunoprecipitation (coIP), chromatin immunoprecipitation (ChIP), and behavioral assays. Mice were administered i.p. saline, acute (1 day), and chronic (14 days) cocaine (15mg/kg) then sacrificed at 6 phases of the light-dark cycle. NAD/NADH ratios were measured using bioluminescence to assess the effects of cocaine on metabolic function. Gene and protein expression was measured from VTA punches using qPCR and Western-blots. Viruses containing mutant CREB (AAV5-mCREB) and SIRT1 overexpression (HSV-SIRT-OX) were delivered to the VTA of wild-type and mutant mice prior to cocaine CPP assays. Cocaine CPP was a biased protocol and the amount of time spent on the cocaine-paired side subtracted from the saline-paired side was used as the CPP score.

Results: CLOCK typically drives circadian rhythms in gene transcription. However, we found that CLOCK is a transcriptional repressor of TH in the VTA through dynamic interactions with P-CREB and SIRT1 at particular diurnal phases. CLOCK and P-CREB bind the TH promoter in antiphase. SIRT1 interacts with CLOCK to inhibit CLOCK-mediated transcription of TH. P-CREB binding and TH expression were constitutively elevated in the VTA of Clock mutants, while SIRT1 protein levels were significantly reduced. Interestingly, mCREB or SIRT1-OX in the VTA of Clock mutants reduced TH expression and attenuated cocaine CPP, suggesting CREB-inactivation and restoring SIRT1 levels in mutant mice reversed the hyperhedonic phenotype. We also found that chronic cocaine completely disrupted diurnal rhythms of NAD/NADH ratios and CLOCK/SIRT1 interactions in the VTA.

Conclusions: Recent evidence suggests cocaine-induced extracellular dopamine enhanced the metabolic demands of astrocytes and neurons in the brain, which can lead to altered mitochondrial function, and are thought to contribute to the long-lasting behavioral changes following chronic cocaine. We demonstrate that the circadian transcription factor CLOCK acts in antiphase with P-CREB to control diurnal rhythms of TH expression. CLOCK recruits SIRT1 to repress TH expression, however, when SIRT1 levels are low, such as in the Clock mutant mice, CLOCK loses the ability to repress transcription. Restoring SIRT1 levels reduces TH expression and attenuates the behavioral response to cocaine reward, which is similar to the effects of inactivating CREB. Metabolic signaling pathways are disrupted by cocaine, which can, in turn, alter the DNA-binding of CLOCK to control circadian-mediated transcription. These studies further demonstrate a link between metabolic and circadian pathways, and how disruption to these pathways are important for behavioral phenotypes relevant to addiction.

Keywords: circadian rhythm, cocaine addiction, Dopamine, CLOCK

Disclosures: Nothing to disclose.

T259. Optical Inhibition of the Infralimbic Cortex Following Unreinforced Lever Presses Increases Ongoing Cocaine-Seeking Behavior in Rats

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Background: The infralimbic cortex (IL) is a component of the neural circuitry that mediates extinction learning and the active suppression of cocaine-seeking behavior. Pre-
vious work has shown that IL inactivation immediately after extinction training sessions impairs the retention of extinction learning for cocaine seeking, while activation enhances extinction learning. However, the precise temporal relationship between IL activity, lever pressing, and extinction learning is unclear. Here, we optically inhibited the IL immediately following each unreinforced lever press during extinction and examined ongoing and subsequent cocaine-seeking behavior.

