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**Poster Session I**
**Monday, December 7, 2015**

## M1. Changes in the Functional Brain Connectivity and Verbal Memory Performance Following Yoga or Memory Training in Older Adults with Subjective Memory Complaints

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**Background:** Mind-body interventions, such as yoga, are used to reduce stress in the community samples. However, clinical efficacy of such interventions for cognition remains unclear. To-date, no studies have explored the effect of yoga on cognition and resting state functional Magnetic Resonance Imaging (rs-fMRI). The present study was to explore neural effects of yoga vs memory training in older adults with subjective memory complaints.
**Methods:** Older participants (age $\geq$ 55 years) with subjective memory complaints were randomized to receive yoga intervention or active "gold-standard" control (memory enhancement training (MET)) for 12 weeks. rs-fMRI was used to map correlations between brain network and memory performance changes over time. Default mode network (DMN), language and superior parietal networks were chosen as networks of interest to analyze the association with changes in verbal and visuospatial memory performance (recall after 30 min. delay).
**Results:** Fourteen yoga and 11 MET participants completed the study. Both groups demonstrated improved memory performance with no group differences. We observed no group differences in the functional connectivity over the course of the study. Increased DMN connectivity correlated with improved verbal memory in the frontal medial cortex, pregenual anterior cingulate cortex, right middle frontal cortex, posterior cingulate cortex, and left lateral occipital cortex. Increased connectivity in the language processing network also positively correlated with verbal memory performance in the left inferior frontal gyrus. However, changes in superior parietal network negatively correlated with visuospatial memory improvements in medial parietal cortex.
**Conclusions:** Our pilot study suggests that yoga can be as effective as MET in improving verbal memory performance in association with increased DMN connectivity. These pilot findings highlight the potential clinical use of yoga for subjective cognitive complaints that should be confirmed in larger studies.
**Keywords:** Non-Pharmacological Therapy, fMRI, cognitive aging, brain connectivity, yoga
**Disclosures:** Funded by the Alzheimer's Research and Prevention Foundation. Other sources of funding: grant from the Forest Research Institute/ Actavis; NIH/ NCCIH; NIMH

## M2. Mental Healthcare Access and the Underserved: Are Our State-of-the-Art Treatments Getting to those who Need It Most?

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**Background:** Disparities in mental health service use by racial/ethnic minority older adults are well-documented. Attention is now turning to the question of whether racial/ethnic disparities in mental healthcare are increasing or diminishing. The net effect of changes in healthcare organization and immigration, along with any explicit policies designed to reduce disparities, is not clear. The purpose of this study is to examine national trends in the use of mental health services by older racial/ethnic minority adults. Specifically, we measured trends in mental healthcare disparities to determine whether racial/ethnic disparities have increased, decreased, or remained constant over time.
**Methods:** Four, two-year longitudinal datasets from Panels 9-14 (2004-2012) of the Medical Expenditure Panel Surveys were combined to estimate trends in racial/ethnic disparities in mental healthcare among older adults (aged 65+) with probable mental health need. The sample included 33,469 older adults, aged 65+ (21,566 non-Latino Whites, 5,526 Blacks; 4,447 Latinos; and 1,930 Asians). Mental health service utilization was defined as engaging in outpatient care or filling a prescription medication. Outpatient care included primary care provider (PCP) or specialist mental health care (services received from a psychiatrist, psychologist, counselor, or social worker). Outpatient care was considered to be a mental health care visit if the treatment was recorded for a disorder covered by ICD-9 codes related to mood, anxiety, psychosis, substance use, personality, behavioral and developmental disorders. Similarly, psychotropic medications were defined as any prescription drug claim with any of the above ICD-9 codes attached to it. Psychotropic drug fill is a prescribed medicine refill without a mental health care visit or outpatient or office-based visits to assess the progress of the medications.
**Results:** Racial/ethnic disparities in any use of mental healthcare persisted from 2004-2012. Black-White and Asian-White disparities remained constant. Latino-White disparities increased over time. A similar pattern was found when type of services was analyzed. Black-White and Asian-White disparities in use of outpatient services and prescription drugs remained constant while Latino-White disparities in the use of these services increased.
**Conclusions:** The mental healthcare system continues to provide less care to racial/ethnic minority older adults than to older Whites, suggesting the need for policy initiatives to

improve services for these racial/ethnic minority groups. Alternative, non-traditional treatments as well as new delivery approaches are two ways to address this persistent problem. For example, health promotion interventions bring mental and physical health benefits to older adults. Moreover, they are non-stigmatizing, culturally relevant and salient. The use of community health workers in the delivery of mental health services is an efficient use of scarce resources and can be an effective tool to engage older racial/ethnic minority adults into mental health services.
**Keywords:** disparities, mental health service use, older adults
**Disclosures:** Nothing to disclose.

### M3. Depletion of Sex Steroid Hormones in Mid-Life Alters Hippocampal Activity during Verbal Memory Encoding: A Population-Based fMRI Study of Sex Differences in Memory Decline

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**Background:** Maintaining intact memory function with aging is one of the most important public health challenges of our time. We know that intervening early with high risk individuals is critical for the attenuation or prevention of disability, but we have yet to identify early targets for treatment. Memory circuitry contains relatively dense populations of sex steroid receptors, with some of the highest concentrations in hippocampus (HIPP). Experimental work in animals and imaging studies in humans have established the role of steroid hormones in regulating hippocampal structure and function. Converging evidence indicates that ovarian decline in women plays a mechanistic role in the neuronal changes that occur early in the aging process. As ovarian sources of estrogens decline, secondary estrogenic support, including adrenal DHEA-S (a weak androgen that can be aromatized to estradiol) may play a role in maintaining hippocampal function and hippocampal-dependent memory performance. Here, we present new human findings characterizing the network-level changes in memory circuitry that occur as a function of reproductive age and endogenous sex steroid levels in midlife men and women.
**Methods:** Healthy mid-life men and women ($N = 142$; age range 46-53), who are part of a 50-year prospective prenatal cohort, were enrolled in a population-based fMRI study. Menstrual cycle histories and hormonal evaluations of fasting serum samples collected on the morning of the scan (0800h) were used to determine the reproductive stage of women per STRAW-10 guidelines. Women were categorized as late reproductive ("premenopause"), menopausal transition ("perimenopause") or early postmenopausal ("postmenopause"). Participants performed a verbal encoding task during fMRI scanning. fMRI data were analyzed in SPM8. Statistical maps representing areas with linear increases in activity across encoding level (novel > repeated word pairs) were generated at the random effects level ($p < 0.001$). Neuropsychological assessments of verbal learning and memory, including the 6-Trial Bushke Selective Reminding Test (Bushke) and Face-Name Associative Memory (FN), were conducted post-scan.
**Results:** Chronological age did not vary appreciably between groups ($F = 1.29$, $p > .25$). However, LC-mass spectrometry and immunoassay results confirmed that serum estradiol ($F = 11.5$, $p < 0.001$) and progesterone ($F = 15.9$, $p < .001$) levels declined while FSH levels rose ($F = 35.0$, $p < 0.001$) over the menopausal transition in women. Functional MRI results revealed robust changes in HIPP BOLD signal as a function of reproductive stage. Postmenopausal women showed an attenuated HIPP BOLD response during verbal encoding compared to premenopausal women (small volume corrected, left HIPP, $p = .005$; right HIPP, $p < .05$). Men also exhibited a weaker HIPP response compared to premenopausal women (left, $p = .002$; right, $p = .005$), a sex difference that was attenuated in postmenopausal women. Neuropsychological testing revealed that Buschke and FN were sensitive to reproductive age. Pre- and perimenopausal women outperformed men on Buschke (Delayed Recall [$F(3,164) = 5.89$, $p = .001$] and FN Cued Recall [$F(3,162) = 7.87$, $p < .001$), a sex difference that was attenuated in postmenopausal women. Finally, across all women, higher levels of DHEA-S were associated with multiple indicators of better verbal memory performance (Bushke Delayed Recall, $r = .30$, $p = .01$, $n = 70$; 30 Min Recall, $r = .29$, $p < .02$; and Total Recall, $r = .26$, $p < .03$). For men, higher testosterone levels were also associated with better performance (Bushke Multiple Choice, $r = .31$, $p < .02$). Finally, greater recruitment of left hippocampus during verbal encoding was related to better FN associative memory performance in women (Free Recall, $r = .29$, $p = .034$) and multiple indicators of verbal memory in men (including Bushke Total Recall, Delayed Recall and FN Free Recall, all $r > .3$, $p < .05$).
**Conclusions:** Our results suggest that the loss of ovarian estradiol during menopause and secondary estrogenic support from DHEAS may play a significant role in shaping memory circuitry function and/or memory performance. We observed HIPP hypoactivation during a challenging verbal encoding task in postmenopausal women. These results mirror the pattern of results seen in traditional cognitive aging studies, with reduced HIPP responding in older versus younger subjects. Importantly, we see an early signature of this in mid-life, as a function of reproductive age in women. These findings underscore the importance of considering menopausal status and mid-life hormonal changes over and above chronological age to improve our understanding of sex differences in memory circuitry aging.
**Keywords:** cognitive aging, menopause, estradiol, Memory Encoding and Retrieval, fMRI
**Disclosures:** Nothing to disclose.

### M4. Positive Effects of Chronic but not Acute Estradiol on Cholinergic-Related Cognitive Performance in Postmenopausal Women

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**Background:** The prophylactic and therapeutic use of gonadal steroids such as estradiol (E2) remains among the

most controversial areas in medicine. While many studies in humans have investigated the effects of E2 and hormone therapy on cognition, the cholinergic system has been implicated in many aspects of the cognitive effects shown after E2 administration including improving attention, working memory, and improved performance on effort-demanding tasks such as verbal memory. The cholinergic system is also an important site of action for estrogen in the brain and E2 appears to modulate cholinergic neurotransmission. Cognitive symptoms reported by postmenopausal women may be linked to changes in the cholinergic system, such as difficulties in memory and attention. While many studies in humans have investigated the effects of estrogen and hormone therapy on cognition, few have proposed a mechanism of action for estrogen in the brain and it is unclear whether the mechanism is neurotropic or pharmacologic. We have shown previously that three months of estrogen administration blunted the detrimental effects of cholinergic antagonists on cognitive function. The hypothesis underlying this study was that estrogen produced trophic effects on basal forebrain cholinergic neurons. Recent studies have suggested that estradiol's effects on these cholinergic neurons may be produced through novel estrogen receptors such as GPR30. GPR30 is a novel G protein-coupled estrogen receptor that is expressed in brain, particularly by cholinergic neurons in the basal forebrain and appears to be an important regulator of basal forebrain cholinergic functioning. However, recent research has shown that E2 has rapid cellular effects. A single dose of estrogen acting via membrane-mediated or receptor-mediated mechanisms could produce pharmacological or functional antagonism of the effects of cholinergic antagonists. To further clarify whether varying durations of E2 administration differentially impact cognitive operations, this study directly compared the acute effects (single dose) of E2 to the chronic effects (3 months) of estrogen in modifying the response to cholinergic blockade. We anticipated that the effects of chronic administration of E2 in postmenopausal women would be greater than acute single-dose estrogen in modifying cognitive performance after cholinergic antagonist challenge.

**Methods:** During the acute pretreatment phase, eighteen non-smoking postmenopausal women (mean age 59.11, SD = 5.81, range 51-69) were randomly and blindly given a single capsule containing either 1mg 17-β estradiol (E2) (n = 10) or matching placebo (n = 8) at four visits. Ninety minutes after the hormone or placebo administration, cholinergic blockade challenges were completed. Subjects received one of the following medications on each challenge day: 2.5 μg/kg of the antimuscarinic drug scopolamine (IV), 20 mg of the antinicotinic drug mecamylamine (oral), a combination of scopolamine and mecamylamine, or placebo. Scopolamine placebo was an injection of saline. Mecamylamine placebo consisted of identical capsules filled with lactose. A double placebo system was used such that on each day, the subject received oral capsules and an injection. These challenge day conditions were randomized among subjects and double blinded. After completing all acute phase challenge visits, all eighteen subjects were placed on oral 17-β estradiol at a dose of 1mg/day for three months. At the end of the three-month chronic treatment phase, subjects completed four additional pharmacological challenge days identical to the acute phase challenges. Multiple domains of cognition were assessed during cholinergic challenge days using the Critical Flicker Fusion Task (CFF), Choice Reaction Time Task (CRT), Buschke Selective Reminding Task (SRT), and the N-Back Task (NBT).

**Results:** Initial analysis utilized the entire model (all drugs, all time points) in a mixed-effects repeated-measures ANOVA. Results showed that single dose of acute E2 or placebo as a pretreatment had no measurable effect on cognitive performance after cholinergic antagonist medication or placebo, thus both groups were combined for acute versus chronic analysis. Comparing acute versus chronic treatment, main effects of chronic E2 treatment appeared in several domains. Chronic E2 improve performance total mean ($p = 0.036$) and motor median ($p = 0.033$) reaction time on the CRT with faster performance in the chronic E2 administration group than acute treatment. For verbal episodic memory, the chronic E2 administration group also showed significantly better performance during the delayed recall trial of the SRT ($p = 0.006$) compared to acute E2 treatment. While main effects of E2 treatment on cognitive performance were seen after chronic administration (but not after acute administration), there were no significant treatment by cholinergic antagonist interactions on the various cognitive domains tested.

**Conclusions:** While rapid effects of single-dose administration of estradiol can be seen in some systems, these results suggest that the effect of estrogen on cognitive performance is likely mediated through long-term trophic effects and less likely to be mediated via rapid, membrane-mediated or receptor-mediated effects on cholinergic systems or other neurotransmitter systems directly related to cognitive performance. Although single administration of estrogen in postmenopausal women has no protective effects on cognitive functions, these results support that chronic administration of estrogen is beneficial to psychomotor speed and verbal recall memory including reducing the cognitively impairing effects of cholinergic antagonists. Knowledge of these differences will assist in further studies using estrogenic compounds for cognitive preservation and/or enhancement, potentially with cholinergic modulation.

**Keywords:** estradiol, Cognition, acetylcholine, menopause, aging

**Disclosures:** Nothing to disclose.

### M5. Severity of Early Life Stress Predicts Thalamic Hyperconnectivity and Multiple Network Disruption: A Transdiagnostic Study of Global Connectivity

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**Background:** Early life stress (ELS) is an established risk factor for psychopathology and is associated with altered functional connectivity within- and between neural networks. The widespread nature of these disruptions suggests that broad imaging measures of neural connectivity, such as global based connectivity (GBC), may be appropriate for

studies of this population. GBC identifies brain regions having maximal functional connectedness with the rest of the brain. Alterations in GBC may reflect a pathological restriction or broadening of network synchronization. In this study, we evaluated whether ELS severity predicted GBC in subjects with a spectrum of ELS exposure.
**Methods:** MRI images from 46 right-handed participants were used for this study. This sample represents a pooled imaging database, comprised of subjects enrolled in studies of ELS, MDD and PTSD at Brown University-affiliated hospitals. This group included $n = 18$ medication-free healthy controls, $n = 14$ medication-free participants with ELS and without psychiatric disorders, and $n = 14$ patients with trauma-related disorders associated with negative affect states. GBC was calculated using AFNI, constrained by each participants grey matter. An initial one-sample, voxel-wise t-test to describe the spatial distribution of GBC, which was followed by a predictor analysis, where we evaluated those voxels in the brain where CTQ score significantly predicted GBC. Brain regions identified by this predictor analysis were evaluated using traditional seed-based functional connectivity. Two group-level analyses were employed. The first compared connectivity in all participants with ELS against those without. The second approach, designed to uncover differences that corresponded to traditional categories and diagnoses, compared each of the three original participant groups against each other. Results of all group contrasts were thresholded at Family-Wise-Error (FWE)-corrected $p < .05$.
**Results:** The spatial distribution of GBC peaked in regions of the salience and default mode networks, and ELS severity predicted increased GBC of the left thalamus (corrected $p < .005$). The thalamus was subsequently evaluated using seed-based connectivity, first to compare groups with and without ELS, and then to compare individual participant groups. This revealed altered connectivity between the thalamus and regions of the default mode and salience networks, particularly the precuneus (corrected $p < .01$) and dorsal anterior cingulate cortex (corrected $p < .005$), in participants with ELS and related disorders, respectively.
**Conclusions:** These findings support a model of disrupted thalamic connectivity in ELS and trauma-related negative affect states, and underscore the importance of a transdiagnostic, dimensional neuroimaging approach to understanding the sequelae of trauma exposure.
**Keywords:** Trauma exposure, Resting State Functional Connectivity, Research Domain Criteria (RDoC)
**Disclosures:** Nothing to disclose.

## M6. Amygdala-Dependent Molecular Mechanisms of the Tac2 Pathway in Fear Learning

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**Background:** Recently we reported that activation of the Tachykinin 2 (Tac2) pathway in the central amygdala (CeA) is necessary and sufficient for the modulation of fear memories. The Tac2 pathway includes the Tac2 gene, which encodes the neuropeptide Neurokinin B (NkB) and its corresponding receptor Neurokinin 3 receptor (NK3R).
**Methods:** In this study, we screened Tac2 pathway-related gene variants in patients with PTSD and explore the action mechanisms of Tac2 pathway in fear memory by combination of optogenetics, pharmacology, immunohistochemistry, qRT-PCR, and electrophysiology in wild-type, Tac2-Cre, and Tac2-GFP mice.
**Results:** In a sample of 3070 individuals with post-traumatic stress disorder (PTSD), we have identified two gene variants of the Tac2 pathway that are significantly associated with PTSD diagnosis. Hence, delineating the Tac2 pathway may be necessary to understand and treat fear disorders such as PTSD. In transgenic mice that express ChR2 solely in Tac2 neurons, in vivo optogenetic stimulation of CeA Tac2-expressing neurons during fear acquisition enhances fear memory consolidation and drives action potential firing in vitro. Notably, Tac2-CeA neurons were found to project to the midbrain reticular nucleus and periaqueductal grey, areas known to play a role in fear memory formation. In addition, Tac2-CeA neurons were shown to co-express striatal-enriched protein tyrosine phosphatase (STEP), which might play an important role regulating Nk3R signaling.
**Conclusions:** Taken together, this study extend previous animal studies to clinical investigation, suggesting that the Tac2 pathway may play a role in PTSD, and we provided data that helps to understand the action mechanisms of the Tac2 pathway.
**Keywords:** fear conditioning, PTSD, Amygdala
**Disclosures:** Nothing to disclose.

## M7. Validation of the Negative Sequelae of Trauma (NeST) Model: A Dimensional Approach to Studying the Neurobiology of Post-Traumatic Stress

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**Background:** Investigation of psychological and behavioral changes in the aftermath of trauma has focused primarily on mechanisms for maintenance and recovery from post-traumatic stress. However, the scope and nature of "core" symptoms is a topic of longstanding debate, resulting in a recent change in diagnostic criteria for post-traumatic stress disorder (PTSD) in the DSM-5. Complicating factors include the fact that responses to trauma vary greatly from individual to individual, and often include depressive symptoms. We propose an alternative approach to study responses to trauma that, in contrast with selecting a set of criteria for a particular disorder (e.g., PTSD, depression), accounts for both anxiety and depressive symptoms in a dimensional manner. This approach is consistent with the NIMH Research Domain Criteria approach to the study of mental illness. We examined symptom severity within data-driven symptom clusters derived using principle components analysis (PCA). We then examined individual differences in declarative memory (behavior and neural

activation) as a test-case for further study linking continuous symptom clusters with intermediate phenotypes.
**Methods:** N = 6248 participants who reported at least one traumatic experience were recruited from the primary care waiting rooms of a public inner-city hospital in Atlanta, GA. Principal components analysis (PCA) was used to identify clusters of post-traumatic anxiety and depression symptoms, as measured by the modified PTSD symptom scale (MPSS) and Beck Depression Inventory (BDI). The PCA included 17 items on the MPSS and 21 items on the BDI, and resulted in six components. The components were interpreted as reflecting Negative Affect, Somatic Symptoms, Re-experiencing, Numbing, Hypervigilance, and Impaired Cognition. Relations between these components and psychological outcome measures were examined. A subset of N = 47 traumatized women completed an fMRI scan measuring declarative memory encoding of complex scene stimuli and completed a delayed cued recall task, and the effects of the 6 PCs on memory encoding were examined.
**Results:** Individuals who were high on one PC had low scores on the other PCs, suggesting sub-types of trauma responses. Of the 6 components, the Negative Affect component most strongly predicted emotion dysregulation (EDS t = 41.6, DERS t = 12.9), current drug abuse (DAST t = 8.6), and was inversely associated with resilience (CD-RISC t = -30.9). The Re-experiencing component most strongly predicted a convergent measure of PTSD severity (CAPS t = 12.6) and PTSD-related problem behaviors (PABQ t = 7.34). Whereas Negative Affect was most strongly related to childhood trauma (CTQ t = 28.7), Re-experiencing was most strongly related to adult trauma (TEI t = 21.6). None of the PCs correlated with cued recall performance, ps > .10. In the fMRI analyses, Negative Affect predicted decreased right amygdala activation to images that were subsequently recalled versus not-recalled, whereas Re-experiencing predicted increased bilateral amygdala and hippocampal activation to recalled versus not-recalled items, p < .05, corrected.
**Conclusions:** The current study identified a novel approach to quantifying post-trauma symptoms, which characterized anxiety and depression symptoms without the need to include one or the other as a "control" variable. This data-driven approach identified symptom clusters that included some similarities with DSM-5 symptom clusters but also diverged in important ways. In particular, avoidance symptoms did not form an independent cluster, but loaded onto several PCs. This may reflect the fact that avoidance is a behavioral coping strategy used by individuals experiencing a variety of different types of trauma responses, or may alternately be causal to several types of symptoms. Whereas trauma-related amnesia is clustered with Negative Affect in DSM-5, here it clustered with concentration difficulties in the Impaired Cognition component. Most interestingly, Negative Affect and Re-experiencing appeared to track very different manifestations of post-traumatic reactions that differed in their neurobiological underpinnings. Further, given that childhood trauma severity was associated with the Negative Affect component, and adult trauma severity was associated with the Re-experiencing component, this analysis points to differential etiology resulting in different clinical and neural signatures. Such transdiagnostic and dimensional approaches are advocated by RDoC and may lead to greater precision in treating the negative sequelae of traumatic experiences.
**Keywords:** posttraumatic stress, Research domain criteria (RDoC), declarative memory, functional neuroimaging
**Disclosures:** Nothing to disclose.

### M8. Does the 'Not Just Right Experience' (NJRE) in Obsessive-Compulsive Disorder Denote a Neurodevelopmental Dimension Characterised by Abnormal Sensory Processing?

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**Background:** Treatment development for obsessive-compulsive disorder (OCD) is compromised by the clinical complexity and heterogeneity of the disorder. It is unclear as to whether this heterogeneity is best understood in terms of categorical subtypes or dimensions. Some OCD symptoms, especially those concerned with ordering, symmetry and arranging, are distinguishable from archetypal harm avoidance (HA) and reflect the need to make the environment feel right. The 'not just right experience' (NJRE) has been proposed as a separate core motivational process in OCD that may adversely impact on clinical outcomes. NJRE is found in non-clinical populations, including at increased levels in the parents of children with autism spectrum disorder (ASD), implicating NJRE as a trans-diagnostic neurodevelopmental risk factor. Moreover, descriptive clinical profiles of OCD patients show parallels to a neurodevelopmental profile characteristic of ASD, including difficulties with social communication, sensory processing and cognitive rigidity. Our objective was to explore whether NJRE defines a neurodevelopmental OCD dimension that is (a) distinct from harm avoidant (HA) OCD, (b) related to autistic traits and/or to a broader phenotype of cognitive rigidity and sensory processing difficulties and (c) associated with an earlier age of OCD onset.
**Methods:** We investigated 25 adult (18-65y) participants from a national OCD service with a primary DSM-IV diagnosis of OCD, using a range of self-report questionnaires, clinician-rated questionnaires and a neurocognitive task. A correlational design investigated whether NJRE and HA, as measured on the Obsessive-Compulsive Trait Core Dimensions Questionnaire, associate together, and explored their relationship with (a) ASD traits measured by the Autism Quotient (AQ), (b) sensory processing measured by the Adolescent /Adult Sensory Profile (AASP), (c) cognitive rigidity measured by the intra-extra dimensional shift (ID-ED) task from the Cambridge Automated Neuropsychological Test Battery and (d) age of OCD onset.
**Results:** HA and NJRE traits did not violate the assumption of normality and were continuously distributed in this sample. NJRE was only moderately (r = .34) correlated to HA and not significant in this study. Consistent with predictions, NJRE was associated with sensory processing difficulties on the AASP (r = .64, p = 0.001) involving low registration, increased sensory sensitivity and increased

sensory avoiding, as well as an earlier age of OCD onset ($r = .59$, $p = 0.002$). The relationship to overall sensory processing was significant even at the rigorous Bonferroni corrected level ($p = .004$), and survived controlling for OCD severity (Yale-Brown Obsessive Compulsive Scale total score; $r = .53$, $p = .008$) and trait anxiety (State Trait Anxiety Inventory; $r = .53$, $p = .008$) using partial correlations. Whilst HA also correlated with sensory processing difficulties, this relationship ceased to be significant once controls were made for anxiety. Contrary to predictions, no significant relationships were found between NJRE (or HA) and ASD traits as measured by the AQ, or with ID-ED set-shifting difficulties.

**Conclusions:** The findings of this study strengthen the understanding of NJRE as a marker of an underpinning neurocognitive dimension rather than a categorical OCD subtype. There was a lack of evidence demonstrating NJRE as a manifestation of core autistic traits as measured by the AQ, or of cognitive rigidity on the ID-ED test. However, NJRE differentiated from HA and was associated with sensory processing abnormalities and early onset of OCD. Thus, the presence of NJRE may indicate an atypical neurodevelopmental trajectory, warranting further research in the quest for alternative treatment approaches for OCD and related disorders.

**Keywords:** OCD, not just right experience, ASD, harm avoidance, sensory processing

**Disclosures:** N. Fineberg consultant for Lundbeck, Glaxo-Smith Kline, Transcept, Novartis and Servier. Received research support from Lundbeck, Glaxo-SmithKline, ECNP, Servier, Cephalon, Astra Zeneca, UK MRC, UK NIHR, Wellcome Foundation. Received honoraria for lectures at scientific meetings from Lundbeck, Servier, Astra Zeneca, Jazz pharmaceuticals, and Bristol Myers Squibb. Received financial support to attend scientific meetings from Janssen, Lundbeck, Servier, Novartis, Bristol Myers Squibb, Cephalon, International College of OC Spectrum Disorders, International Society for Addiction, ECNP, BAP, WHO, and Royal College of Psychiatrists.

## M9. Plasticity of MAOA Methylation: An Epigenetic Correlate of Therapy Response in Panic Disorder?

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**Background:** Treatment resistance in anxiety disorders is considerable with a high individual and socioeconomic burden. This emphasizes the urgent need for better understanding predictors and mechanisms of action of therapeutic interventions in order to inform expert treatment decisions towards a more personalized medicine in anxiety disorders. Epigenetic signatures such as methylation of the monoamine oxidase A (MAO-A) gene have been found to be altered in panic disorder (PD) and are increasingly suggested to possibly mediate treatment success and resistance, respectively. Hypothesizing temporal plasticity of epigenetic processes to constitute a key mechanism of successful fear extinction, the present psychotherapy-epigenetic study for the first time investigated MAO-A gene methylation changes during the course of a psychotherapeutic intervention in PD.

**Methods:** MAO-A methylation at 13 CpGs in the exon I / intron I gene region was analyzed at baseline and after a standardized 6-week cognitive behavioral therapy (CBT) in a discovery sample ($N = 28$; age: age: $34.57 \pm 8.51$ years) as well as in an independent replication ($N = 20$; age: $33.55 + 11.15$ years) sample of somatically healthy female Caucasian patients with PD via direct sequencing of sodium bisulfate treated DNA extracted from blood cells. Possibly confounding factors such as age, smoking behavior, psychotropic medication, comorbidities and MAO-A VNTR genotype were considered.

**Results:** In both samples, a significant negative correlation between MAO-A methylation and baseline panic disorder severity was discerned. In the discovery sample, responders and non-responders to CBT showed differential dynamics of MAO-A methylation during the course of treatment, with responders displaying a significant increase in average MAO-A methylation ($+3.4\%$), while in non-responders average methylation decreased (-2.0%) ($p = 0.001$). This pattern held true for eight individual CpG sites ($p = 0.001$-$0.04$) and survived the most conservative correction for multiple testing at four CpGs. In the replication sample, increase in methylation during therapy also correlated significantly with symptom improvement ($r = -0.42$--$0.57$).

**Conclusions:** The present psychotherapy-epigenetic study supports previous evidence for MAO-A DNA hypomethylation as a risk marker of PD and for the first time suggests restitution of altered MAO-A methylation patterns as a potential epigenetic correlate of treatment response to CBT in PD. The emerging notion of epigenetic signatures as a core mechanism of action of response to psychotherapeutic interventions is hoped to contribute to a more effective treatment of anxiety disorders, e.g. by promoting psychotherapeutic or pharmacological options inducing epigenetics changes for lasting extinction effects.

**Keywords:** Epigenetics, anxiety disorders, Psychotherapy, CpG Methylation

**Disclosures:** Nothing to disclose.

## M10. Within-Session Salivary Cortisol Reactivity during Psychotherapy is Associated with Treatment Outcome for Post-Traumatic Stress Disorder

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**Background:** Convergent evidence suggests that hypothalamic-pituitary adrenal (HPA) axis function is disrupted in post-traumatic stress disorder (PTSD). Further, release of cortisol may enhance extinction, and normalization of HPA axis may be associated with symptom improvement. However, little research has examined how HPA axis function changes over the course of treatment, and no research to date has examined how HPA axis reactivity during treatment session is related to outcome. Thus, the

current study was designed to test the association between HPA axis reactivity and treatment response in individuals undergoing trauma-focused treatment for post-traumatic stress disorder.
**Methods:** 30 OEF/OIF veterans were randomly assigned to receive 10-12 sessions of Prolonged Exposure Therapy or Present Centered Therapy. PTSD symptoms were assessed at baseline and posttreatment using the Clinician-Administered PTSD scale. Salivary cortisol was collected during each of three therapy sessions (3, 6, and 10). Cortisol collections occurred at the start of session, 45 minutes into the session, and at the end of the session (90 minutes from start). Saliva was collected into salivettes via passive drool, and cortisol levels were determined by chemiluminescent enzyme immunoassay (IMMULITE). Cortisol reactivity was calculated by area under the curve with respect to ground (AUC). Statistical analyses were conducted using hierarchical linear modeling, a multilevel modeling technique that accounts for the inherent nested nature of data generated by treatment studies. We modeled longitudinal change in salivary cortisol (level one) nested within patients (level two). We then tested the effects of treatment responder status at both levels. We hypothesized that high responders would show a greater reduction in salivary cortisol output over the course of treatment.
**Results:** Models of longitudinal cortisol reactivity were based on 66 points of measurement (level one) nested within 28 patients (level two). The unconditional model estimated variance components for level one ($\sigma 2 = 57.91$) and level two units ($\tau = 124.87$). The value of $\tau$ was significantly different from zero ($\chi 2(29) = 177.73$, $p < .001$), indicating the presence of patient-level effects on outcomes. The intra-class correlation (ICC) for between-patient variability was .68, indicating that 68% of variance in cortisol could be accounted for by factors associated with the patient or external factors that varied by patient (R2-between). The remaining variance (32%) could be attributed to within-patient effects such as time in treatment or other factors not included in the model. In the final model, the significant effect of session number indicated linear increases in cortisol output over the course of treatment (across sessions) ($\beta = 1.06$, $t = 2.45$, $df = 40$, $p = .02$). In addition, responder status significantly predicted slope of cortisol level across sessions ($\beta = -1.35$, $t = -2.10$, $df = 40$, $p = .04$) but not overall cortisol level, indicating that responder status was specifically related to change in cortisol over the course of treatment. Compared to low responders, high responders exhibited a 1.35 ($SE = 0.64$) decrease in cortisol level, on average, between sessions. Responder status accounted for 6% of the previously unexplained variance in cortisol level.
**Conclusions:** As compared to low treatment responders, high treatment responders showed greater decreases in salivary cortisol output over the course of treatment. These results indicate that reductions in HPA axis reactivity over the course of psychotherapy may be associated with better treatment response. Future work is needed to investigate how modulation of HPA axis reactivity may be targeted in order to optimize PTSD treatment outcomes.
**Keywords:** PTSD, Cortisol, HPA axis, Psychotherapy, Exposure therapy
**Disclosures:** Nothing to disclose.

## M11. Signaled Avoidance Learning Recruits a Prefrontal-Hippocampal System for the Suppression of Innate Defensive Behavior

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**Background:** The ability to master challenging circumstances promotes resilience to trauma. Signaled active avoidance (SigAA) in rodents nicely models the therapeutic mastery experience. SigAA involves a response performed during an auditory CS that prevents an aversive US. Early in training, the CS elicits innate defensive reactions (freezing) that conflict with the avoidance response. As training proceeds, the subject moves from reactive to active CS-evoked behavior. This transition leads to a robust suppression of freezing, even when tested in environments that do not allow the avoidance response. We hypothesized that active avoidance learning recruits a neural circuit that negatively regulates the expression of aversive Pavlovian memory. This pathway could provide important insights into the biological process by which self-efficacy promotes resilience to trauma. Aversive Pavlovian memory requires the amygdala, which can be regulated by other forebrain circuits. The ventro-medial prefrontal cortex (vmPFC) is known to attenuate the expression of conditioned freezing, while the dorsal hippocampus (DH) has a role in the resolution of response conflict. These two regions are interconnected by the nucleus reuniens (NR) of the thalamus. Using a combination of pharmacological and pharmacogenetic techniques, we explored the role of a vmPFC-NR-DH circuit in the suppression of freezing that occurs as a function of active avoidance learning.
**Methods:** SigAA behavior: rats were trained in standard shuttle boxes. A 15 second tone CS predicted a footshock US. If the animal shuttled (crossed the divided chamber) during the CS, the tone terminated immediately and the US was omitted. This was counted as an avoidance response. If the animal shuttled during the US, it was terminated immediately. This was counted as an escape response. Training consisted of 3 daily sessions with 30 trials each. After training, the CS was tested in a distinct chamber that did not allow for shuttling, in order to isolate freezing from the competing avoidance response. In all experiments, drugs (CNO or muscimol) were administered prior to this CS test. Pharmacogenetic manipulations: in separate groups of animals, vmPFC and DH were infected with a virus containing the gene construct for the inhibitory hm4d(Gi) receptor. Animals were allowed to recover before SigAA training. Prior to the CS test, 5mg/kg of CNO (ligand of the hm4d(Gi) receptor) or vehicle was injected up. Pharmacological manipulations: cannula were aimed at NR and animals were allowed to recover prior to SigAA training. Prior to the CS test, 0.02 micrograms of muscimol or vehicle was infused into NR.
**Results:** Pharmacogenetic manipulations of vmPFC and DH had a profound effect on the expression of CS-evoked freezing at test. Compared to controls, inactivation of DH or vmPFC with CNO caused a significant increase in conditioned freezing. Intriguingly, the inhibition of both structures produced levels of freezing comparable to poor

performers, which are a subset of animals that fail to suppress Pavlovian reactions to the CS. CNO inactivation of the dorsal mPFC and the ventral hippocampus had no effect, suggesting that avoidance learning selectively recruits vmPFC and DH to attenuate CS-evoked freezing. Muscimol inactivation of the NR, which interconnects vmPFC and DH, also enhanced freezing after SigAA training. Thus, control animals were able to suppress CS-evoked freezing as a function of the avoidance learning. However, inactivation of vmPFC, NR or DH reversed the effect of SigAA training and caused animals to freeze at high levels, comparable to poor performers that did not express the avoidance response.
**Conclusions:** SigAA models therapeutic processes by which an internal locus of control is used to regulate fear and anxiety. The acquisition of SigAA involves a transition from reactive to active CS-evoked responses. This process recruits a vmPFC-NR-DH circuit for the inhibition of freezing. Intriguingly, freezing is suppressed by this pathway even in environments that do not allow the avoidance response. Thus, by recruiting this circuit, SigAA produces an inhibition of conditioned defensive reactions that is not tied to a particular context, suggesting that these results may be relevant to therapeutic concepts of resilience.
**Keywords:** Hippocampus, Medial Prefrontal Cortex, resilience
**Disclosures:** Nothing to disclose.

### M12. Sex- and Sert-Mediated Differences in Stimulated Serotonin Revealed by Fast Microdialysis

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**Background**: Serotonin is essential for encoding emotionally salient information. Recently, we greatly improved the temporal resolution of online microdialysis for the investigation of in vivo serotonin neurotransmission. In vitro probe recovery demonstrated that 2-min sampling is sufficient to capture rapid serotonin dynamics. We developed an in vivo stimulus paradigm that uses brief, serially delivered high K+ stimuli via reverse dialysis, which we used to study serotonin release capacity and stimulus sensitivity in behaving mice. Five high K+ pulses (120 mM K+ in artificial cerebrospinal fluid (aCSF)) were delivered at 20-min inter-stimulus intervals with 1-min increases in stimulus pulse duration to examine sensitivity and stimulation dose-response. Two sets of three repeated stimulations (6-min pulses) were then delivered with long (54 min) vs. short (24 min) inter-stimulus intervals to investigate responses to repeated maximal stimulation.
**Methods:** Separation and detection conditions on commercially available high-performance liquid chromatography instrumentation were optimized to achieve 2-min resolution for online serotonin microdialysis. Male and female siblings were randomly assigned to the following groups based on sex and genotype: wildtype male (WT-M, N = 7), wildtype male with acute local SERT inhibition by reverse microdialysis of 1.2 μM S-citalopram in aCSF (WT-M-ESC, N = 8), constitutive SERT deficient male (KO-M, N = 6), and wildtype female mice. Female subjects were further divided into two groups based on estrous phase: metestrus/diestrus (WT-F-MD, N = 5) vs. proestrus/estrus (WT-F-PE, N = 5), determined by vaginal smear immediately following microdialysis testing. We investigated SERT-mediated effects on serotonin clearance by reverse dialysis of serotonin for 1 h in a separate cohort of male mice (N = 7 mice each for WT-M, WT-ESC, and KO-M). Basal serotonin was measured in all subjects prior to high-K+ challenges. All mice were generated at the University of California, Los Angeles (UCLA), which is fully accredited by AAALAC. All animal care and use met the requirements of the NIH "Guide for the Care and Use of Laboratory Animals." The UCLA Chancellor's Animal Research Committee pre-approved all procedures.
**Results**: Basal serotonin in the ventral striatum was significantly higher in WT-F-MD mice (0.20 ± 0.009 nM; $P < 0.001$) compared to WT-F-PE (0.14 ± 0.005 nM) and WT-M subjects (0.15 ± 0.005 nM). Significantly elevated basal serotonin concentrations were also observed in the WT-M-ESC and KO-M groups (0.67 ± 0.02 and 0.91 ± 0.02 nM, respectively; $P < 0.001$) relative to the WT-M group. We observed transient, stimulated serotonin overflow following high K+ pulses as short as 1-min. Throughout the 1-5 min K+ stimulations, induced serotonin overflow was similar in WT-M and WT-F-PE mice, and higher in WT-F-MD and WT-M-ESC groups ($P < 0.05$). Stimulated serotonin overflow was greatest in the KO-M group compared to all other groups, and in particular, the WT-M-ESC group, indicating neuroadaptive potentiation of serotonin release after constitutive SERT loss. Groups with higher levels of stimulated serotonin overflow (i.e., WT-F-MD, WT-M-ESC, and KO-M), showed diminishing responses ($P < 0.05$) to repeated maximal stimuli in conjunction with the shorter inter-stimulus interval. Exogenous serotonin perfusion (500 nm) produced stable, elevated dialysate serotonin concentrations that were significantly different between WT-M, WT-M-ESC, and KO-M groups ($P < 0.001$). The KO-M mice exhibited the highest dialysate serotonin levels (498 ± 1 nM) associated with minimal (0.4%, or 2 ± 1 nM) tissue extraction of perfused serotonin, indicating that other mechanisms of serotonin clearance, including diffusion, contribute minimally to removal of extracellular serotonin in vivo. Tissue extraction was 4% (19 ± 1 nM) in WT-M-ESC and 15% (76 ± 3 nM) in WT-M groups demonstrating SERT-dependence for serotonin clearance during exogenous serotonin perfusion.
**Conclusions:** Brief high K+ stimulation in conjunction with fast online microdialysis sampling is a powerful approach for elucidating biologically important differences in serotonin neurotransmission. Previously, we showed that fast sampling during online microdialysis improved estimates of basal serotonin levels. Furthermore, the overall times needed for basal dialysis and, particularly, no net flux estimates of serotonin concentrations were greatly decreased. Improved temporal resolution prevented undersampling, thereby enabling differentiation of brief stress- or circadian-induced increases in endogenous serotonin levels. The current findings illustrate that sex, female hormonal cycles, and pharmacologic vs. genetic loss of SERT modulate measureable differences in basal and stimulated

serotonin levels. These results further indicate that the magnitude of differences between female mice in different estrous phases is similar to differences associated with acute, pharmacological SERT inhibition in male mice. Future studies should include female subjects taking into account estrous status.
**Keywords:** Serotonin Transporter, sex differences, Microdialysis, circadian rhythm, anxiety disorders
**Disclosures:** Support from the National Institute of Mental Health (MH064756, MH086108), the Brain & Behavior Research Foundation (formerly NARSAD), the Shirley and Stefan Hatos Foundation, and the UCLA Weil Endowment Fund are gratefully acknowledged. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Mental Health or the National Institutes of Health. Declaration of competing financial interest(s): A. Andrews, consultant Forest Laboratories (Actavis).