**Methods:** The light-sensitive outward proton pump eArchT3.0 was selectively expressed in glutamatergic pyramidal neurons of male Sprague-Dawley rats by injecting the adenovirus-associated virus encoding for eArchT3.0 under the CaMKIIα promoter bilaterally into the IL. Fiber optics were implanted immediately dorsal to the area in which eArchT3.0 was expressed. Rats were implanted with indwelling jugular catheters and trained to self-administer cocaine. Rats underwent a minimum of 12 days of 2 hr cocaine self-administration sessions, during which each active (right) lever press resulted in an infusion of cocaine and the presentation of a light and tone cue. After each right lever press, the lever was retracted for 20 s. Rats then underwent 5 days of shortened (30 min) extinction sessions, during which active lever presses did not produce cocaine infusions or cues. During these shortened extinction sessions, the IL was optically inhibited for 20 s following each unreinforced active lever press. This was followed by 7 days of full-length (2 hr) extinction sessions without optical inhibition, which served as retention tests for the extinction learning. In order to examine whether IL activity was required in a temporally precise manner, another experiment was conducted in which similar 20 s periods of optical inhibition were applied non-contingent upon lever pressing. In an additional control experiment, illumination was applied for 20 s following each unreinforced lever press during the first 5 days of extinction in rats that expressed eYFP but not eArchT3.0. Following extinction, all rats underwent cue-induced reinstatement tests, in which active lever presses resulted in the delivery of the light and tone cue, but no optogenetic manipulations were given.

**Results:** Optical inhibition applied for 20 s after each active lever press increased active lever pressing during the 5 sessions in which the inhibition occurred but had no effect on lever pressing during the 7 full-length extinction sessions. IL inhibition that was applied in a manner not contingent upon lever pressing did not increase lever pressing during the session itself or on the subsequent extinction sessions. Rats that had received IL inhibition during extinction showed potentiated cue-induced cocaine seeking, whereas rats that had received non-contingent IL inhibition did not show any change in cue-induced reinstatement. IL illumination in rats expressing eYFP had no effect on either active lever presses during extinction or reinstatement sessions.

**Conclusions:** These results suggest that IL activity immediately following an unreinforced lever press contributes to the suppression of ongoing cocaine-seeking behavior and is important for suppression of subsequent cue-induced reinstatement.

**Keywords:** cocaine addiction, Medial Prefrontal Cortex, optogenetics

**Disclosures:** Nothing to disclose.

**T260. Deficient Encoding Leads to Reduced Delayed Recall among Young Adult Marijuana Users**

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**Background:** Early onset marijuana use, i.e., initiation of use at age ≤16 years, is associated with more neurocognitive compromise than later onset use, controlling for cumulative exposure (e.g., 1). This may be due to cannabinoid effects on neurodevelopmental processes occurring before age 17 (2), including maturation in the prefrontal cortex, limbic system and white matter association fibers (3). The most consistent neurocognitive finding is poorer delayed recall in marijuana users compared to non-users. However, memory is multi-factorial, involving encoding, consolidation, and retrieval. Thus, we examined which specific aspects of memory were different among non-users (CON), early onset marijuana users (EMJ), and late onset users (LMJ).

**Methods:** This study included a community sample of 48 young adults who used marijuana ≥1 time a week (LMJ, n = 21; EMJ, n = 27) and 48 matched controls who used marijuana <5 times in their life with no recent use, all aged of 18-25 years. No participants had abuse or dependence for any substance other than marijuana. Participants completed the California Verbal Learning Test, Second Edition (CVLT-II; 4), which involves the presentation of a 16-word list consisting of 4 non-adjacent words from 4 semantic categories. The list is presented 5 consecutive times, and participants are asked to recall the words after each trial. After a 20-minute delay, participants are again asked to recall as many words as they can remember. Outcome variables included: trial encoding, total encoding, delayed recall, and percent retention (delayed recall/Trial 5 recall). We also evaluated encoding strategies used to learn the word list, inferred by the order in which words were recalled. Semantic clusters were when 2 adjacently recalled words were from the same semantic category, and serial clusters were when 2 adjacently recalled words were in the original presentation order. Each cluster-type was adjusted by what would be expected by chance, with higher values indicating greater reliance on that respective strategy. Semantic and serial clustering scores were calculated for each recall and averaged across all 5 encoding trials.