### M13. Interleukin-6 as a Peripheral Fear Signal and a Regulator of Fear Memory Maintenance

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**Background**: To date, no treatment effectively alleviates the debilitating fear memories for trauma that are characteristic of post-traumatic stress disorder (PTSD). While fear memories are useful for avoiding previously encountered threats, PTSD patients continue to experience profound aversive responses to fear memory for trauma even after repeated false alarms. Most treatments directed at profound and persistent fear memories in PTSD target the central nervous system (CNS), but the observation that many individuals with PTSD exhibit elevated levels of circulating proinflammatory immune molecules suggests a role for peripheral signaling. While some studies have demonstrated that increasing the proinflammatory immune response can alter fear memory maintenance, these findings are difficult to interpret because levels of inflammation far exceed those induced endogenously by psychological stress.
**Methods:** We have developed a modified traumatic version of Pavlovian fear conditioning in mice that induces a persistent proinflammatory response and a strong conditioned fear phenotype. Proinflammatory cytokines (IL-1β, IL-6, IFN-γ, TNF-α) were measured in plasma using a multiplex ELISA. In order to assess the relevance of cytokine signaling to behavior, mice were systemically treated with neutralizing antibodies to the cytokines. Studies were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all procedures were approved by the Institutional Animal Care and Use Committee at Emory University.
**Results:** Days after fear conditioning, conditioned animals showed no differences in cytokine levels compared to naïve controls. However, recall of the fear memory by re-exposure to the conditioned stimulus rapidly increased plasma levels of the proinflammatory cytokine interleukin-6 (IL-6). This IL-6 response to the conditioned stimulus was abolished after the fear response was extinguished. Neutralizing IL-6 prior to fear memory recall reduced the maintenance of both contextual and cued fear memory the following day.
**Conclusions:** Our findings indicate that traumatic experiences can induce a conditioned inflammatory response in addition to a conditioned fear response. The observation that fear memory recall, and not just fear conditioning itself, induced an IL-6 response perhaps explains mixed reports of elevated IL-6 in PTSD patients. Our observations are not the first to demonstrate a relationship between psychological memory and the immune system. However, the observation that neutralizing IL-6 resulted in less fear memory after recall provide the first endogenous model to suggest that the fear-induced IL-6 response contributes to the maintenance of fear memory after it is recalled. While mounting evidence indicates a role for proinflammatory cytokines in depression, few studies have adequately addressed whether inflammation contributes to disorder of fear and anxiety. Together, our findings suggest that peripheral inflammation, through IL-6, contributes to the persistence of fear memory in PTSD and other anxiety disorders characterized by impaired fear learning (i.e. phobia). The authors declare no conflicts of interest pertinent to this abstract.
**Keywords:** Fear conditioning, cytokines, inflammation, Interleukin-6
**Disclosures:** Nothing to disclose.

### M14. Limbic and Hypothalamic Intrinsic Connectivity Correlates with Cortisol Reactivity to Stress in Inhibited Children

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**Background**: Studies in animal models have shown that a number of brain regions, including the amygdala, hypothalamus, and hippocampus, can induce cortisol release in response to stress. In rodents, altered cortisol release is associated with altered neurocircuitry; however, this has yet to be tested in humans. Dysregulated cortisol release is associated with anxiety disorders and may be related to individual differences in limbic intrinsic connectivity in humans.
**Methods:** Thirty-four children ages 8-10 were recruited across a range of inhibited temperament, a risk factor for anxiety disorders. Three salivary cortisol samples were collected over the course of an MRI scan, a mildly stressful situation. Cortisol area under the curve with respect to ground (AUCg) was calculated. Intrinsic connectivity was measured by "resting state" functional MRI. Correlations between amygdala, hypothalamus, and hippocampus limbic connectivity and cortisol reactivity were calculated using a regression analysis.
**Results**: More inhibited temperament was correlated with increased cortisol release (AUCg, $r = .41$, $p = .02$). Increased cortisol was positively correlated with positive connectivity between the amygdala and the dorsal anterior cingulate cortex and motor cortex (all findings $p < .05$, FWE corrected). Increased cortisol was associated with less

positive connectivity between limbic regions, including the amygdala, hypothalamus, and hippocampus, with regulation regions, including the ventromedial and ventrolateral prefrontal cortex, subgenual anterior cingulate, and medial prefrontal cortex.
**Conclusions:** This is the first study in inhibited children to demonstrate that alterations in a broad network of limbic connectivity are associated with individual differences in cortisol reactivity. We propose that altered limbic connectivity may lead to altered cortisol stress response, resulting in anxiety symptoms. Interventions which strengthen limbic-ventral prefrontal cortex connectivity may prevent the development of anxiety symptoms in inhibited children.
**Keywords:** Cortisol, neurobiology, stress, depression, anxiety, adolescence, Resting State Functional Connectivity, BNST, Amygdala
**Disclosures:** Nothing to disclose.

## M15. Amygdala Endocannabinoids Promote Resiliency to Traumatic Stress Exposure

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**Background**: Posttraumatic stress disorder (PTSD) is a complex syndrome characterized in part by pathological conditioned fear responses, fear generalization, and fear sensitization. Furthermore, not all people exposed to traumatic stress will develop PTSD, suggesting resiliency mechanisms may buffer the development of PTSD in some individuals. Cannabis use rates are very high in PTSD populations, and symptom coping motives are highly cited by chronic cannabis users with PTSD. Based on these data we tested the hypothesis that endogenous cannabinoids promote resiliency to traumatic stress exposure, and that enhancing endocannabinoid signaling could represent a novel approach to the treatment of PTSD and stress-related psychiatric disorders.
**Methods:** We developed and validated a novel repeated measures novelty-induced hypophagia (NIH) assay to determine inter-individual differences in susceptibility to fear generalization in repose to foot-shock stress, and responsively to pharmacological manipulations in mice. We generated constitutive and conditional diacylglycerol lipase alpha (DAGLa) KO mice to reduce 2-arachidonoylglycerol mediated endocannabinoid signaling and utilized pharmacological inhibition of monoacylglycerol lipase to enhance 2-AG signaling. Whole-cell patch clamp recordings from BLA pyramidal neurons were conducted from susceptible and resilient mice.
**Results:** ~30% of mice exhibited generalized anxiety responses in the modified NIH assay after acute traumatic stress exposure, and were termed susceptible, with the rest were termed resilient. Acute pharmacological augmentation of 2-AG signaling reversed anxiety in susceptible mice, but had no effect in resilient mice, however blocking CB1 receptors increases generalized anxiety in resilient mice only. In contrast, global and BLA-specific deletion of DAGLa, which decreased 2-AG levels, increased the proportion of mice showing susceptibility to acute stress. Electrophysiological studies revealed susceptible mice have increased glutamate signaling in the BLA relative to resilient mice, an effect which was reversed by ex vivo 2-AG augmentation.
**Conclusions:** We conclude that 2-AG mediated endocannabinoid signaling promotes resiliency to acute traumatic stress exposure via reductions in BLA glutamatergic signaling. These data suggest pharmacological approaches aimed at enhancing 2-AG signaling could represent novel treatment approaches for stress-related psychiatric disorders, and provides insight into the neural basis for high rates of cannabis use in PTSD populations.
**Keywords:** Posttraumatic stress disorder, endocannabinoid, anxiety, depression, cannabis
**Disclosures:** S. Patel, research support from Lundbeck Pharmaceuticals, however, none of the data presented in the abstract were supported by Lundbeck.

## M16. Cortical Glx Concentrations in Cholecystokinine-Tetrapeptide (CCK-4) Induced Panic

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**Background**: A dysbalance in excitatory-inhibitory neurotransmitter systems has been suggested to play an important role in the pathogenesis of panic disorder. In addition, the neuropeptide S (NPS) system has been implicated in modulating GABA and glutamate neurotransmission in animal models and to genetically drive altered fear circuit function and an increased risk of panic disorder in humans.
**Methods:** In order to elucidate the role of glutamate in anxiety and panic in humans, brain glutamate + glutamine (Glx) levels were measured during a pharmacological cholecystokinin-tetrapeptide (CCK-4) panic challenge paradigm using 3T magnetic resonance spectroscopy (MRS). In a first step, 18 healthy subjects underwent CCK-4 challenge. MR spectra were obtained in the bilateral anterior cingulate cortex (ACC) using a single voxel point resolved spectroscopy method (PRESS) and analyzed using LCModel. A combined fitting of glutamine and glutamate (Glx) was performed. Panic was assessed using the Acute Panic Inventory (API) and/or Panic Symptom Scale (PSS) scores. Moreover, hypothalamic-pituitary-adrenal (HPA) axis stimulation was monitored throughout the challenge. Probing a multi-level imaging genetic risk model of panic, the functional neuropeptide S receptor gene (NPSR1) rs324981 A/T variant was investigated with regard to its impact on cortical glx levels in an enlarged sample of 35 healthy male subjects.
**Results:** A significant panic response following CCK-4 as revealed by a marked increase in both panic scores (API: $F[1,17] = 149.41$; $p < .0001$; PSS: $F[1,17] = 88.03$; $p < .0001$) and a significant increase in heart rate (HR: $F[1,17] = 72.79$; $p < .0001$) was found in the first study. MRS measures showed a significant increase of brain glutamate + glutamine/creatine (Glx/Cr) levels peaking at 5-10 minutes

after challenge ($F[1,17] = 15.94$; $p = .001$). There was also a significant increase in CCK-4 related cortisol release ($F[6,11] = 8.68$; $p = .002$). Finally, significant positive correlations were found between baseline Glx/Cr and both APImax ($r = .598$; $p = .009$) and the maximum heart rate during challenge HRmax ($r = .519$; $p = .027$). In the extended sample, a significant time x genotype interaction was detected ($F[5,27] = 3.936$; $p = .008$), with significantly lower ACC Glx/Cr levels in T risk allele carriers as compared to AA homozygotes five minutes after injection ($F = 5.628$; $p = .003$). CCK-4 induced significant HPA axis stimulation, but no effect of genotype was discerned.

**Conclusions:** The present pilot data add to the hypothesis of a disturbed inhibitory-excitatory equilibrium in panic attacks. Moreover, our results suggest NPSR1 gene variation to modulate Glx levels in the ACC during acute states of stress and anxiety, with blunted, i.e. possibly maladaptive ACC glutamatergic reactivity in T risk allele carriers. Our results underline the notion of a genetically driven rapid and dynamic response mechanism in the neural regulation of human anxiety and further strengthen the emerging role of the NPS system in anxiety.

**Keywords:** panic attacks, CCK-4, glutamate, NPSR1, magnetic resonance spectroscopy

**Disclosures:** Nothing to disclose.

### M17. Olfactory Challenge Test in Combat Veterans: Evidence for Enhanced Olfactory-Visual Functional Connectivity with Avoidance and Hyperarousal Symptoms in PTSD

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**Background:** According to DSM-IV criteria, posttraumatic stress disorder (PTSD) is characterized by re-experiencing with intrusive images, thoughts, or perceptions of the trauma, avoidance of stimuli and contextual reminders, as well as hyperarousal or increased startle, vigilance, and focused attention to potential threat cues. While task-based functional connectivity studies in PTSD have mainly focused on emotion processing (e.g. amygdala-prefrontal circuitry), little is known about primary sensory processing of trauma cues and how that relates to PTSD symptomatology. We previously reported that burning-related odors elicit significant distress in combat veterans with PTSD (Cortese et al., 2015), who compared to healthy combat veterans, show a blunted odor-elicited BOLD response in olfactory cortex (Cortese et al., 2014). In this odor challenge test, we sought to extend those findings by assessing the relationship between burned rubber odor-elicited functional connectivity between olfactory cortex and other brain areas as a function of PTSD symptomatology.

**Methods:** Twenty Operation Enduring Freedom/Iraqi Freedom/New Dawn (OEF/OIF/OND) combat veterans (CV) with PTSD (CV + PTSD) and 24 healthy combat-trauma controls (CV-PTSD) participated in an fMRI odor challenge test. An odor specific to traumatic combat experiences (burned rubber, BR) and odorless propylene glycol (PG) were systematically delivered by an MRI-compatible olfactometer. Task-based functional connectivity analyses were conducted using SPM12 and the CONN 14 toolbox. The mean time course across voxels within 4 anatomically-defined olfactory seed regions of interest [ROIs: bilateral piriform (primary olfactory) and orbitofrontal (secondary olfactory) cortices; Seubert et al., 2013] were determined during BR and PG. Activation contrasts (BR minus PG) for each of the ROIs were covaried to all other voxels within the whole brain mask to derive connectivity estimates for each individual and then entered into group-level as well as symptom severity correlational analyses. Results were corrected for multiple comparisons at the cluster-level using $p < .005$/ $p < .05$ FWE cluster-level.

**Results:** Demographics: The Participants were nearly all male (CV + PTSD: 19 M/1 F, CV-PTSD: 23M/1 F) and of similar age [CV + PTSD: $M = 31.4$ ($SD = 9.6$), CV-PTSD: $M = 31.0$ ($SD = 7.1$)]. The groups differed significantly on the total score of the Clinician Administered PTSD Scale (CAPS) [CV + PTSD: $M = 59.9$ ($SD = 23.0$), CV-PTSD: $M = 14.8$ ($SD = 12.7$), $p < 0.01$], but did not differ with respect to the level of combat exposure [CV + PTSD: $M = 21.6$ ($SD = 8.5$), CV-PTSD: $M = 19.7$ ($SD = 10.1$), $p > 0.1$], the number of additional traumatic events experienced during their lifetime [CV + PTSD: $M = 2.8$ ($SD = 2.1$), CV-PTSD: $M = 2.4$ ($SD = 1.5$), $p > 0.1$], or years of education [CV + PTSD: $M = 14.0$ ($SD = 1.3$), CV-PTSD: $M = 14.6$ ($SD = 2.4$), $p > 0.1$].

Functional Connectivity: Group analyses revealed no evidence of enhanced BR odor-elicited connectivity between the olfactory ROIs and any other brain region in CV + PTSD compared to CV-PTSD. However, CV + PTSD, compared to CV-PTSD, demonstrated reduced odor task-based connectivity between right anterior piriform cortex and left middle ($z = 4.27$, x, y, $z = 62, 4, 15$, $k = 604$, $pcorr < .05$) and right inferior ($z = 3.74$, x, y, $z = -54, 0, -4$, $k = 971$, $pcorr < .01$) frontal gyri. Regression analyses showed that CAPS re-experiencing and hyperarousal severity inversely related to BR odor-elicited connectivity between right anterior piriform cortex and left inferior frontal gyrus ($z = 4.09$, x, y, $z = -46, 34, -10$, $k = 1038$, $pcorr < .01$; $z = 4.14$, x, y, $z = -42, 10, 16$, $k = 925$, $pcorr < .01$, respectively). Conversely, CAPS re-experiencing was positively related to connectivity between left orbitofrontal olfactory cortex and a cluster encompassing posterior cingulate (e.g. retrosplenial cortex) and precuneous ($z = 4.46$, x, y, $z = -15, -42, 18$, $k = 1172$, $pcorr < .01$), while higher CAPS avoidance and hyperarousal scores related to greater connectivity between right orbitofrontal olfactory and primary visual cortices ($z = 4.56$, x, y, $z = -12, -102, -16$, $k = 1121$, $pcorr < .01$; $z = 3.79$, x, y, $z = -28, -94, -14$, $k = 823$, $pcorr < .01$, respectively).

**Conclusions:** While these olfactory task-based connectivity results add to a consistent finding of dysregulated (e.g. reduced coupling) prefrontal circuits in PTSD, they also point toward other important brain circuits. Specifically, results showed PTSD-related increased functional coupling between secondary olfactory cortex and retrosplenial cortex, a brain region involved in self-referential processing and the establishment of autobiographical, including

trauma, memories (Sartory et al., 2013). Moreover, increased olfactory-retrosplenial functional connectivity was directly related to increased severity of re-experiencing. While olfactory-visual functional connectivity was not significantly related to re-experiencing as initially hypothesized, connectivity between these sensory processing cortices increased as a function of avoidance and hyperarousal. These results extend the dysregulated amygdala-prefrontal circuits traditionally associated with PTSD and suggest that sensory circuits might be targeted as either a risk factor for developing PTSD or as a functional consequence of PTSD that perpetuates symptoms.
**Keywords:** PTSD, olfactory, task-based functional connectivity, odor-related trauma, combat veteran
**Disclosures:** Nothing to disclose.

### M18. Pilot Trial of a Brief Course of Exposure-Based CBT in Extending IV Ketamine's Effects in OCD

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**Background**: A single subanesthetic intravenous (IV) dose of ketamine leads to rapid anti-obsessional effects in obsessive-compulsive disorder (OCD) patients with near-constant intrusive obsessions, but these effects usually do not persist. We tested whether a brief course of exposure-based cognitive behavioral therapy (CBT) could extend ketamine's effects in a two week pilot open trial and if this effect was maintained (without additional treatment) two weeks later. Our rationale was: 1) ketamine is reported to enhance plasticity and extinction learning in rodents, and 2) enhanced extinction learning may facilitate CBT gains, as reported in trials that combined CBT with agents thought to facilitate extinction learning (e.g. D-cycloserine). Mimicking those trials, CBT was abbreviated (i.e. 10 one-hour exposure sessions) but delivered during the putative time interval when ketamine facilitates extinction learning (within 14 days).
**Methods:** With IRB approval, ten unmedicated OCD outpatients (aged 18-55) with near-constant intrusive obsessions ($>8$ hours/day) were recruited (3/2014-3/2015). They provided written informed consent. Participants met DSM-IV and DSM-5 criteria for OCD with at least moderate symptoms (Yale-Brown Obsessive-Compulsive Scale [Y-BOCS] score $\geq 16$). Exclusion criteria included severe depression (Hamilton Depression Rating Scale [HDRS] $>25$), current CBT, and comorbid psychiatric or medical conditions that made participation unsafe.
In an open-label design, participants received a single 40-minute IV infusion of ketamine (dose $= 0.5$ mg/kg), followed by 10 one-hour exposure sessions delivered over two weeks. The CBT treatment was planned in a 90-minute session the day before the ketamine infusion.
At baseline, during the infusion, at 20, 90, 110, 230 minutes post-infusion, patients rated their obsessional severity using the OCD-VAS. We focused on obsessions because the patients were supine and connected to stationary monitoring equipment during the infusion. At baseline and weekly for four weeks post-ketamine, an independent evaluator, blind to study design, evaluated patients using the Y-BOCS, which appraises obsessive and compulsive symptoms over the prior week. Treatment response was defined a priori as $\geq 35\%$ Y-BOCS reduction at week 2. Y-BOCS outcomes were analyzed using mixed-effects regression to model symptoms as a function of time.
**Results:** Of the 10 patients who started ketamine, nine completed the infusion. Eight reported a rapid reduction in obsessive severity as measured by the OCD-VAS, which persisted up to 230 minutes post-infusion in seven patients. Eight completed the 10 hours of exposure and the two week follow-up and were included in the Y-BOCS analyses. From baseline to four weeks post-infusion, OCD severity, as measured by the YBOCS, was significantly decreased over time ($F = 14.36$, $df = 4,28$, $p < .0001$; Figure 1). Compared to baseline, the mean estimated Y-BOCS score was significantly lower at week 2 (difference = -10.75 points, $SE = 1.44$, $p < .0001$) and at week 4 (difference = -6.88, $SE = 2.61$, $p = 0.01$); there was a trend-level increase between week 2 and 4 (difference = 3.63, $SE = 1.97$, $p = 0.07$). At the end of CBT (week 2), 63% of patients demonstrated treatment response ($\geq 35\%$ Y-BOCS reduction). Importantly, individuals varied in their response, with one subject having no benefit, the majority benefitting for up to two weeks, and one no longer meeting criteria for OCD (i.e., achieving minimal symptoms post-infusion that persisted throughout the CBT and for up to 6 months in naturalistic follow-up).
**Conclusions:** These results corroborate prior findings that IV ketamine can rapidly reduce obsessions in unmedicated OCD patients. The data suggest that a brief course of CBT may help some individuals maintain the improvement they experienced from ketamine; however, this needs to be formally tested in a randomized controlled trial to determine whether the improvement seen after two weeks of CBT is due to the addition of CBT, or whether the effects of ketamine persist longer in some than previously described.
**Keywords:** Ketamine, OCD, CBT, Exposure therapy, NMDA Antagonists
**Disclosures:** Nothing to disclose.

### M19. PTSD Severity is Associated with Anterior Hippocampal Dysconnectivity: A Graph-Based Whole-Brain Data-Driven Analysis

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**Background**: Global brain connectivity (GBC) is a recently developed graph-based measure of nodal strength in functional connectivity networks. Using fully data-driven approaches, GBC has been shown to identify major intrinsic brain networks (e.g. default mode network), to correlate with normal brain functions (e.g. intelligence), and to be disrupted in major depressive disorder (MDD), bipolar

disorder, schizophrenia, and obsessive-compulsive disorder (OCD). In treatment-resistant MDD, our group has demonstrated prefrontal GBC abnormalities, which rapidly normalized 24 hours post ketamine treatment. These prior data highlight the potential utility of GBC as a replicable measure for data-driven identification of brain functional connectivity biomarkers. Here we investigated whether GBC is altered in US veterans with severe symptoms of posttraumatic stress disorder (PTSD).

**Methods:** Sixty-eight combat-exposed US veterans (age mean ± SEM = 34.5 ± 1.6, 8 females) completed the Clinician-Administered PTSD Scale (CAPS) and resting-state functional connectivity magnetic resonance imaging (rs-fcMRI) scans. Whole brain GBC analyses were conducted. The GBC value of each voxel is the average of its time series correlation with all other voxels, providing a measure of nodal strength in a graph-based network. Voxel-wise correlations between GBC values and CAPS scores were performed, along with cluster-level type I error correction ($p < 0.05$).

**Results:** We found negative correlations between CAPS scores and GBC in the left superior temporal cortex (Brodmann's Area [BA]22; an area involved in complex sounds processing) and bilateral medial temporal regions, including primarily the anterior bilateral hippocampi and the right amygdala such that participants with severe PTSD symptomatology showed reduced amygdala and anterior hippocampal GBC. Two small clusters of positive correlations between CAPS scores and GBC were found in left visual cortex (BA 17 & 18) and left precuneus (BA 7; a hub in the default mode network).

**Conclusions:** In contrast to findings by our group, and others, showing that GBC abnormalities in depression are predominantly in the prefrontal cortex, GBC alterations in PTSD were primarily found in mediotemporal structures, highlighting the potential disease specificity of the derived GBC measure. GBC dysconnectivity in the anterior hippocampus and amygdala may contribute to previously reported alterations in limbic system functioning in PTSD. In addition, it is believed that the posterior hippocampus is involved in spatial navigation and memory, while the anterior hippocampus mediates fear, reward, and motivation. Thus, the localization of the dysconnectivity to the anterior, but not posterior, hippocampus may have essential theoretical, clinical and therapeutic implications.

**Keywords:** PTSD, fMRI Functional Connectivity, ventral hippocampus

**Disclosures:** Nothing to disclose.

### M20. Relevance and Function of Long Non-Coding RNAs in Fear Memory Formation

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**Background**: An increasing body of evidence suggests that the non-coding transcriptome critically contributes to the development of psychiatric disorders e.g., via the regulation of chromatin structure, gene transcription, post-transcriptional regulation and epigenetic modifications. Several landmark studies have identified an involvement of non-coding RNAs such as micro RNAs (miRNAs) in the development of behavioral phenotypes in mice including depressive-like behavior and the formation of fear memories. However, little is known about the vast majority of other non-coding RNA species such as piwi interacting RNAs (piRNAs), long non-coding RNA (lncRNAs) and circular RNAs (circRNAs).

Here, we focus on the molecular function of lncRNAs during the formation of fear memories in mice. LncRNAs are a group of non-coding RNAs that is defined by a length of >200nt and lack a coding potential. Given the arbitrary definition of lncRNAs, this group likely comprises RNAs with different molecular modes of action, which vary as a function of their genomic location and strand direction. In an unbiased screening approach, we identified several hundred lncRNAs differentially regulated two hours after fear conditioning in the mouse amygdala. Here we present the molecular characterization of candidate lncRNAs potentially involved in the formation of long lasting fear memories.

**Methods:** Fear conditioning paradigm, mouse behavior, genome-wide lncRNA array, qPCR, stereotaxic surgery, antisense oligonucleotides, small RNA sequencing, DNA methylation.

**Results:** Using an unbiased screening approach, we identified several hundred lncRNAs significantly regulated two hours after fear conditioning in the mouse amygdala. However, the molecular function of most lncRNAs identified here is unknown. In a proof-of-concept design, we therefore focused on the antisense lncRNA Nespas on chromosome 2, which is involved in the transcriptional regulation of the complex imprinted Gnas locus. The Gnas gene encodes for the stimulatory G-protein alpha subunit (Gs-alpha). However, in addition to Gnas, Nespas affects the regulation of other transcripts encoded by the Gnas locus. We were able to replicate the downregulation of Nespas after fear conditioning in independent experiments by RT-qPCR ($p<0.05$ compared to homecage and context controls). Subsequently, we designed and established an antisense oligonucleotide (ASO) that specifically targets Nespas. Using intra-amygdala infusions of this Nespas ASO prior to fear conditioning, we were able to provide further evidence that the regulation of Nespas is critical for the formation of fear memories.

**Conclusions:** The present data illustrate the extensive involvement of non-coding RNAs, in particular lncRNAs, in the transcriptional regulation accompanying fear memory formation. The example of Nespas as an antisense lncRNA to the sense protein-coding and imprinted Gnas locus exemplifies the role of lncRNAs as critical regulators of fear memory formation. Given the complex interaction of the coding and non-coding genome, this dataset may also provide a mechanistic base for the involvement of genetic variants in non-coding regions of the genome in the development of psychiatric disorders through regulation of non-coding RNA.

**Keywords:** long noncoding RNA, Fear conditioning, anxiety disorders

**Disclosures:** Nothing to disclose.

### M21. Pharmacotherapy Relapse Prevention with Escitalopram in Body Dysmorphic Disorder: A Double-Blind Placebo-Controlled Trial

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**Background**: Body dysmorphic disorder (BDD), an often-delusional preoccupation with perceived defects in one's physical appearance, is common, with a current prevalence of about 2%. BDD is distressing, often severely impairing, and associated with high rates of suicidality. SRIs appear to be selectively efficacious for BDD, including delusional BDD. However, very few pharmacotherapy studies have been conducted, and all studies have been short-term (no longer than 4 months). No relapse prevention studies or continuation phase studies have been conducted. Here we report results from the first relapse prevention study in BDD. For the study's primary aim, we compared time to BDD relapse and relapse rates in responders to acute phase open-label escitalopram treatment who were subsequently randomized to placebo versus continuation treatment with escitalopram for six additional months. The acute treatment phase is also of interest, because few prior acute-phase studies have been done in BDD, and all contained relatively small samples (N = 15-34 medication-treated subjects).
**Methods:** Across two sites, 100 adults with DSM-IV BDD received open-label escitalopram for 14 weeks (Phase 1). Fifty-eight escitalopram responders were then randomized to double-blind continuation treatment with escitalopram or switched to placebo for six additional months (Phase 2). Outcome measures were the BDD-YBOCS (primary outcome), BDD-PSR, Brown Assessment of Beliefs Scale (BABS), HAM-D, LIFE-RIFT (psychosocial functioning), and Q-LESQ Short Form (quality of life). For the study's primary aim, which examined group differences in time to relapse (and proportion relapsing), Kaplan-Meier survival curves were generated, and Cox's proportional hazards regression was employed (with the likelihood ratio p-value reported). Change in secondary outcome measures was also examined.
**Results:** Phase 1 (acute-phase treatment): Overall, 67.0% of treated subjects and 81.1% of completers achieved response of BDD with escitalopram treatment (p's $< 0.0001$). Median time to first response was 7.9 weeks (95% CI = 6.9, 8.9). BDD severity, BDD-related insight, depressive symptoms, psychosocial functioning, and quality of life significantly improved from baseline to the end of Phase 1 (all p's $< 0.0001$).
Phase 2 (placebo-controlled discontinuation phase): Regarding the study's primary aim, time to relapse was significantly longer for subjects receiving escitalopram than for those receiving placebo (hazard ratio = 2.72, 95% CI = 1.01, 8.57, p = .049). Phase 2 relapse proportions were 18% for the escitalopram group versus 40% for the placebo group. There were no statistically significant between-group differences in BDD severity, BDD-related insight, depressive symptoms, psychosocial functioning, or quality of life. During six additional months of continuation treatment with escitalopram, BDD severity significantly decreased over time ($p = 0.036$); improvement in BDD symptoms during the continuation phase occurred in 35.7% of the escitalopram group. The medication was generally well tolerated. In Phase 2, 25.0% of escitalopram-treated subjects versus 46.7% (n = 14) of placebo-treated subjects experienced at least one adverse event considered at least possibly related to study medication ($p = 0.11$). No serious adverse events occurred during either treatment phase.
**Conclusions:** Among those subjects who responded to acute-phase escitalopram treatment, continued treatment with escitalopram significantly delayed time to relapse of BDD compared to treatment with placebo, thus confirming our primary hypothesis. Moreover, more than twice as many placebo-treated subjects relapsed compared with escitalopram-treated subjects. BDD symptom severity significantly improved with six additional months of escitalopram treatment following acute response, with more than one-third of escitalopram-treated subjects experiencing further improvement in BDD symptoms. Additional studies with larger samples and longer follow-up are needed.
**Keywords:** Body Dysmorphic Disorder, Escitalopram, Pharmacotherapy, Obsessive-Compulsive and Related Disorders, Relapse Prevention
**Disclosures:** Study was by a Collaborative R01 grant from the National Institute of Mental Health to Dr. Phillips and Dr. Wilhelm. Study medication and matching placebo provided by Forest Laboratories.

### M22. Lower Posterior Cingulate Cortex Glutathione Levels in Obsessive-Compulsive Disorder

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**Background**: Obsessive-compulsive disorder (OCD) is grouped by the DSM-5 within a family of obsessive compulsive and related disorders. Despite substantial prior research into these conditions, neither their pathogenesis nor their pathophysiology is well understood. Several lines of evidence support the hypothesis that lower cerebral levels of glutathione (GSH), associated with increased oxidative stress, may contribute to obsessive compulsive and related disorders. Moreover, interventions that enhance GSH levels, such as the GSH-precursor N-acetylcysteine, have demonstrated efficacy in several obsessive compulsive and related disorders, including OCD, trichotillomania, and excoriation disorder. Proton magnetic resonance spectroscopy (MRS) enables the noninvasive measurement of brain GSH levels. Although a number of MRS studies have been conducted in people with OCD, none to date has reported on GSH levels, primarily because previous MRS protocols were not optimized for GSH detection. We developed a 2D J-resolved proton MRS protocol to examine differences in cerebral GSH levels between individuals with OCD and non-OCD comparison individuals. We previously used this MRS sequence in the pregenual anterior cingulate cortex and did not detect any metabolite abnormalities in OCD individuals. Because hypermetabolism has also been reported in the

posterior cingulate cortex (PCC) of individuals with OCD during both cognitive challenge and at rest, the degree of which predicted clinical response to medication or ablative surgery, we used 2D J-resolved proton MRS to determine whether PCC GSH or other metabolite abnormalities are detectible in individuals with OCD. We hypothesized that: 1) OCD individuals would demonstrate lower GSH levels in PCC compared with non-OCD individuals and 2) GSH levels in PCC would be inversely associated with OCD symptom severity, as measured by the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS).

**Methods:** Thirty individuals with OCD and 25 age-, sex-, and race-matched comparison individuals without OCD underwent single voxel 2D J-resolved proton magnetic resonance spectroscopy (MRS) at 3 Tesla on a Siemens TIM Trio system, using a 32-channel head coil. Anatomical images were used as a guide to position MRS voxels (2 x 2 x 2 cm = 8cc) medially in the PCC. MRS data were analyzed using LCModel and a simulated basis set. Group metabolite differences, as well as relationships between metabolite levels and Y-BOCS scores, were analyzed using linear regression adjusted for age, sex, and race.

**Results:** One OCD participant failed to produce usable PCC MRS data. We found significantly lower PCC GSH levels in OCD participants compared with non-OCD participants ($\beta$ = -0.03 [95% CI: -0.05 to -0.006]; P = 0.014). PCC GSH levels were not significantly associated with total Y-BOCS score in the OCD group ($\beta$ = 0.0006 [95% CI: -0.005 to 0.006]; P = 0.83).

**Conclusions:** Using 2D J-resolved MRS, we found lower GSH levels in PCC in individuals with OCD than in individuals without OCD. Lower PCC GSH levels may be indicative of increased oxidative stress secondary to hypermetabolism in this brain region in OCD. Peripheral oxidative stress has been reported in children and adults with OCD, suggesting that both peripheral and brain oxidative stress could contribute to the disorder. Future MRS studies are warranted to investigate GSH levels in other brain regions that comprise the cortico-striato-thalamo-cortical circuit thought to be abnormal in OCD and to determine whether lower cerebral GSH levels may represent a neurobiological diagnostic marker across other obsessive-compulsive and related disorders.

**Keywords:** obsessive-compulsive disorder, OCD, glutathione, MRS, oxidative stress

**Disclosures:** Dr. Brennan has received grant support from Eli Lilly and Transcept Pharmaceuticals. Dr. Pope has received consulting fees from Pronutria. Dr. Hudson has received consulting fees from Genentech, Pronutria, Roche, Shire, and Sunovion; and has received grant support from Genentech and Shire. Dr. Rauch has received research funding from NIMH, the US Army, and royalties from APPI and Oxford University Press. He further receives honoraria for advisory board service from the Harvard Football Players Health Study. He is employed by, and receives salary from McLean Hospital/Partners Healthcare. Dr. Rauch also holds leadership roles with the SOBP, APA, NNDC and ADAA. Dr. Kaufman receives compensation for travel expenses as a member of the Board of Directors for the College on Problems of Drug Dependence, and has a patent filing under review for the use of xenon as an adjunct to psychotherapy for psychiatric disorders. All other authors have no disclosures to report.

### M23. Modeling Cognitive Therapy in Rats: Fear Extinction Reverses the Chronic Stress-Induced Shift from Active to Passive Coping Behavior

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**Background**: Stress-related psychiatric disorders, like depression or post-traumatic stress disorder (PTSD), are highly prevalent yet poorly treated. These disorders share many dimensions, including cognitive flexibility deficits associated with hypoactivity in the medial prefrontal cortex (mPFC), and maladaptive avoidant coping strategies, which may also be modulated by mPFC activity. Psychotherapies that invoke mPFC activity can be efficacious even in pharmacotherapy-resistant patients, although, as with pharmacotherapies, the response to psychotherapy can be incomplete, some patients do not respond, and relapse remains an issue. Identifying the neurobiological mechanisms underlying its efficacy could lead to more rapid, efficacious, or long-lasting treatments. Pre-clinically, chronic unpredictable stress (CUS) induces cognitive inflexibility and a shift from active to passive coping behavior in rats, similar to the cognitive inflexibility and avoidant coping seen in patients with stress-related psychiatric illness. We have previously shown that the extinction of conditioned fear, which engages the mPFC and closely resembles prolonged exposure therapy used for PTSD treatment, can model cognitive behavioral therapy (CBT) in rats by improving their performance in a test of cognitive flexibility, and restoring active coping behavior in the shock probe defensive burying (SPDB) test, that have been compromised by chronic stress (SfN Abstract 468.07, 2014). In this study, we tested the hypothesis that mPFC activity during extinction is necessary for its beneficial effect in reversing the CUS-compromised coping behavior in the SPDB.

**Methods:** Fos immunohistochemistry was used to demonstrate activation of the mPFC by extinction, with dual labeling of cell type-specific markers to identify mPFC cell types that were activated. To test the necessity of activating the glutamatergic pyramidal cells in mPFC during extinction for its therapeutic impact, rats received AAV microinjections into the infralimbic cortex (IL) to express the Gi-coupled designer receptor activated exclusively by designer drug (DREADD) hM4Di or control GFP protein, driven by a CamKII promoter. After four weeks of viral expression, including 2 weeks of CUS or control treatment, rats received an IP injection of the designer drug clozapine-n-oxide (0.5mg/kg) followed by extinction training or control treatment 30 min later. They were tested on the SPDB test 24 hr later. A separate cohort of rats was sacrificed immediately after fear extinction, the mPFC and lateral septum (LS) were dissected, and changes in phosphorylation of ribosomal protein S6 were analyzed by Western blot. All procedures were in accordance with NIH guidelines and

approved by the UTHSCSA Institutional Animal Care and Use Committee.
**Results:** Extinction training induced Fos protein in the IL of the rat mPFC that colocalized with CaMKIIα, indicating activation of glutamatergic cells. By contrast, no Fos protein colocalized with GAD65/67, indicating extinction did not activate GABAergic cells in the IL. Western blot analysis of LS, a sub-cortical target of mPFC that mediates active coping behavior on the SPDB test, showed elevated phosphorylation of ribosomal protein S6 after extinction, suggesting the induction of protein synthesis. However, inhibiting the mPFC by the Gi DREADD during extinction did not impact its effect on CUS-compromised coping.
**Conclusions:** Extinction training reversed the CUS-induced shift from active to passive coping on the SPDB. Further, extinction activated the mPFC, and increased pS6 in the LS, an indicator of activity-dependent protein translation and plasticity. The effect of extinction on coping behavior was not affected by inhibiting the mPFC, suggesting that this effect may not be due to mPFC modulation of LS, but that a different brain region activated during extinction influences LS function, underlying the effect of extinction on coping behavior. Overall, this study further shows that fear extinction is a useful model of CBT, allowing us to investigate neural mechanisms responsible for its therapeutic effects. Supported by: NIH training grant T32NS082145, Owen's Foundation Research Grant, and NIMH research grant R01MH072672.
**Keywords:** cognitive behavioral therapy, Medial Prefrontal Cortex, coping, PTSD, DREADDs
**Disclosures:** Nothing to disclose.