**Results:** There was a group difference in delayed recall (X2 (2) = 10.98, p = .004): EMJ recalled fewer words (Md = 13) after a delay than LMJ (Md = 15; p = .007) and CON (Md = 15; p = .002). Delayed recall was not different between LMJ and CON (p = .78).

A multilevel mixed-effects ordered logistic regression with random effects for intercept and slope was fit to the data to examine if encoding across 5 learning trials varied by group. EMJ encoded less than LMJ or CON (B = .20, p = .002; LMJ vs. CON, B = .26, p = .74). All groups acquired more words with each successive learning
tial (p's < .0001), demonstrating improvement with repetition, and this pattern of change did not vary by group (p's > .44).

There were no group differences in percent retention (X2 (2) = .88, p = .63; 95.2%, 97.8% and 98.4% retention for EMJ, LMJ and CON, respectively), demonstrating that poorer delayed recall among EMJ was due to reduced encoding, and that consolidation and retrieval was intact. We then examined whether organizational encoding strategies were weaker among EMJ. EMJ were less likely to use semantic clustering than CON (p = .05; EMJ vs. LMJ and CON vs. LMJ, p's > .09). Serial clustering was comparable across groups (p's > .15). Total strategy use (semantic + serial) also varied by group (F (2,93) = 3.50, p = .03). EMJ used fewer total strategies (M = 2.76 SD = 1.84) than CON (M = 4.03 SD = 2.00, t(73) = 2.73, p = .008), and there were no differences between LMJ and EMJ or CON in total strategies used (M = 3.70 SD = 2.25, p's > .12).

Finally, we examined whether less use of organizational strategies among EMJ explained observed encoding differences. To show that semantic but not serial clustering was key in encoding, we conducted 2 binary mediation analyses in the full sample. Semantic and serial clustering were separate predictors, total encoding was the mediator and delayed recall was the binary dependent variable. Semantic clustering predicted total encoding (p < .0001) and total encoding predicted delayed recall (p < .0001). Semantic clustering predicted delayed recall (p = .001) but this relationship was not significant after controlling for total encoding (p = .18), consistent with full mediation (z' = 3.62, p < .0001). Approximately 64% of the variance in delayed recall was accounted for by the predictors. In contrast, serial clustering was not associated with total encoding or delayed recall (p's > .95).

Conclusions: Results argue for poor encoding as a 1° deficit and intact consolidation and retrieval among EMJ. EMJ had poor encoding due to reduced efficiency organizing information into meaningful categories. EMJ also had poorer delayed recall (commonly cited in the literature), but this was 2° to reduced encoding. That is, less use of semantic organization worsened encoding, which may serve as a causal pathway for reduced delayed recall among EMJ. Finally, LMJ were comparable to CON on all memory indices, suggesting that earlier use onset may represent a key vulnerability to later neurocognitive compromise.

Findings point to a deficit in executive functioning with marijuana use. Marijuana use may disrupt frontal instead of hippocampal processing, particularly when use begins at an early age when the frontal lobes are undergoing significant development. Phase II of this research will examine neural correlates of these effects and will determine if abstinence reverses marijuana’s effects on encoding.

Keywords: cannabis use, Memory and Learning, Adolescence, neuropsychology

Disclosures: Nothing to disclose.
T262. Alcohol Sensitivity and Sex Effects on Cardiac Reactivity During Acute Intravenous Alcohol Exposure in Non-Dependent Drinkers

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Background: Cardiac dysfunction such as low heart rate variability and high heart rate, have been have been reported in chronic alcoholics after moderate doses of alcohol. We have previously shown that acute intravenous (IV) alcohol resulted in decreases in heart rate and increases heart rate variability during the ascending phase; these effects were associated with subjective alcohol responses. Previous studies have reported that low sensitivity to the subjective effects of alcohol is associated with greater alcohol use and abuse and has shown to predict future alcohol dependence. The relationship between cardiac reactivity and alcohol sensitivity is less clear in non-dependent drinkers. The objective of this study was to examine the effect of acute IV alcohol on cardiac physiology and to explore the relationship of cardiac reactivity with alcohol sensitivity in healthy non-dependent drinkers.