### M24. Brain Markers of Late Adolescent Academic Functioning

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**Background**: Academic performance in adolescence strongly influences adult prospects, such as options for college, graduate school, and beyond. IQ has historically been considered a strong predictor of academic performance. Less objectively explored has been brain development and morphometry in relation to academic performance. Qualitatively, the role of memory, attention, and planning have been thought to influence success in high school, but brain morphometry has not been explored quantitatively as a predictor of academic performance. We seek to determine regions of the brain whose morphometry in early adolescence predicts academic performance over the course of high school.
**Methods:** We collected high resolution brain magnetic resonance imaging (MRI) data and high school grade point averages (GPAs) from 71 subjects in the Youth at Risk project (R01 AA13419 PI: Tapert). Participants were recruited from local schools at a mean age of 13.3 years. We extracted surface areas, cortical thickness, and subcortical volumes as input training data to distinguish those with exemplary academic performance from those struggling academically in high school. Exclusionary criteria included left-handedness, psychiatric disorder, use of psychoactive medications, history of neurological abnormality, and, for this study, incomplete GPA data through high school. Subjects were divided into high (GPA $\geq 3.5$; $n = 42$) and low (GPA $< 3.5$; $n = 31$) performers. To identify a manageable number of predictive features from the 340 available, we applied multiple algorithms for selection and reduction of attributes (Chi-squared with Ranker for Attribute Selection, CfsSubset with GreedyStepwise for Attribute Reduction, Information Gain Analysis with Ranker for Attribute Selection) using the Weka machine-learning software suite. Twelve morphometric attributes that were shared across these multiple methods were used to make predictions using a naïve Bayes classifier of high and low academic performance that was then 10-fold cross-validated. Subcortical and white matter volumes were normalized by means of the proportion method using the intracranial volume of each subject to account for intrasubject intracranial volume differences.
**Results:** Of the 15 features examined, 12 distinguished high versus low academic performance using the Bayesian classifier approach, with a sensitivity of 0.70 and specificity of 0.70 with 10-fold cross validation. These included: larger right postcentral white matter volume fraction; larger left superior temporal sulcus volume fraction; larger left nucleus accumbens surface area, larger right caudate volume fraction; greater right parahippocampal gyrus cortical thickness; larger bilateral lateral and 3rd ventricles volume fraction; smaller left pericalcarine white matter volume fraction; smaller left lingual gyrus volume fraction and surface area; smaller right fusiform gyrus surface area; and anterior corpus callosum volume fraction. Comparisons were made from high to low performers and were statistically significant in independent t-tests ($p < 0.05$) with the exception of the anterior corpus callosum.
**Conclusions:** Many regions have historically been associated with learning and memory formation. The importance of the visual cortex (lingual gyrus) found above is consistent with what we expected given its known role in memorization, encoding complex images, word processing, and nonverbal logic. Also highlighted by the above results is the importance of the parietal lobe (left postcentral gyrus and pericalcarine cortex). The postcentral gyrus is well established as the primary somatosensory cortex. The pericalcarine cortex is known to be involved in visual processing and long term working verbal memory in those who are congenitally blind. It was not surprising to see various regions of the temporal region (superior temporal sulcus and fusiform gyrus) highlighted given its importance in understanding language, learning, memory, and recognition. The limbic system also demonstrated predictive power at the level of the parahippocampal gyrus. The importance of parahippocampal thickness was not surprising given its importance in memory encoding and retrieval. The left accumbens and right caudate regions were also found to be predictive, consistent with the role of basal ganglia in skill learning, habit formation, and reward systems. We also saw regions of the corpus callosum highlighted as might be predicted because of its role in passing information between hemispheres. However, the predictive value of the left lateral and 3rd ventricles was a surprising finding. The ventricular

system may provide a possible structural role in allowing other forebrain structures specific space for development based on their volumes in adolescence. Future studies will provide further insight into understanding regions implicated in mediating attention and planning behavior function in the classroom setting.
**Keywords:** Structural MRI, Adolescence, Memory and Learning
**Disclosures:** Nothing to disclose.

### M25. Human Perinatal Choline Supplementation Decreases Preschool Parent-Reported Attentional and Social Withdrawal Symptoms via an Alpha7 Nicotinic Receptor Mediated Effect on Infant Development of Sensory Gating

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**Background**: Most neuropsychiatric illnesses—including ADHD, anxiety disorders, autism, bipolar, and schizophrenia—are neurodevelopmental disorders, where onset illness is the end result of brain developments changes which begin prenatally. Thus, one potential window for primary prevention is the perinatal period. In animal models, prenatal stimulation of the alpha7 nicotinic cholinergic receptor with dietary choline supplementation leads to improved development of sensory gating, improved memory, and decreased anxiety. Previously published work has supported perinatal choline supplementation's positive impact on human infant sensory gating development at 1 month of age. This report is a follow-up of these same children to 40 months of age.
**Methods:** Randomized controlled trial of perinatal choline supplementation in 100 healthy mothers (phosphatidylcholine 6300 mg QD) and infants (phosphatidylcholine 700 mg QD). Outcomes of P50 sensory gating (1 months of age) and parent-reported behavior utilizing the Child Behavior Checklist (CBCL; 40 months of age). All infants were genotyped for a schizophrenia-associated SNP in CHRNA7, rs3087454.
**Results:** Infant sensory gating predicted the 40-month CBCL total problems score. Homozygosity for a schizophrenia risk allele in either CHRNA7 is associated with delayed development of infant cerebral inhibition and increased CBCL total problems at 40 months of age. Both genetic effects are reversed by perinatal choline supplementation, with a particular benefit of perinatal choline supplementation on 40-month-old attention and social withdrawal.
**Conclusions:** Prenatal choline supplementation compensates for genetic vulnerability's impact on the infant development of sensory gating and the 3-year-old development of behavior. The effect of choline is moderated by CHRNA7 genotype supporting perinatal stimulation of the alpha7 nicotinic receptor as the mechanism of action. Universal prevention strategies have a role in preventing major mental illnesses.
**Keywords:** infant, choline, alpha-7 nicotinic acetylcholine receptor, P50, pregnancy
**Disclosures:** Nothing to disclose.

### M26. Neural Network Predictors and Correlates of PTSD Symptom Reduction during TF-CBT among Adolescent Girls with PTSD

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**Background**: Adolescent girls exposed to physical or sexual assault are at significantly elevated risk for PTSD across the lifespan. Trauma-focused cognitive-behavioral therapy (TF-CBT) is the gold standard treatment for adolescent PTSD, but is associated with high variability in treatment outcome. Here, we use functional neuroimaging pre- and post-TF-CBT to identify predictors of subsequent PTSD symptom reduction during TF-CBT and changes in brain function from before to after therapy that correlates with symptom reduction.
**Methods:** Adolescent girls, aged 11-16, with a history of physical or sexual assault and a current diagnosis of PTSD were enrolled ($N = 34$). Participants completed a common emotion processing task during fMRI, in which they made gender decisions while viewing faces displaying emotional or neutral facial expressions, before and after receiving 12 standardized sessions of TF-CBT. Twenty girls completed all TF-CBT sessions and had usable pre- and post-treatment imaging data. Imaging analyses focused on large-scale (using an 883 ROI parcellation) functional modularity; i.e., the degree to which the brain organizes into discrete modules of information processing in response to the emotional or neutral facial expressions. We tested functional modularity that predicted subsequent response, and changes from before to after treatment correlated with symptom reduction. We also recruited a separate sample of 17 healthy control adolescent girls who underwent the same task in order to identify normative functional modularity patterns.
**Results:** Pre-treatment large-scale modularity in response to fear expressions (relative to neutral expressions) significantly predicted subsequent PTSD symptom reduction ($p = .01$). Specifically, adolescent girls who demonstrated high modularity (more specialized information processing) in response to fear expressions demonstrated significantly greater PTSD symptom reduction relative to adolescent girls with less modularized network organization during fear processing. The functional modularity patterns of the healthy controls girls were similar to the adolescent girls with PTSD who responded well to treatment and were greater than the adolescent girls with PTSD who demonstrated less symptom reduction. By contrast, pre- to post-treatment changes in large-scale functional modularity were unrelated to degree of symptom reduction. Instead, we found that greater PTSD symptom reduction during TF-CBT was related to changes in the integration of specific nodes within and between modules: greater symptom reduction was related to greater increases in cross-module

integration of the right insular cortex and greater within-module integration of the left inferior frontal gyrus and superior frontal gyrus.
**Conclusions:** The present results suggest that individual differences in large-scale functional modularity patterns predict PTSD symptom reduction during TF-CBT. Adolescents responding better had modularity patterns more akin to healthy adolescent girls characterized by more specialized patterns of information processing. By contrast, symptom reduction did not appear to be mediated by large-scale changes in modularity and was instead related to changes in how specific nodes were integrated within and between functional modules. Overall, the results highlight 1) individual differences in functional network organization among adolescent girls with PTSD, demonstrating that a common neurocircuitry model does not apply equally to all, and 2) that PTSD symptom reduction is more closely linked with subtle changes within existing functional modularity patterns rather than changes in overall functional modularity patterns.
**Keywords:** Adolescent, Childhood trauma, Posttraumatic stress disorder, Cognitive Neuroscience, cognitive behavior therapy
**Disclosures:** Nothing to disclose.

### M27. Volume Reduction in the Associative Striatum in Adolescents at Genetic Risk for Schizophrenia

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**Background**: The striatum is a key component of the neural circuitry underlying the cognitive and emotional processes that are altered in schizophrenia. It can be subdivided into 5 anatomic subregions (pre and post-commissural caudate, pre and post-commissural putamen and the ventral striatum). These 5 ROIs can be grouped into 3 functional zones defined by their cortical connections. Specifically, the limbic striatum (LST) is comprised of the ventral striatum, the associative striatum (AST) is comprised of pre and post-commissural caudate and pre-commissural putamen, and the sensorimotor striatum (SMST) is comprised of the post-commissural putamen. Prior studies report that the volume of the caudate is enlarged in chronic schizophrenia but, in more recent reports, unchanged in first-episode schizophrenia (SZ). To disentangle possible medication enlargement effects even with the minimal neuroleptic exposure of first-episode SZ patients, we assessed neuroleptic-naïve first-degree relatives of SZ patients. Specifically, we measured striatal volume in adolescents at genetic risk of SZ, which can facilitate differentiation between "trait" effects caused by genetic predisposition and "state" effects caused by symptoms of illness, medication or chronicity.
**Methods:** Participants were 26 patients adolescents with a parent or biological sibling with schizophrenia and 28 healthy controls (HCs) matched for age and gender. 1.5mm MP-RAGE images were acquired on a Siemens 1.5T MR scanner. 5 ROI anatomic subregion and 3 functional subregion volumes were computed, and corrected for head size, using total intracranial contents.
**Results:** A mixed-model ANOVA was performed that compared functionally segmented striatal subregion volume in adolescents-at risk with HCs with group as the 'between-subjects' factor, and side (left, right) and subregion (right and left AST, right and left SMST, right and left LST) as the 'within-subjects' factors. Results showed a main effect for group for functionally segmented striatal subregion volume ($F(1,52) = 4.5$, $p = 0.038$). Post-hoc t-tests showed reduced right ($t = -2.1$, $df = 45.9$, $p = 0.042$) and left ($t = -2.2$, $df = 41.8$, $p = 0.037$) AST in Adolescents-at risk compared with HCs. We performed the same analyses for anatomically segmented striatal subregion volume with group as the 'between-subjects' factor, and side (left, right) and subregion (right and left pre-commissural caudate, right and left pre-commissural putamen, right and left post-commissural caudate, right and left post-commissural putamen, right and left ventral striatum) as the 'within-subjects' factors. Results similarly showed a main effect for group for anatomically segmented striatal subregion volume ($F(1,52) = 4.5$, $p = 0.038$). Post-hoc t-tests showed reduced right ($t = -2.5$, $df = 40.2$, $p = 0.016$) and left ($t = -2.4$, $df = 38.1$, $p = 0.022$) pre-commissural caudate volume and right ($t = -2.4$, $df = 52$, $p = 0.023$) and left ($t = -2.4$, $df = 52$, $p = 0.021$) post-commissural caudate volume in Adolescents-at risk compared with HCs.
**Conclusions:** The finding of a reduction in left and right anatomic caudate volume and in left and right functional associative striatum volume in adolescents at genetic risk of schizophrenia is in agreement with, and extends, the finding from a previous report (Rajarethinam et al., 2007). Further, these data support that this finding, indeed, is a trait effect of SZ and a potential endophenotypic marker for the disorder. In the future, we plan to correlate AST striatal volume with measures of cognitive function subserved by the striatal associative loop to test for its behavioral predictive value.
**Keywords:** striatum, adolescence, high risk psychosis, schizophrenia
**Disclosures:** Nothing to disclose.

### M28. Effects of Binge-Like Exposure to Alcohol on Cognitive Flexibility and Dopaminergic Neurotransmission in the Adult Prefrontal Cortex

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**Background**: The prefrontal cortex (PFC) is a brain region that is critically involved in cognitive function and inhibitory control. Adolescence represents a critical period of continued development of this region that parallels the maturation of its cognitive function. This extended period of developmental plasticity is thought to render the PFC, and its underlying circuitry, especially vulnerable to environmental insult that may result in deficits that persist well into adulthood. Alcohol drinking typically begins during adolescence when consumption of large quantities,

in binge-like episodic patterns, is common. A primary function of dopamine (DA) in the PFC is to maximize the efficient processing and transfer of information within the neurocircuitry that mediates decision-making. Dopamine innervation of the medial PFC peaks in early adolescence and then undergoes pruning and changes in DA receptor function during the transition to adulthood. These changes appear to play a critical role in the maturation of the executive function of the PFC.
**Methods:** Rodent model of adolescent binge-like exposure: Rats received 4-14 hour cycles of intermittent vapor exposure to alcohol and all outcome measurements were taken in adulthood at P90-P110.
Electrophysiology: We performed whole-cell patch clamp recordings in acute brain slices of prelimbic prefrontal cortex. Recordings targeted layer V pyramidal neurons labeled with a retrograde tracer to isolate specific prefrontal projections to subcortical structures (BLA, NAcc core and NAcc shell).
Behavior: We utilized the operant-based set-shifting task to test for differences in behavioral flexibility in adult rats. In addition, we tested anxiety behavior using the marble burying task.
**Results:** The present study investigated the effects of adolescent intermittent ethanol (AIE) exposure during postnatal days 28-42 by vapor inhalation on dopaminergic function in the prelimbic region of the adult PFC. Specifically, the functionality of DA D1 and D2/D4 receptors of layer V pyramidal neuron in the prelimbic PFC was examined in the adult acute slice preparation. These studies revealed that AIE exposure resulted in a loss of D1 receptor modulation of intrinsic excitability and synaptic transmission, but had no effect on D2 or D4 receptor function. Interestingly, treatment with the D2 agonist eticlopride during AIE exposure prevented the loss of D1 receptor function. In contrast, eticlopride treatment had no effect in the control air exposed rats.
**Conclusions:** Taken together, these findings demonstrate that binge-like alcohol exposure during early to mid-adolescence compromises D1 receptor function, but co-administration of a DA D2 receptor agonist during AIE exposure can protect against these deficits and may prevent AIE induced deficits in the cognitive function of the PFC.
**Keywords:** prefrontal cortex, Dopamine, Cognitive Enhancement, Alcohol, Adolescence
**Disclosures:** Nothing to disclose.

## M29. WITHDRAWN

## M30. Transcranial Magnetic Stimulation Potentiates Glutamatergic Neurotransmission in Depressed Adolescents

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**Background:** Noninvasive brain stimulation technologies such as repetitive transcranial magnetic stimulation (rTMS) may have promise as enduring therapeutic interventions in adolescents. Abnormalities in glutamate neurotransmission most likely play a role in the pathophysiology of adolescent depression. This study sought to examine changes in cortical glutamate neuorchemsitry in depressed adolescents receiving high-frequency rTMS. We hypothesized that the glutamine to glutamate (Gln/Glu) ratio would increase with treatment and that this change would relate to symptom improvement.
**Methods:** Ten adolescents with treatment-refractory major depressive disorder received up to 30 sessions of 10-Hz repetitive transcranial magnetic stimulation at 120% motor threshold with 3,000 pulses per session applied to the left dorsolateral prefrontal cortex. Baseline, posttreatment, and 6-month follow-up proton magnetic resonance spectroscopy scans of the anterior cingulate cortex (ACC) and left dorsolateral prefrontal cortex (L-DLPFC) were collected at 3T with 8-cm3 voxels. A FAST 3D SPGR sequence was used to acquire volumetric data for cerebrospinal fluid (CSF) correction (axial acquisition; repetition time [TR] = 12.6 ms, echo time [TE] = 5.6 ms, flip angle = 15, voxel dimensions = 0.49 x 0.49 x 1.5 mm). A TE-optimized PRESS sequence was used to measure Glu and Gln (PROBE-P PRESS; TE = 80 ms, TR = 2000 ms, No. of excitations = 8, No. of acquisitions = 128). A 2-dimensional J-resolved averaged PRESS sequence was obtained with the aim of collecting an optimized measure of Glu (2DJ PRESS; TE = 35-195 ms in 16 steps, TR = 2000 ms, No. of excitations = 8). The SPGR anatomical data were segmented into gray matter, white matter, and CSF using a technique modified from a previous study. Tissue volume–corrected metabolite concentrations were then calculated.
**Results:** The study sample included 6 males and 4 females, with a mean (SD) age of 15.4 (1.2) years. A linear mixed model analyses of repeated measures showed that Gln/Glu ratios increased in the ACC and L-DLPFC during acute rTMS treatment as well as through the 6-month follow-up period. Over the 6-month follow-up, the increase in the Gln/Glu ratio PRESS in the ACC was significant ($F = 5.32$; $df = 2,10$; raw $P = .02$; FDR $P = .04$), but the increases in the Gln/Glu ratios PRESS and 2DJ in the L-DLPFC did not reach significance (both FDR $P = .08$). There was no significant increase in the Gln/Glu ratio 2DJ in the ACC. To evaluate the timing and pattern of increase in the Gln/Glu ratios in the ACC and L-DLPFC, we examined the LS mean contrasts and respective effect size estimates (Hedges' g) of the Gln/Glu ratio change during the acute rTMS treatment (baseline to posttreatment) and 6-month follow-up periods (posttreatment to follow-up and baseline to follow-up). The Gln/Glu ratios in the ACC and L-DLPFC increased during the acute rTMS treatment and continued to increase throughout follow-up. The Gln/Glu ratio PRESS in the ACC had the largest increase throughout the 6-month follow-up, with effect sizes (Hedges' g) of 1.064 (baseline to posttreatment), 1.242 (posttreatment to follow-up), and 1.474 (baseline to follow-up). We examined the relationship between each Gln/Glu ratio and CDRS-R total score (depressive symptoms) during the treatment and follow-up periods. The linear mixed model repeated measures analysis revealed a negative (inverse) linear relationship between the CDRS-R total score and the ACC Gln/Glu ratio 2DJ ($b\square = -0.00014$; 95% CI, $= -0.00045$ to 0.00018; raw $P = .37$;

FDR P = .37), the ACC Gln/Glu ratio PRESS (b□ = –0.00123; 95% CI, = –0.00277 to 0.00029; raw P = .10; FDR P = .20), the L-DLPFC Gln/Glu ratio 2DJ (b□ = –0.00017; 95% CI, –0.00043 to 0.00008; raw P = .16; FDR P = .21), and the L-DLPFC Gln/Glu ratio PRESS (b□ = –0.00127; 95% CI, –0.00207 to –0.00048; raw P = .003; FDR P = .01). The direction (inverse relationship) of the regression coefficients suggests that the mean change in each Gln/Glu ratio increased as depression severity decreased. In other words, throughout the 6-month follow-up period, we estimated that a 1–scale unit increase (or decrease) in the CDRS-R total score (depression severity) was related to a mean decrease (or increase) in each Gln/Glu ratio.

**Conclusions:** In summary, this is the first study, to our knowledge, to demonstrate increases in the cortical Gln/Glu ratio in adolescents with treatment-refractory depression undergoing high-frequency rTMS. The Gln/Glu ratios also had an inverse relationship with symptom severity throughout the 6-month follow-up—Gln/Glu ratios increased as depression severity decreased. Successful rTMS treatment of adolescent depression may modulate glial cells and Glu neurochemistry. Adolescents with lower baseline cortical Gln/Glu ratios in the context of treatment-refractory depression may be preferred candidates for high-frequency rTMS. Further work with larger samples and control groups will be necessary to develop a biomarker for validation.

**Keywords:** glutamate/glutamine cycle, glutamate, Glutamine, rTMS, Adolescent Depression

**Disclosures:** Neuronetics, Inc. provided supplies (disposable SenStar shields) for the study.

## M31. Prenatal Stress Induces Maturational Delay in Dorsal Forebrain Inhibitory Cell Populations Postnatally

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**Background**: Prenatal stress is a risk factor for childhood behavioral and emotional disorders. GABAergic neuronal pathology is implicated in these same disorders, and the prenatal development and migration of inhibitory neurons is significantly delayed by prenatal stress. In order to evaluate the trajectory of these delays during postnatal development of inhibitory cell populations, we evaluated dorsal forebrain regions at multiple juvenile and adult ages.

**Methods:** We used CD1 GAD67GFP knock-in mice exposed to three times daily restraint stress during the last week of gestation. Brain tissue of offspring was collected at multiple postnatal time points, and GAD67GFP + and parvalbumin + cell populations in the medial frontal cortex (mFC) and hippocampus were assessed using immunohistochemistry and stereology. The expression of inhibitory neuron genes in these regions was also assessed with qPCR.

**Results:** In prenatally-stressed mice at postnatal day 0 (P0), total inhibitory neuron numbers were deficient in mFC and hippocampus as compared to non-stressed controls. By P24, inhibitory neuron density was increased in the mFC of prenatally-stressed offspring but was normalized within three weeks after. mFC and hippocampus of prenatally-stressed juvenile mice showed deficits in the proportion of GABAergic cells that were parvalbumin + with no significant difference in the ratio of parvalbumin to GAD1 gene expression. By adulthood, a parvalbumin + to GAD67 + cell deficit in mFC was accompanied by an increased ratio of parvalbumin to GAD1 gene expression. No significant differences were seen in hippocampus. Behavioral abnormalities were also observed in adulthood with more inhibited behavior in a social preference test, open field and elevated plus maze after prenatal stress. The levels of anxiety-like behavior were correlated with inhibitory neuron densities and the proportion of parvalbumin + cells in both mFC and hippocampus.

**Conclusions:** This model demonstrates that following delayed prenatal development, GABAergic population development may continue to be delayed by prenatal stress. While much of this maturation normalizes, small inter-individual GABAergic variations in multiple brain regions may be related to persistent behavioral inhibition.

**Keywords:** prenatal stress, GABAergic interneurons, parvalbumin, Anxiety, Medial frontal cortex

**Disclosures:** Nothing to disclose.

## M32. Enhanced Fronto-Subcortical Connectivity Following Childhood Adversity as a Protective Mechanism Against Internalizing in Adolescence

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**Background:** Much research has focused on the deleterious neurobiological effects of childhood adversity that may underlie anxiety and depressive disorders. The good news, however, is that most youth are capable of adaptation in the face of adversity and do not develop mental illness. Furthermore, work in animal models suggests that mild to moderate levels of early life stress can even have inoculating effects, protecting against later life stress exposure. While these findings are intriguing, the neurobiological mechanisms conferring adaptation following childhood adversity remain largely unknown.

**Methods:** We examined the associations among childhood family adversity, adolescent internalizing symptoms and functional connectivity of the amygdala and hippocampus during emotion regulation in 132 adolescents (69 female) participating in a longitudinal community study. Childhood family adversity (maternal depression, financial stress, marital/family conflict, parental role overload) was measured prospectively by maternal report from the child's infancy to age 11. Adolescent internalizing symptoms were obtained by mother, teacher, and self-report from ages 15-18. At age 18, adolescents completed an emotion processing task in which they viewed positive, negative, and neutral images (IAPS) during fMRI. Regional brain activation and functional connectivity of the amygdala and hippocampus were examined in the negative-neutral and positive-neutral contrasts. Group models included childhood adversity,

adolescent internalizing, and their interaction, with results corrected for multiple comparisons.
**Results:** Consistent with prior work, childhood adversity was associated with heightened adolescent amygdala reactivity to negative vs. neutral images, however amygdala reactivity itself was not related to internalizing symptoms. At the same time, childhood adversity was associated with increased connectivity between the amygdala and hippocampus to dorsomedial prefrontal cortex (BA 9, 10). However, adversity-related increases in fronto-amygdala and -hippocampal connectivity were attenuated or absent in higher internalizing adolescents (i.e. adversity by internalizing interaction). Adversity-related enhancement of connectivity was not accounted for by adolescent stress, suggesting specificity of stress effects to childhood. Finally, these effects were specific to negative emotional stimuli.
**Conclusions:** Together, these findings suggest that adaptation to childhood adversity is associated with a strengthening of fronto-subcortical circuits important in the regulation of fear and anxiety. On the other hand, insufficient recruitment of regulatory circuits, leaving increasing amygdala reactivity unchecked, may represent a neural signature of vulnerability for internalizing by late adolescence. Furthermore, our findings implicate early childhood as a critical developmental period in determining the brain's adaptation to adversity. These findings point to neural mechanisms of stress adaptation and vulnerability which could be used in the prediction of risk for psychopathology following childhood adversity, and as treatment targets in those suffering from internalizing and stress disorders.
**Keywords:** neurobiology, stress, depression, anxiety, adolescence, early life stress, prefrontal-amygdala-connectivity, emotion regulation
**Disclosures:** Nothing to disclose.

### M33. Adolescent Social Stress Produces an Enduring Activation of the Locus Coeruleus and Impairs its Communication with the Prefrontal Cortex

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**Background**: Early life stress has been implicated in diverse psychiatric diseases. Adolescence is a unique period of stress vulnerability because brain circuits regulating emotion and decision-making are still in development. Social stressors may have a particularly potent impact because adolescence is a socially dynamic time characterized by independence from parental social bonds and the beginning of sexual maturity. The locus coeruleus (LC)-norepinephrine system is a major stress response system that is pivotal in an emotional arousal circuit. The LC receives afferents from the central amygdalar nucleus, including afferents containing the stress-related neuropeptide, corticotropin-releasing factor. In turn it projects massively to the prefrontal cortex (PFC) and through this projection it can modulate executive functions such as cognitive flexibility that are impaired in many psychiatric disorders. This study tested the hypothesis that social stress has enduring effects on LC activity and its regulation of the PFC that are unique to adolescents.
**Methods:** Adolescent (PND 42-55) and adult (PND $>70$) male rats were implanted with a multiwire bundle into the LC to record single unit activity and local field potentials (LFPs). Rats were then exposed to 5 consecutive days of the resident-intruder social stress. Spontaneous and auditory-evoked LC neuronal activity was compared between adult and adolescent rats immediately before and during stressor exposure on days 1 and 5. Local oscillatory activity quantified from LFPs was also compared. In some rats an additional electrode was implanted into the medial PFC (mPFC) for simultaneous recording of LC and mPFC LFPs, thereby providing a measure of LC-mPFC coherence.
**Results:** An initial exposure to social stress produced a robust tonic activation of LC neurons, while simultaneously attenuating LC responses to discrete auditory stimuli in rats of both ages. Oscillatory rhythms within the LC were also shifted during the stress towards a prominent theta rhythm and LC-mPFC coherence within the theta range was also increased. By the fifth exposure to social stress LC neurons of adolescent rats were tonically activated and oscillatory rhythms remained prominently in theta, even in the absence of the stressor. These LC neurons of adolescents with a history of repeated social stress did not mount a response upon stressor re-exposure and their responses to discrete auditory-evoked stimuli were similarly diminished as seen during the first stress exposure. Additionally, LC-PFC coherence in the high frequency range (beta and gamma) was reduced in these rats while coherence in theta remained robust. In direct contrast, LC neurons of adult rats exposed to repeated social stress were relatively inhibited in the absence of the stressor, were able to mount robust responses upon stressor presentation and were responsive to auditory stimuli. Moreover, LC-mPFC coherence was not decreased by repeated social stress in adult rats.
Analogous studies in female rats are ongoing and preliminary results suggest sex differences in the effects of an initial social stress exposure on the pattern of LC oscillations with a shift to theta and decrease in beta frequencies occurring selectively in males and enhanced alpha activity induced by the stressor in females.
**Conclusions:** The results suggest that adaptive mechanisms that promote stress recovery and maintain basal activity of the brain norepinephrine system in the absence of stress are not fully developed in adolescence. The sustained LC activation and muted responses to discrete sensory stimuli that are consequences of adolescent social stress could underlie adolescent hyperactivity. The inability to mount a response to a subsequent social stress challenge may translate to impaired coping with social challenges. Finally, given the modulatory function of the LC on mPFC activity, social stress-induced changes in LC-PFC coherence that are selective to adolescents have the potential to adversely impact on cognition and behavior of adolescents and these effects may endure into adulthood.
**Keywords:** adolescent stress, oscillation, Social defeat stress, Medial Prefrontal Cortex, Locus coeruelus
**Disclosures:** Nothing to disclose.

### M34. In Utero Exposure to Clinically Relevant Concentrations of Paliperidone Does Not Affect Several Measures of Development or Early Measures of Cognition in Rats

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**Background:** There is increasing utilization of atypical antipsychotics as mood stabilizers and adjuncts to antidepressant therapy, contributing to an increase in fetal exposure to these medications. Because of large differences in the pharmacokinetics of many drugs between humans and rodents, we have demonstrated that "behaviorally active" doses of many psychiatric drugs in laboratory animals do not accurately reflect/model actual human clinical exposure. Previous studies from our group demonstrate the need for clinically relevant and sustained drug administration in animal studies that more closely mirror human exposures. Using a rat model, this study provides rigorous control of clinically relevant fetal exposure to paliperidone, the major active component of risperidone treatment. We tested our hypothesis that prenatal paliperidone alters developmental trajectory in the offspring using behavioral assessments of development (motor and cognitive) prior to weaning and as juveniles (postnatal days 23-35).
**Methods:** Pregnant female rats, with (dose-finding experiments) or without (experiments generating pups for further study) chronic indwelling catheters, were administered either paliperidone or vehicle (2% acetic acid adjusted to pH 5.0) using subcutaneous osmotic minipumps. Following parturition, both male and female offspring (4 per litter) were transferred to foster dams. At least 7-8 independent litters from each treatment group were produced. Assessment of developmental milestones and behavior before weaning (postnatal day 1 through 21) and again in preadolescence (PND23-35) were obtained. Preweaning developmental milestones examined included: forelimb placing and grasping, cliff aversion, fur, ear opening, eyes open, incisor eruption, negative geotaxis, righting, adult gate, pre-pulse inhibition of the acoustic startle response and locomotor activity. Testing of cognitive development (Morris water maze and spontaneous alternation), locomotor activity and acoustic startle response in preadolescence were conducted.
**Results:** Following initial dose-finding in nonpregnant rats, we implanted chronic indwelling jugular catheters and followed serum paliperidone concentrations throughout gestation. In round #1 (N = 16 rats), serum paliperidone concentrations were maintained near the 90th percentile of human exposure (mean concentrations of ~45 ± 7 ng/ml) based upon women (N = 68) within the Emory Women's Mental Health Program. Drug administration began 4 days before mating and catheter placement on estimated gestational day 3, however only one rat became pregnant. We wondered whether this was due to not allowing sufficient time for mating and/or surgical anesthesia and repeated the experiment (round #2). These animals were housed with male breeder rats for 84 hours vs. 60 hours in round #1. These animals had mean concentrations of drug throughout gestation of ~35 ± 5 ng/ml (human 80th percentile of exposure) but also failed to get pregnant. Although atypical in nature and dosed in a clinically relevant manner, we hypothesized that drug-induced increases in prolactin may have been responsible. In round #3, we chose to begin drug administration and catheter placement on either estimated gestational day 4, 5 or 6. Fourteen of 15 rats became pregnant and drug concentrations were maintained throughout gestation at an average of ~40 ± 6 ng/ml (~85th percentile of human exposure). This finalized our dosing strategy for paliperidone. Behavioral assessment, as noted above, on separately treated litters with paliperidone exposure throughout gestational days G4-G21 (mean 44 ± 6 ng/ml; ~85th percentile of human exposure) revealed, in preliminary analyses, no differences between treatment groups in any of these measures.
**Conclusions:** A clinically relevant prenatal dosing paradigm has been developed for paliperidone (and by association, risperidone). Paliperidone clearance in rats does not appear to be substantially altered during pregnancy and will not be a confound to future studies during pregnancy. Preliminary analyses reveal that, when dosed in a clinically relevant manner, no deleterious effects on external measures of early development or cognitive behaviors in preadolescence are observed.
Grant Support NIH: R01HD074486
**Keywords:** Atypical antipsychotics, developmental trajectories, Pharmacokinetics
**Disclosures:** Nothing to disclose.

### M35. Early-Life Exposure to the SSRI Paroxetine Disrupts DNA Methylation in the Early Postnatal Hippocampus

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**Background:** Selective serotonin reuptake inhibitors (SSRIs) are the most common pharmacological treatment for major depression in pregnant women. SSRIs are generally considered safe as they do not increase risk for miscarriage or major birth defects, although the long-term effects of perinatal SSRI exposure on exposed children's emotional health are largely unknown owing to insufficient study. In rodents, perinatal SSRI exposure elicits life-long adverse outcomes, elevating adult anxiety- and depression-like behavior. Furthermore, certain individuals appear to be more susceptible to adverse effects of perinatal SSRI exposure, but the mechanisms driving this differential vulnerability are completely unknown. We recently showed that rats selectively bred for low behavioral response to novelty (Low Novelty Responders, LRs), which also exhibit high levels of spontaneous anxiety- and depression-like behavior, are vulnerable to the adverse effects of perinatal SSRIs. When LR mothers were treated with SSRIs through pregnancy and the postnatal lactation period, their exposed offspring grew up to display even higher than normal levels of depression-like behavior (Forced Swim Test FST immobility) as adults, while High Novelty Responder rats (HRs) were unaffected by the treatment. A transcriptome

study also revealed widespread gene expression alterations in the early postnatal hippocampus of perinatal SSRI-exposed LR offspring, with striking SSRI-induced down regulation of several DNA methylation-related genes (Glover et al, Neurosci 2015). Our new experiments are testing the working hypothesis that the adverse behavioral consequences of perinatal SSRI exposure are mediated by perturbed DNA methylation in the early postnatal hippocampus.

**Methods:** In our first study, HR and LR females received the SSRI paroxetine (10 mg/kg/day) via drinking water or normal tap water throughout mating, pregnancy and the 3-week postpartum lactation period. Brains were harvested and hippocampal tissue dissected from SSRI- and vehicle-exposed offspring on postnatal day 15 (P15; n = 6/phenotype/condition), a time point when we previously observed dramatic perinatal SSRI-induced gene expression differences. Global DNA methylation (5-methylcytosine) levels were assessed using an Epigentek Methylflash assay. SSRI-induced site specific methylation changes in the LR hippocampus are currently being assessed by methylated DNA immuno-capture coupled with next-generation sequencing (MethylCap-Seq). In a second experiment, we manipulated DNA methylation in developing LR pups to test whether it produced behavior changes reminiscent of the effects of perinatal SSRI exposure. To do so, we manipulated dietary methyl donor content for pregnant and postpartum LR rat mothers. On the 15th day of pregnancy (beginning of the third trimester), LR rat mothers received: (1) a diet deficient in methyl donors; or (2) standard food (n = 8 mothers/condition). We monitored maternal behavior during the postnatal lactation period and will examine anxiety- and depressive-like behaviors in adult LR offspring exposed to each diet.

**Results:** Perinatal SSRI exposure led to decreased hippocampal DNA methylation levels in LR offspring relative to vehicle-exposed LR controls; no changes were observed in other limbic brain regions examined (amygdala and prefrontal cortex). Interestingly, HR offspring were not affected by SSRI exposure, with both SSRI- and vehicle-exposed HR groups showing similar DNA methylation levels in the P15 hippocampus. Next generation sequencing analyses are currently underway to assess gene-specific methylation changes in perinatal SSRI-exposed offspring. Behavioral analyses are also underway in methyl diet-manipulated animals to determine whether perinatal methyl depletion mimics the effects of perinatal SSRI exposure on adult LR behavior.

**Conclusions:** Our previous work showed that LR rats exhibit high levels of behavioral inhibition, high anxiety and learned helplessness together with diminished social interaction and sexual motivation – all hallmarks of a depression-like syndrome. We recently found that "depression-prone" LR offspring are particularly susceptible to the deleterious effects of perinatal SSRI exposure, leading exposed LR offspring to display even higher than normal levels of depression- and anxiety-like behavior in adulthood. We also showed that perinatal SSRI exposure triggered substantial gene expression changes in the early postnatal and adult LR hippocampus, including suppressed expression of several genes involved in DNA methylation (e.g. Dnmt3a, Mecp2). Our new data provide additional evidence that early-life SSRI exposure perturbs DNA methylation in the developing brain, which may dysregulate brain development in exposed offspring and thereby mediate its deleterious long-term effects on adult emotional behavior.

**Keywords:** Antidepressant, Brain development, Epigenetics
**Disclosures:** Nothing to disclose.

### M36. Characterization of Olfactory and Gait Behaviors in the Balb/c Mouse Model of Autism Spectrum Disorders

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**Background:** Abnormalities of olfaction and gait in autism spectrum disorders (ASDs) may reflect involvement of the cerebellum and nodes related to olfaction in neural circuits subserving social, cognitive, and motor domains of psychopathology. There is anatomic evidence for pathological involvement of the cerebellum in autopsied brains of persons with ASDs. Further, some of the stereotypic behaviors of patients with ASDs, especially younger patients and patients with co-morbid intellectual disability, include sniffing of objects. Therefore, we hypothesized that the Balb/c mouse model of ASDs would show differences in gait and olfaction, relative to the Swiss Webster comparator strain.

**Methods:** 4-week old male, Swiss Webster and Balb/c mice (n = 18 per group) were placed in the center of a 3-compartment apparatus containing suspended cotton swabs on opposing left or right compartments. During a 5-minute acclimation, test mice were free to sniff/explore swabs saturated with water, providing measures of general exploratory behavior and locomotor activity. Following acclimation, preference for olfactory stimuli was assessed by measuring the amount of time test mice spent sniffing cotton swabs saturated with either water or a floral scent (Trial 1); a familiar or novel floral scent (Trial 2); and a novel floral scent or a salient social odor (mouse urine) (Trial 3). For Trials 1, 2 and 3, scents were presented simultaneously over a 2-minute period. There were 3-minute breaks between each trial to clean, remove residual odors and set-up the apparatus for the following trial. Importantly, the scent of each swab was randomly assigned to the left or right compartment within each trial and counterbalanced throughout the experiment. Floral scents chosen because they elicited a similar amount of olfactory interest (i.e., jasmine, plumeria, and rose) were randomly presented in each trial. The social scent was obtained by rubbing the cotton swab in week-old saturated bedding from group-housed, male B6.129S2-Alox15 mice, a non-obese diabetic (NOD) mouse strain congenic for a targeted deletion of 12/15-lipoxygenase. Each trial was recorded in order to measure initial latency to approach and time spent exploring/sniffing the cotton swabs. Following testing of olfactory preferences, gait was assessed quantitatively. The soles of the front and hind paws were painted with nontoxic red and brown paint, respectively. Mice walked down an enclosed runway lined with white paper, with three

repetitions per mouse (n = 20 per group), to obtain footprint tracks. Footprints were analyzed by measuring the length of three strides for each front and hind leg and the front leg and hind leg base-width from the middle portion of each run.

**Results:** Balb/c and Swiss Webster mice showed no preference for olfactory exploration of cotton swabs saturated with either a floral scent or water. However, in Trial 1, seven out of 18 Balb/c mice failed to approach either the floral scent or water-saturated cotton swab ($p = 0.013$), whereas all of the Swiss Webster mice made initial approaches to within 2 cm of either cotton swab. Moreover, in Trial 3, when a novel floral scent and salient social odor were presented simultaneously, 15 out of 18 Balb/c mice did not engage in olfactory exploration of either condition, whereas 13 out of 18 Swiss Webster mice made an initial approach to within 2 cm of the salient social odor ($p < 0.0001$).

Balb/c mice were not impaired in exploratory/locomotor behavior during Acclimation. 2-way ANOVA revealed significant main effects for strain (i.e., Balb/c vs Swiss Webster) ($F_{1,136} = 45.63$, $p < 0.0001$), condition (i.e., Acclimation, Trials 1, 2 and 3) ($F_{3,136} = 4.822$, $p = 0.0032$) and their interaction ($F_{3,136} = 25.23$, $p < 0.0001$) on initial latency to approach a cotton swab. Post-hoc comparisons showed that the Balb/c strain had a significantly shorter initial latency to approach the cotton swabs than Swiss Webster mice during Acclimation ($p < 0.01$). However, in the presence of a floral scent (Trial 1), the initial latency to approach the cotton swabs was significantly increased in the Balb/c strain compared to the Swiss Webster strain ($p < 0.001$). Similarly, simultaneous presentations of a familiar and novel floral scent (Trial 2) and a novel floral and salient social scent (Trial 3) significantly increased the initial latency of the Balb/c strain to approach the cotton swabs compared to the Swiss Webster strain ($p < 0.0001$). Moreover, compared to the Acclimation condition, Balb/c mice showed longer initial latencies to approach cotton swabs saturated with either floral or salient social odors in Trials 2 ($p < 0.001$) and 3 ($p < 0.0001$).

Balb/c and Swiss Webster mice did not differ in measures of gait. Thus, the reduced olfactory exploratory behavior and locomotor activity of the Balb/c strain in the presence of either the floral scent alone (Trial 1) or simultaneous presentations of a familiar and novel floral scent (Trial 2) and a novel floral and salient social scent (Trial 3) were not due to motor incoordination (ataxia) or an inability to move.

**Conclusions:** Balb/c and Swiss Webster mice differ in olfactory exploratory behavior. Floral scents and the simultaneous presentation of a floral scent with a salient social odor suppress the olfactory exploratory behavior of the Balb/c strain, relative to the Swiss Webster comparator strain. Importantly, concentrated mouse urine has greater salience for the Swiss Webster strain than for the Balb/c strain. The diminished exploratory behavior of the Balb/c strain was not an epiphenomenon of a primary disturbance of gait. Diminished olfactory exploration of a salient social odor by the Balb/c strain is consistent with reports of its impaired sociability. Future experiments will explore the sensitivity of the Balb/c strain to elicitation of a fear response by a predatory scent (e.g., bobcat urine). We will also examine whether NMDA receptor agonist interventions increase olfactory exploration of a salient social odor by the Balb/c strain.