Methods: Healthy non-dependent drinkers (N = 86) completed a Computer-Assisted Self-infusion of Ethanol (CASE) session that allows individuals to self-administer alcohol while controlling the breath alcohol concentration (BrAC) using a physiologically-based pharmacokinetic (PBPK) model-based algorithm. Each session consisted of a priming phrase, where participants were required to push a button for four standardized ethanol infusions, followed by a 120-min free-access IV alcohol self-administration (IV-ASA) phase, where participants could push a button to receive the same IV ethanol infusions. IV-ASA measures included peak (PEAK) and average (AVG) BrAC and total ethanol (EtOH) infused during the session. Heart rate was continuously measured throughout the session. Heart rate measures included mean heart rate (HR), root mean square of mean squared difference of successive NN intervals (RMSSD), NN50 count divided by the total number of all NN intervals (PNN50), total power (TP), and low frequency (LF), high frequency (HF), and very low frequency (VLF) power. Subjective response was serially measured using the Drug Effects Questionnaire (DEQ). Participants completed the Alcohol Sensitivity Questionnaire (ASQ), and were stratified, based on median split, into low sensitivity (N = 43) high sensitivity (N = 45) groups.

Results: Acute IV alcohol had significant effects on cardiac function during IV-ASA. Across the session, the low sensitivity group had greater mean heart rates, SDNN, VLF, LF, HF, and TP. To account for sex differences in exposure, the total ethanol infused (EtOH) was included as a covariate in all analyses. Immediately after the priming dose of alcohol there was a main effect of alcohol sensitivity, with the low sensitivity group showing a decrease from baseline for SDNN and RMSSD. There was a main effect of sex, with males showing a decrease from baseline in RMSSD, VLF and HF and females showing an increase from baseline. There was an interaction showing that females in the low sensitivity group had less change from baseline in PNN50 while males had an increase in change. However, females in the low sensitivity group had greater change than males from baseline in VLF, RMSSD, HF and TP. During the self-administration session there was an interaction between sex and alcohol sensitivity in that males with low sensitivity had greater average NN50 and average PNN50 while low sensitivity females had lower average NN50 and average PNN50. This effect was trending for Peak measures of NN50 and PNN50. Across the entire sample, lower subjective effects of “feeling” the alcohol, “high”, and “intoxication” during the priming phase predicted increased IV-ASA during the session. The low sensitivity group showed significantly lower DEQ effects of “high”, “feel”, and “intoxicated” during the priming phase, and also had greater PEAK and AVG during the IV-ASA session. Males reported great feelings of “high” during the priming, suggesting that they felt the stimulating effects of the alcohol more than females (all p values < 0.05).

Conclusions: These results demonstrate a significant effect of acute IV alcohol on cardiac reactivity, and influences of alcohol sensitivity and sex on heart rate and heart rate variability measures and subjective responses during IV-ASA in non-dependent drinkers. Overall, participants with low sensitivity to alcohol showed greater alcohol self-administration during the session. These individuals also showed significantly lower heart rate variability during the session, which was more pronounced in females. The results also showed that males who feel the effects of alcohol less have lower heart rate variability during IV-ASA, suggesting that cardiac reactivity to IV-ASA in male and female non-dependent drinkers depends on their alcohol sensitivity. Thus, alcohol sensitivity may be an important marker for those that are at risk for alcohol-related cardiac dysfunction and alcohol use disorders. Future analyses will try to further characterize how drinking patterns and tolerance in males and females are related to differences in alcohol-induced cardiac measures and risk for alcohol problems.

Keywords: Cardiac Reactivity, Heart Rate Variability, IV Alcohol, Alcohol Sensitivity

Disclosures: Nothing to disclose.