**Keywords:** Balb/c mouse, autism Spectrum Disorders, Olfaction, gait, sociability

**Disclosures:** Nothing to disclose.

## M37. CNV Analysis and Exome Sequencing in Japanese Autism Spectrum Disorder Subjects

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**Background:** Twin and family studies indicate a predominantly genetic basis for Autism Spectrum Disorders (ASD). Linkage and candidate gene association studies have implicated several genes and chromosomal regions in autism. However, consistent picture of a common susceptibility loci in autism is still lacking. Hence there is paradigm shift away from the previously held "common disease – common variant" hypothesis to a "common disease – rare variant" model, for the genetic architecture of autism. There is a growing consensus among geneticists that rare structural variants including genomic copy number variations (CNVs) may contribute to the autism etiology. Most recently, exome sequencing (ES) studies have revealed the importance of rare single nucleotide variations (SNVs) also. To date, almost all of the CNV and ES studies in autism have focused predominantly on the Caucasian populations, with little representation of the Asians and the Africans. In this study, for the first time as per our knowledge, we have examined the global CNV, with special emphasis on rare de novo and inherited CNVs in a total of 778 Japanese samples, which includes 203 autism families and 163 control subjects. We have also sequenced the exomes of 60 individuals which include 20 patients with sporadic autism and their parents, reasoning that these families with no previous history of ASD or related phenotypes would be enriched for de novo mutations.

**Methods:** Samples were recruited on collaboration with a non-governmental organization, Asperger Society Japan (http://www.as-japan.jp/) and various university hospitals in central Japan. The Affymetrix Genome-Wide Human SNP Nsp/Sty 6.0 array was used to screen the samples. PennCNV (University of Pennsylvania) and Birdsuite package (Broad Institute) were used to identify autosomal CNVs from the genome wide SNP data. For exome sequencing, Genomic DNA was captured using SureSelect Human All Exon v5 kit (51 Mb; Agilent, Santa Clara, CA, USA), and sequenced on HiSeq2000 (Illumina, San Diego, CA, USA). Image analysis and base calling were performed by sequence control software real-time analysis and CASAVA software v1.8 (Illumina). Single-nucleotide variants and small indels were identified using the GATK UnifiedGenotyper and filtered according to the Broad Institute's best-practice guidelines v3.

**Results:** The overall CNV burden and the average size of CNVs of probands did not differ significantly from their parents and controls. There was no significant difference in the total CNV burden or average size of CNVs between male and female probands, and it had no correlation with the parental age at conception. Among a

total of 7305 CNVs detected in affected children after all QC corrections, 3728 were found to be affecting genes. 89.6% of these were confirmed to be inherited from parents. Among the remaining 378 CNVs, 17 novel/ultra-rare de novo events were identified through a series of filtering and validating strategies. Among the de novo events, there were four novel, five partially novel and eight ultra-rare CNVs; many of these regions has genes involved in neurodevelopmental pathways. Majority (72.7%) of the de novo CNVs were of paternal in origin. Among the inherited CNVs too, we found 53 ultra-rare events, with $<0.1\%$ frequency in various databases. None of the ultra-rare/novel de novo and inherited variants observed in probands was present in the control samples analyzed. All of the ultra-rare/novel CNVs were re-confirmed by relative qPCR. A total of 32 rare novel de novo events were observed though ES too. This includes 15 missense mutations, a nonsense mutation, 2 frameshift mutations caused by indels, one splice site mutation, and 7 synonymous mutations. All the de novo events, except for the synonymous mutations, were validated by Sanger's sequencing. 25 de novo events had occurred within the coding sequences, while the remaining 7 de novo events were located within 20 bp of the exon/intron boundary. Majority of these mutations were predicted (PolyPhen or SIFT) to have a damaging effect on the respective protein structure/function. Several of these genes have been implicated in neural functions.

**Conclusions:** We discovered several novel/ultra-rare de novo and inherited CNVs and SNVs, many of them are potentially deleterious, in the first ever genome wide study in Japanese autism subjects; further highlighting the genetic heterogeneity of the disorder. Many of these variations affect genes that play major roles in crucial neurodevelopmental processes. Though our study has limited statistical power due to comparatively smaller sample size, we do support some of the previous hypotheses and findings in genome wide studies in autism in larger populations, predominantly Caucasian. Another limitation of the present study is we failed to observe any significant correlation between detected variations and the available phenotypic data like IQ and ADIR scores. Estimation of the exact functional consequences of CNVs and rare mutations, and the translation of this knowledge to support clinical decisions towards personalized pharmacological interventions is a real future challenge. Much greater wealth of available data from different populations concerning CNVs, rare variants, common variants and gene pathways involved in autism is indeed spearheading this ultimate aim.

**Keywords:** autism spectrum disorders, copy number variation, de novo, exome sequencing

**Disclosures:** Nothing to disclose.

### M38. Suppression of Autistic Self-Injurious Behavior by Deep Brain Stimulation

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**Background:** Over a third of autistic individuals display self-injurious behavior (SIB) ranging from head banging to self-directed biting and punching. Sometimes, these behaviors are extreme and unresponsive to pharmacological and behavioral therapies. We have found electroconvulsive therapy (ECT) can produce life-changing results, with more than 90% suppression of SIB frequency. However, these patients typically require frequent maintenance ECT (mECT), as often as every 5 days, to sustain the improvement gained during the acute course. Long-term consequences of such frequent mECT started as early as childhood in some cases are unknown. Accordingly, there is a need for alternative forms of chronic stimulation for these patients.

**Methods:** To evaluate the feasibility of deep brain stimulation (DBS) for autistic SIB, we utilized two genetically distinct mouse models demonstrating excessive self-grooming, namely the Viaat-Mecp2-/y and Shank3B-/- lines. In preliminary studies, we found that a single ECS significantly suppresses the excessive self-grooming of Viaat-Mecp2-/y mice, similar to the positive effect of ECT observed in the clinic. In order to test whether DBS could also suppress excessive self-grooming, we targeted the subthalamic nucleus (STN) due to its central role in response inhibition, regulating output nuclei from the basal ganglia. Moreover, STN-DBS is effective in suppressing repetitive stereotyped behaviors in both monkeys and humans suffering with severe obsessive compulsive disorder. We delivered bilateral monopolar stimulation for up to 3 hours daily over 3 days. Mice were either knockouts or controls, and received either active or sham stimulation.

**Results:** We found that STN-DBS significantly suppressed autistic SIB in both the Viaat-Mecp2-/y line and in the genetically distinct Shank3B-/- mouse model, suggesting STN-DBS could be broadly effective for autistic SIB in a heterogeneous autistic population. Unexpectedly, we found that suppression of excessive self-grooming by STN-DBS not only occurs acutely when stimulation is switched on, but is also persistent for several days after DBS is turned off. After excessive self-grooming returns to elevated baseline levels following cessation of STN-DBS, the stimulation retains its efficacy once it is reapplied to Viaat-Mecp2-/y mice. The effect of STN-DBS on excessive self-grooming is also selective. Locomotor activity is not affected by STN-DBS indicating the suppression in excessive self-grooming is not due to hypoactivity. Also, the heightened social interaction of Viaat-Mecp2-/y mice does not appear to be corrected by STN-DBS.

**Conclusions:** We have explored the feasibility of DBS for autistic SIB as an alternative to long-term frequent mECT, and our data suggest STN-DBS could be a candidate site for clinical trials. However, focal brain stimulation engages neural elements that form part of distinct, parallel-organized, functional basal ganglia-thalamocortical circuits. Accordingly, in future studies it will be important to evaluate DBS for autistic SIB at other sites, including cortical regions that project to the STN and globus pallidus interna, such as the orbitofrontal cortex and supplementary motor area, which have also been implicated in mediating autistic SIB. Translational studies using optogenetic techniques could also help optimize targeting in patients. Such studies might provide clues about brain circuitry which could potentially be harnessed by less invasive techniques such as epidural cortical stimulation as well as non-invasive,

nonconvulsive neuromodulatory techniques such as transcranial magnetic stimulation and transcranial direct current stimulation.
**Keywords:** Autism, stereotypy, self-injury, electroconvulsive therapy, deep brain stimulation
**Disclosures:** Nothing to disclose.

### M39. Childhood Maltreatment and Methylation of FKBP5

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**Background:** A growing body of evidence suggests that alterations in the stress response system may be a mechanism by which childhood maltreatment alters risk for psychopathology. FK506 binding protein 51 (FKBP5) binds to the glucocorticoid receptor and alters its ability to respond to stress signaling. Previous work has shown that methylation of the FKBP5 gene (FKBP5) at intron 7 is decreased in adults with a history of childhood maltreatment and that a genetic variant of FKBP5 affects the degree of methylation. Decreased FKBP5 intron 7 methylation is associated with increased glucocorticoid induction of FKBP5 expression and decreased glucocorticoid receptor sensitivity. Studies of subjects with later traumas did not exhibit FKBP5 methylation changes, suggesting a role of developmental stage at the time of exposure in FKBP5 methylation. The aim of the present study was to examine the impact of childhood maltreatment and an FKBP5 genetic variant on FKBP5 methylation in a sample of impoverished preschool-aged children. This is the first study to specifically examine FKBP5 epigenetic changes with maltreatment exposure in this age group.
**Methods:** One hundred seventy-four families, including $n = 69$ with child welfare documentation of moderate-severe maltreatment in the past six months, participated in this study. Families of children with no indicated case of maltreatment within the past six months served as the control group. Children ranged in age from 3 to 5 years, and were racially and ethnically diverse. Structured record review and interviews in the home were used to assess a history of maltreatment, other traumas, and contextual life stressors, and a composite variable assessed the number of exposures to these adversities. Methylation of FKBP5 intron 7 CpG sites was measured from saliva samples via sodium bisulfite pyrosequencing.
**Results:** Maltreated children had lower levels of methylation at intron 7 FKBP5 CpG sites ($p < .05$). Children with lifetime contextual stress exposure showed lower levels of methylation at CpG1 ($p = .064$) and an interaction with the FKBP5 polymorphism that approached significance ($p = .082$). A composite adversity variable was associated with lower levels of CpG1 methylation ($p < .05$).
**Conclusions:** These data are consistent with previous work in adult populations and suggests that epigenetic changes associated with childhood maltreatment may start soon after the exposure. It has been proposed that FKBP5 methylation may be a mechanism through which adverse exposures in young children contribute to alterations in the stress response system and to bio-behavioral outcomes. Our results suggest that childhood maltreatment decreases FKBP5 methylation, which would be consistent with this proposed mechanism. Future extensions of work with this population could examine the relationship of FKBP5 methylation to glucocorticoid levels and responsiveness and behavioral outcomes.
**Keywords:** Epigenetics, childhood maltreatment, FKBP5, HPA axis, early life stress
**Disclosures:** A. Tyrka support for research from Neuronetics, NeoSync, and Cervel Neurotech. The other authors declare no biomedical financial interests or potential conflicts of interest.

### M40. Childhood Poverty Affects Brain White Matter Integrity

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**Background:** Brain development is critically shaped by early life experiences, including poverty. One fifth of America's children grow up in families that suffer poverty. While there is good evidence that low socioeconomic status (SES) is harmful to development, health, achievement, and socio-emotional adjustment, very little is known about the role of brain microstructure. Thus, we aim to determine how the chronic stress of childhood poverty prospectively influences adult brain structural connectivity. We hypothesize that early childhood poverty disrupts white-matter brain microstructural integrity.
**Methods:** Participants were recruited from an ongoing 16-year, longitudinal research program of low and middle-income individuals focused on childhood poverty, physiological stress, and socio-emotional development with brain imaging and coordinated psychometrics. Here, we examine childhood poverty at age 9 and white matter integrity in the same individuals at age 23-25 using diffusion tensor imaging (DTI) and tract-based spatial statistics (TBSS). Brain imaging was performed in a Phillips 3T scanner with 32 diffusion-weighted volumes (one per gradient direction), $b = 1000$ s/mm2 and one volume with $b = 0$ acquired for each scan. Data were analyzed using the fMRI Software Library (FSL v5.0; www.fmrib.ox.ac.uk/fsl). Each subject's raw image data was preprocessed with FDT (FMRIB's Diffusion Toolbox) and examined before proceeding to further analyses to detect any outliers in the data due to signal drop-outs, poor signal-to-noise ratio, and image artifacts such as banding. 4 of 53 subjects' were excluded because of 3 or more diffusion volumes with significant image quality problems. Thus, voxel-wise whole brain FA (fractional anisotropy) maps were generated with a permutation-based (10,000 permutations) non-parametric inference test using FSL's randomise on 49 high quality DTI TBSS data sets. For preliminary analyses, poverty threshold at age 9 was defined as above ($n = 26$) and below ($n = 23$) income-to-needs ration of 1.3 Significance threshold for the whole brain set at $p < .005$ (uncorrected) in a GLM of SES

grouping, controlled by current SES. Regions-of-interest (ROI) analysis was also conducted to explore the continuous relationship between income to needs ratio at age 9 and brain regions from the literature, including the hippocampus, amygdala, dorsolateral prefrontal cortex (dlPFC), and ventrolateral prefrontal cortex (vlPFC).
**Results:** Childhood SES at age 9 was associated with white matter structure as measure by DTI FA in several brain regions, controlling for concurrent adult income measures. These included the hippocampus, parahippocampal gyrus, dlPFC, vlPFC, corpus callosum and thalamus. Furthermore, adult brain ROI DTI FA measures were significantly correlated with SES at age 9 in the dlPFC ($P < 0.01$) and arcuate fasciculus ($p < 0.05$), controlling for concurrent SES. Thus, we demonstrate apparent deficits in white matter integrity associated specifically with early-life poverty, including brain regions that support executive function, social cognition, memory and language processing. This is consistent with established literature on poverty and emerging allied brain-imaging studies.
**Conclusions:** This study is among the first longitudinal analyses of objective childhood poverty to suggest a neuroanatomical basis for long-term SES-related effects on adult brain structural integrity. These preliminary white matter differences point to possible structural connectivity deficits for individuals affected by childhood poverty. Future planned analyses will include individual executive function, memory, language and sex, plus longitudinal measures of early life circumstances, cumulative risk, cognitive development and 3-dimensional modelling of neural tracts. This work promises structural brain endophenotypes to disambiguate environmental mediators of brain development toward possible early detection and amelioration of adverse brain development trajectories.
**Keywords:** poverty, DTI, neurocircuitry, social neuroscience, Structural MRI
**Disclosures:** Nothing to disclose.

### M41. Characterization of Cognitive Function with the Cantab in Individuals with Amnestic MCI in Relation to Hippocampal Volume, Amyloid and Tau Status: Preliminary Baseline Results from the PharmaCog/European-ADNI Study

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**Background:** MCI is a heterogeneous condition with differential underlying pathophysiologies. Accumulation of beta amyloid (Abeta42) and/or Tau in the brain is associated with greater neurodegeneration and cognitive decline and a prelude to Alzheimer's disease (AD). Understanding MCI populations for hippocampal specific memory deficits and biomarker abnormalities will help identify a more homogeneous population with a greater risk of developing AD.
**Methods:** Participants were recruited from the PharmaCog (E-ADNI; work package 5), European multicentre study. 150 individuals underwent clinical and cognitive evaluation using the CANTAB tests, high resolution 3T MRI with MPRAGE and lumbar punctures for the assessment of cerebrospinal fluid (CSF) levels of Abeta42, tau and p-tau. Individuals were divided into Abeta+ (CSF-POS) and Abeta- (CSF-NEG) based on CSF Abeta42 levels (cut off; 550 pg/ml). The data reported here is the preliminary analysis of the baseline data.
**Results:** At baseline, CSF-POS individuals showed worse performance relative to CSF-NEG individuals on hippocampal dependent memory tasks (effect sizes ranging from -0.12 to -0.66). Age and education adjusted performance on the paired associate learning (PAL) task of episodic memory was associated with hippocampal volume (Right hippocampus $\beta = -0.03$, $p = < 0.01$; $R2 = 0.26$; Left hippocampus $\beta = -0.03$, $p = < 0.01$; $R2 = 0.20$) and CSF levels of tau ($\beta = 0.04$, $p = < 0.01$; $R2 = 0.12$) and p-tau ($\beta = 0.24$, $p = 0.02$; $R2 = 0.06$) with greater errors on the PAL task associated with reduced hippocampal volume and higher CSF levels of tau and p-tau. Similarly, worse performance on the spatial recognition memory (SRM) task was associated with low CSF levels of Abeta42 ($p = 0.01$) and higher CSF levels of tau ($p = 0.05$), p-tau ($p = 0.03$) while worse performance on the pattern recognition memory (PRM) task (delayed) was associated with reduced left ($p = 0.04$) and right hippocampal ($p = 0.05$) volume.
**Conclusions:** These findings show associations between hippocampal dependent memory performance assessed using the CANTAB tests, CSF biomarkers and hippocampal volume in biomarker positive amnestic MCI individuals. The findings have implications for identifying MCI patients at risk of developing AD and enriching a more homogenous population for clinical trials with episodic memory deficits, neurodegeneration and A□/tau biomarker abnormalities consistent with prodromal AD populations.
**Keywords:** Cognition, Alzheimer's disease, Biomarker, CANTAB, mild cognitive impairment due to AD
**Disclosures:** Nothing to disclose.

### M42. Chemogenetic Suppression of Neural Activity in Dorsolateral Prefrontal Cortex Impairs Spatial Working Memory in Rhesus Monkeys

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**Background:** The DREADD (Designer Receptors Exclusively Activated by Designer Drugs) methodology allows for expression of an artificial receptor system in an anatomically and/or neurochemically defined population of neurons. These designer receptors are derived from human muscarinic G-protein coupled receptors, allowing for physiologically relevant manipulations in activity. DREADDs remain quiescent until activated by an otherwise inert pharmacological agent, clozapine-N-oxide (CNO). This system has tremendous potential for interrogating the functions of neural circuits in the nonhuman primate brain.
**Methods:** We placed multiple injections of AAV5 vector containing a construct for the inhibitory hM4Di DREADD receptor, under control of the human synapsin promoter, bilaterally into the dorsolateral prefrontal cortex of male

rhesus monkeys. Monkeys performed the delayed response test of spatial working memory in a manual test apparatus.
**Results:** Task performance was unaffected by the surgery or by vehicle injections, but was dramatically impaired by intramuscular injection of CNO. Functional DREADD receptor expression lasted at least a year postsurgery and was verified histologically. The performance of monkeys that had not received DREADD AAV injections was unimpaired by CNO. The effect of CNO on task performance in monkeys that had received DREADD AAV injections into dorsolateral PFC was dramatic, resulting in near-chance levels of task performance under CNO.
**Conclusions:** These initial observations support the effectiveness of this chemogenetic system for neurobiological investigations in macaque monkeys, and open new experimental modalities in this species.
**Keywords:** DREADD, dorsolateral prefrontal cortex, monkey, working memory
**Disclosures:** Nothing to disclose.

### M43. The Alzheimer's Prevention Initiative Genetic Testing, Disclosure and Counseling Program

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**Background:** The Alzheimer's Prevention Initiative (API) was established to evaluate preclinical Alzheimer's disease (AD) treatments in cognitively unimpaired people who, based on age and genetic background, are at imminent risk for developing symptoms of AD. The ongoing API Autosomal Dominant AD is enrolling mutation carriers and non-carriers so as to not require disclosure of mutation status. By contrast, the API APOE4 Trial, if approved by health authorities, will only enroll apolipoprotein E (APOE) e4 homozygotes whose genotype will be disclosed. In order to support this trial and later ones, the API established an APOE Genetic Testing and Counseling Program (GTCP), which will define the manner in which many thousands of people will undergo genetic testing and disclosure. We believe that this program will define the paradigm for future Alzheimer's prevention trials requiring genetic disclosure, a practice common in other disease states but new to this research space.
**Methods:** The progress of GTCP thus far includes (1) 2 online studies of attitudes toward and responses to APOE genetic testing and disclosure, using the Alzheimer's Prevention Registry (with 130,000 members to date); (2) a pilot study of APOE genotyping using saliva collection tubes sent via US Postal Service; (3) establishment of the interdisciplinary GTCP Committee, which has defined the genetic testing and counseling program based in part on review of best practices in other diseases, and (4) updating lifetime and relative risk estimates for developing mild cognitive impairment (MCI) or AD dementia by APOE genotype using data from multiple cohort studies: Framingham Heart Study, National Alzheimer's Coordinating Center, Rotterdam Study, and the Sacramento Area Latino Study on Aging.
**Results:** Online studies of attitudes regarding genetic testing and disclosure indicated that a majority of online Prevention Registry members queried would be willing to participate in a program of this nature, structured as follows. APOE genotyping will be conducted through the Alzheimer's Prevention Registry (www.endALZnow.org) using a buccal swab kit sent via mail, a method shown to be feasible based on our pilot study. APOE4 homozygotes and a random sample of non-homozygotes who consent and appear to meet basic trial eligibility criteria will be invited to trial sites for additional screening and disclosure of APOE genotype and associated risk of developing symptoms due to AD. Given limited availability of genetic counselors specializing in AD, we will evaluate outcomes of two remote post-test counseling approaches in a randomized sub-study of telephone versus real-time videoconference counseling, as well as longitudinal outcomes of APOE disclosure including psychological, behavioral and cognitive effects in all genetic groups.
**Conclusions:** The GTCP is a key element of the API program, facilitating the enrollment into the API APOE4 Trial and establishing processes for clearly and safely communicating genetic information. We anticipate that the program will also identify a large pool of prospective participants for future trials. Results from the counseling program will provide crucial information regarding the implementation and delivery of APOE genotype results relevant to clinical practice and precision medicine, as well as lessons learned for future trials in genetically-enriched populations.
**Keywords:** genetic, Prevention of Alzheimer's disease, Alzheimer's
**Disclosures:** Nothing to disclose.

### M44. Prenatal Exposure to Toxoplasmosis and Risk for Childhood Autism

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**Background:** Several maternal infections during the prenatal period have been associated with neurodevelopmental disorders, such as childhood autism. However, the association with autism and Toxoplasma gondii remains unclear. The authors examined whether serologically confirmed maternal exposure to toxoplasmosis and the degree of exposure is associated with risk of childhood autism in offspring.
**Methods:** The study is based on a nested case-control design of a large national birth cohort (N = 1.2 million) and the national psychiatric registries in Finland. There were 771 cases of childhood autism and controls matched 1:1 on date of birth, sex, birthplace and residence in Finland. Maternal sera were prospectively assayed from the national serum biobank for antibody titers to T. gondii IgG and IgM to quantify exposure to this parasite.

**Results:** Exposure to maternal Toxoplasma gondii IgM antibody was associated with a significantly decreased risk of childhood autism. Maternal IgG antibody at the equivocal level relative to the unexposed level was associated with significantly increased risk of childhood autism. Maternal IgG antibody in seropositive subjects, overall and by tertile, relative to the unexposed, was not significantly associated with risk of childhood autism.
**Conclusions:** The findings suggest that recent maternal exposure to Toxoplasma gondii indicative of higher IgM antibody levels is related to a decrease in offspring risk for childhood autism. Past maternal exposure to Toxoplasma gondii indicative of equivocal IgG antibody levels was associated with an increased risk of childhood autism. Based on our findings, we posit two mechanisms to account for the associations: an inadequate maternal Toxoplasma gondii IgM response to acute infection and insufficient control of latent infection due to low circulating maternal Toxoplasma gondii IgG levels.
**Keywords:** autism Spectrum Disorders, prenatal, toxoplasmosis
**Disclosures:** Nothing to disclose.

### M45. Age-Related Differences on Cognitive Functions of Children and Adolescents with Typical and Atypical Development: Results from a Large Community-Based Study

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**Background:** To understand age-related differences in cognitive functions in children with typical and atypical development may shed light on disrupted brain mechanisms that underlie psychiatric disorders in children and adolescents. Our objective here is threefold. First to investigate age-related differences on six domains of cognitive functions. Second, to look for differences in cognitive functions among four common groups of mental disorders in childhood. Third, to investigate whether age related differences may vary among diagnostic groups.
**Methods:** This study is part of the 'High Risk Cohort Study for Psychiatric Disorders in Childhood' (HRC). Data from 2153 subjects (6-14 years old) was available from the HRC for this study The Development and Well Being-Behavior Assessment (DAWBA) was used to classify subjects into five groups: Typical Developing Controls, TDC ($n = 1780$), fear ($n = 105$), distress ($n = 74$), Attention-Deficit/Hyperactivity Disorder (ADHD; $n = 140$), Oppositional Defiant/Conduct (ODD/CD; $n = 54$). All comorbid cases were excluded from the analysis. A cognitive model was constructed using 13 tasks evaluating declarative memory, processing efficiency, speed-accuracy trade-off, working memory, inhibitory control and temporal processing. Factor scores from each model were saved as observed variables and each hypothesis was investigated with a series of general linear models. First, we investigated linear, quadratic and cubic effects of age on each neurocognitive function selecting only TDC. Second, we tested diagnosis main effects using the TDC as a single contrast. Lastly, we investigate diagnosis by age interactions. The institutional review board of the University of São Paulo approved the study.
**Results:** First, age-related associations for memory fitted a quadratic model ($r2 = 0.180$), whereas speed-accuracy trade off ($r2 = 0.204$) and inhibitory control ($r2 = 0.223$) fitted a cubic model, representing a decrease in the strength of the association between age and each domain with increasing age (plateau around 12-13 years of age). Processing efficiency ($r2 = 0.054$), working memory ($r2 = 0.084$) and temporal processing ($r2 = 0.111$) showed linear relationships with age. Second, omnibus tests showed that the neurocognitive domains differ among diagnostic groups. Post-hoc ANOVAs and simple contrasts with TDC showed that only the ADHD group showed lower performance if compared to TDC in all neurocognitive domains (all p-values $< 0.05$). No other psychopathological group (except for ADHD) differed from TDC in any neurocognitive domain. Lastly, three diagnosis by age emerged from the analysis for three neurocognitive domains: processing efficiency, speed-accuracy trade off and inhibitory control. Stratified analysis by diagnostic groups reveled that processing efficiency was only associated with age in TDC, but not in any other psychopathological group. For inhibitory control, all children showed an association between inhibitory control and age, except for children with distress disorders. For speed accuracy trade off, the internalizing groups (fear and distress) showed a lower slope whereas externalizing groups (ADHD and odd/conduct) show a higher slope after adolescence.
**Conclusions:** As expected, cognitive functions improve with increasing age, but show somewhat distinct age-related differences. ADHD seems to be impaired in several neurocognitive functions that are intact in other common mental disorders. The emergence of age by diagnosis interactions revealed that some deficits might be present in specific developmental periods. These observations strengths the notion of time-sensitive periods for both the malicious effects of risk factors and for the beneficial effects of preventive strategies.
**Keywords:** Cognition, Developmental Psychopathology, epidemiology
**Disclosures:** Nothing to disclose.

### M46. Preclinical Evidence to Demonstrate that Lisdexamfetamine Prevents Impulsivity in Binge-Eating

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**Background:** Binge-eating disorder (BED) is a common psychiatric condition, affecting $\sim 2\%$ of the adult population and presents as the frequent, compulsive, excessive consumption of highly palatable foods. We have recently developed and pharmacologically characterized a rat model of BED in which rats that are freely-fed on standard chow are given irregular, limited access to chocolate along with normal chow. Over a period of 4 weeks, the rats develop robust, binge-eating (BE) of the chocolate with concomitant

reductions in their consumption of normal chow. Body weights remained at the same level as control rats maintained on normal chow. We have, therefore, proposed that this paradigm models BED without obesity (Vickers et al, 2013). Given that impulsivity and a loss of inhibitory control are important factors in BED (Schag et al, 2013) and that lisdexamfetamine (LDX), a prodrug of d amphetamine, was recently approved for the treatment of moderate to severe BED, we have now investigated whether BE rats show impulsive responding in a delay-discounting test and the influence of LDX on this behaviour.

**Methods:** Forty-two, adult, female, Wistar rats were given continuous access to chow and water and trained to lever press for chocolate pellets in a delay discounting procedure. One lever was assigned to deliver a single chocolate pellet while the other delivered a larger 3 pellet reward with increasing delays, ie 0, 4, 8, 16, 32 sec. The rats were then divided into 2 groups, ie BE rats that were given intermittent access to chocolate over 28 days and non-binge (NB) controls that were presented with an empty pot on these occasions. Both groups of rats were tested in the delay-discounting test. LDX (0.8 mg/kg po [d amphetamine base]) was evaluated in the BE rats. Results are reported for the cohorts of rats which met the acceptance criterion of $>70\%$ responding on the 3 pellet lever during the first block of the final two baseline delay discounting trials. Results are mean ± SEM ($n = 8$-12 rats/group).

**Results:** At Days 17/18 part way through the establishment of binge eating, both the BE and NB groups of rats showed an incrementally decreasing preference for lever pressing to receive the larger delayed food rewards as the delay interval was increased from 0 32 sec. However, the BE rats responded significantly less on the 3 pellet lever than the NB controls at the 16 sec delay interval (NB = 38.9 ± 6.7 vs BE = 23.5 ± 5.7, $p<0.05$). In addition, when the percentage choice for the 3 pellet lever was expressed as an average over the entire test session (including block 1: no delay), there was a significant reduction in preference for larger delayed food reward lever when the BE rats were compared against the NB controls (NB = 46.1 ± 3.4 vs BE = 37.3 ± 4.1, $p<0.05$).

Animals were re-tested when bingeing was fully established in the BE group. The NB control rats showed a decreasing preference for the larger 3 pellet reward as the delay was increased [% responses on 3 pellet lever: 0 sec = 66.5 ± 7.9; 4 sec = 45.3 ± 9.8; 8 sec = 30.4 ± 9.1; 16 sec = 32.3 ± 7.4; 32 sec = 25.5 ± 9.5; overall = 39.4 ± 7.9]. BE rats showed significantly lower preference for the larger reward at several delay intervals [% responses on 3 pellet lever: 4 sec = 19.5 ± 4.1, $p<0.05$; 8 sec = 10.7 ± 4.1, $p<0.05$; overall = 19.6 ± 3.6, $p<0.05$; all significances vs NB controls). LDX increased the percentage responding by BE rats for the larger, delayed 3 pellet rewards at almost all delay intervals [% responses on 3 pellet lever: 4 sec = 38.2 ± 7.5, $p<0.01$; 8 sec = 42.5 ± 9.5, $p<0.001$; 16 sec = 28.0 ± 7.2, $p=0.052$; 32 sec = 28.4 ± 7.6, $p<0.05$; overall = 35.2 ± 5.4, $p<0.001$; all significances vs vehicle-treated BE group]. The possibility that reduced appetite and/or increased satiety played a role in LDX's effects on operant responding for chocolate flavoured, sucrose pellets in the delay discounting test can be disregarded because the BE rats treated with LDX (0.8 mg/kg po) consumed the same number of food reward pellets as the vehicle treated BE rats (Vehicle/BE = 48.7 ± 2.6 vs LDX/BE = 51.7 ± 3.5).

**Conclusions:** BE rats showed an unequivocal lack of tolerance to delay for larger chocolate rewards in the delay-discounting task when compared against NB controls. This intolerance of delayed reward resulted in BE rats preferring to press for the smaller immediate reward. The impulsive responding of BE rats was abolished by LDX pretreatment demonstrating its ability to increase inhibitory control in BE rats when given access to chocolate.

**Keywords:** rat, impulsivity, lisdexamfetamine

**Disclosures:** P H Hutson is an employee and holds shares and share options in Shire Pharma.

**References:**

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## M47. A Double-Blind, Placebo-Controlled, Randomized-Withdrawal Study of Lisdexamfetamine Dimesylate in Adults with Moderate to Severe Binge Eating Disorder

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**Background:** Lisdexamfetamine dimesylate (LDX) is approved in the United States for the treatment of adults with moderate to severe binge eating disorder (BED). In two 12-week, double-blind, placebo-controlled trials, dose-optimized LDX (50 or 70 mg) produced clinically meaningful and statistically greater reductions than placebo in binge eating days per week in adults with moderate to severe BED. Here, the maintenance of efficacy of LDX in adults with moderate to severe BED, as assessed by time to relapse, is reported.

**Methods:** This double-blind, placebo-controlled, randomized-withdrawal study consisted of a screening phase (up to 4 weeks); a 12-week open-label phase (4 weeks of dose optimization [week 1, 30 mg LDX; week 2, 50 mg LDX; weeks 3 and 4, 50 or 70 mg LDX] followed by 8 weeks of dose maintenance); a 26-week double-blind, randomized-withdrawal phase; and a 1-week follow-up visit. Adults (18–55 years of age) meeting Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision criteria for BED were eligible. Participants were required to have BED of at least moderate severity ($\geq 3$ binge eating days per week in the 14 days before open-label baseline) and a Clinical Global Impressions–Severity (CGI-S) score $\geq 4$ (at least moderately ill) at screening and open-label baseline. Efficacy endpoints, binge eating days per week assessed by clinical interview based on daily binge eating diaries and CGI-S scores, were assessed at all study visits. At the end of dose maintenance, protocol-defined LDX responders (participants reporting $\leq 1$ binge eating day per week for 4 consecutive weeks [28 days] and having CGI-S scores $\leq 2$

[borderline ill or less] at week 12) entered the double-blind phase and were randomized to placebo or continued treatment with dose-optimized LDX. The primary efficacy endpoint was time to relapse in the double-blind, randomized-withdrawal phase, with relapse defined as the occurrence of $\geq 2$ binge eating days per week for 2 consecutive weeks prior to the visit and an increase in CGI-S score of $\geq 2$ points from randomized-withdrawal baseline. Treatment differences in time to relapse were assessed in the full analysis set (FAS; participants taking $\geq 1$ study drug dose during the double-blind, randomized-withdrawal phase and having $\geq 1$ postrandomization CGI-S assessment) using a stratified log-rank test stratifying for 4-week cessation status (Yes/No). Safety and tolerability assessments included treatment-emergent adverse events (TEAEs) and vital signs.

**Results:** Of 418 enrolled participants, 275 responded to LDX (per protocol) and were randomized at the end of open-label treatment; 138 participants were randomized to placebo and 137 continued on dose-optimized LDX from the open-label phase. LDX demonstrated superiority over placebo ($P < 0.001$) for time to relapse in the FAS ($n = 267$) during the double-blind, randomized-withdrawal phase. Additionally, the proportion of participants who met relapse criteria was 3.7% (5/136) for LDX and 32.1% (42/131) for placebo. During open-label treatment, 82.2% (338/411) of participants reported TEAEs (serious TEAEs, $n = 3$; TEAEs leading to discontinuation, $n = 22$). TEAEs reported by $>5\%$ of participants during open-label treatment were dry mouth (33.8%), headache (16.1%), and insomnia (11.2%), decreased appetite (9.2%), nausea (8.5%), anxiety (7.1%), constipation (6.8%), hyperhidrosis (5.6%), feeling jittery (5.1%), and diarrhea (5.1%). Mean (SD) changes from open-label baseline in systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse, respectively, during open-label treatment were 1.14 (9.937) mmHg, 1.79 (7.532) mmHg, and 6.64 (9.948) bpm at week 12/early termination (ET). During the double-blind, randomized-withdrawal phase, 46.3% (62/134) of placebo and 60.3% (82/136) LDX participants reported TEAEs (serious TEAEs: placebo [$n = 0$], LDX [$n = 2$]; TEAEs leading to study discontinuation: placebo [$n = 0$], LDX [$n = 6$]). TEAEs reported by $\geq 5\%$ of participants in either treatment group during the double-blind, randomized-withdrawal phase were nasopharyngitis (placebo, 6.7%; LDX, 9.6%), headache (placebo, 6.7%; LDX, 8.8%), upper respiratory tract infection (placebo, 3.7%; LDX, 8.1%), dry mouth (placebo, 1.5%; LDX, 5.1%), and fatigue (placebo, 5.2%; LDX, 2.9%). Mean (SD) changes from open-label baseline in SBP, DBP, and pulse (placebo vs LDX) during the double-blind, randomized-withdrawal phase were –0.28 (9.643) vs 2.07 (9.960) mmHg, 0.38 (7.883) vs 0.85 (7.232) mmHg, and 1.96 (9.501) vs 6.63 (9.423) bpm at week 38/ET.

**Conclusions:** This randomized withdrawal study demonstrated that, following initial response to treatment with LDX, the risk of relapse to binge eating over a 6-month period was markedly lower in those continuing treatment with LDX compared with placebo. In this study the safety and tolerability profile was generally consistent with previous LDX studies in adults with moderate to severe BED.

**Keywords:** binge-eating disorder, eating disorders, randomised clinical trial, Lisdexamfetamine
**Disclosures:** This study was sponsored by Shire.
J. Hudson, consulting fees and grant support from Shire; consulting fees from Genentech, Pronutria, Roche, and Sunovion, and has also received grant support from Genentech.
S. McElroy, consultant to and grant support from Shire, consultant to or member of the scientific advisory boards of Alkermes, Bracket, Corcept, F. Hoffmann-LaRoche Ltd, MedAvante, Myriad, Naurex, Novo Nordisk, Sunovion, and Teva; grant support from the Agency for Healthcare Research & Quality (AHRQ), Alkermes, AstraZeneca, Cephalon (now Teva), Forest, Lilly, Marriott Foundation, National Institute of Mental Health, Orexigen, Pfizer, Takeda, and Transcept. She is also an inventor on United States Patent No. 6,323,236 B2, Use of Sulfamate Derivatives for Treating Impulse Control Disorders and, along with the patent's assignee, University of Cincinnati (Cincinnati, OH), has received payments from Johnson & Johnson, which has exclusive rights under the patent.
C. Cornwell, J. Radewonuk, and M. Gasior are employees of Shire and hold stock and/or stock options in Shire.

## M48. Reduced Expression of GAD65/67 Mrna and Dopamine D1 and D2 Receptors in Binge-Eating Rats

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**Background:** Binge-eating disorder (BED) is a common psychiatric condition, affecting $\sim 2\%$ of the adult population and presents as the compulsive, excessive consumption of highly palatable foods. We have recently developed a model of BED in which rats are given irregular, limited access to chocolate along with normal chow. Over a period of 4 weeks, rats develop robust, binge-eating (BE) of the chocolate with concomitant reduction in their intake of normal chow. In addition, BE rats showed increased impulsive behaviors. A recent study has demonstrated reduced glutamate decarboxylase (GAD65/67) expression in the nucleus accumbens of rats selected for high impulsivity. Therefore in this study, we quantified the expression of glutamate decarboxylase GAD65 and GAD67 mRNAs using in situ hybridization as well as dopamine (DA) D1 and D2 receptor levels using autoradiography in frontal cortex, nucleus accumbens and caudate putamen of binge-eating and control rats.

**Methods:** Adult, female, Wistar rats were given irregular, limited access to chocolate over 28 days and non-binge (NB) controls were presented with an empty pot on these occasions. Access to normal chow and water was provided throughout the study period. Brains were collected from BE and NB control rats ($N = 8$/group). In situ hybridization: Sections were fixed in 4% paraformaldehyde, dehydrated in 100 % EtOH, and dried at room temperature. Two 49-base-long sequences from the rat GAD65 or GAD67 mRNA sequence were chosen to avoid regions with known homology to other mRNAs and regions with greater than expected homology to rat structural RNA. Probes (5pmol)

were labeled by 3'-tailing of 35S-dATP (1250 Ci/mmol,) with terminal deoxynucleotidyl transferase in a hybridization buffer. Sections were incubated overnight at 37°C, washed and air-dried then quantified using an image analyzer (MCID-M4) and [14C] standards. Data was expressed as fmol/mg tissue. Receptor autoradiography: D1 receptors were determined with 1 nM [3H]SCH-23390 and 100 nM ketanserin to mask 5HT2A/2C receptors. Non-specific binding (NSB) was determined with 1μM cis-fluphenthixol. D2 receptors were determined with 1nM [3H] nemonapride and 0.5μM DTG and 0.1uM pindolol to mask sigma1/2 receptors and 5HT1A receptors. NSB was determined with 10μM sulpiride. Radiolabeled slides and [3H] standards were exposed to Kodal Biomax MR films then quantified. The amount of ligand bound within each area was expressed as fmol bound/mg tissue.

**Results:** Chocolate consumption in the BE rats increased markedly over the 28 day period. GAD65 mRNA expression was significantly reduced in medial prefrontal cortex (MPC) (by 20%), dorsal frontal cortex (DFC) (21%), nucleus accumbens-core (NAc-C) (33%) and -shell (NAc-S) (34%) in BE vs. control rats (all $p < 0.05$). Similarly, GAD67 mRNA expression was significantly reduced in MPC (18%), DFC (20%), NAc-C (34%) and NAc-S (33%) of BE vs. control rats (all $p < 0.05$). There were no changes in expression of either GAD65 or GAD67 mRNA in caudate putamen-medial (CP-M) or -lateral (CP-L) of BE vs. control rats. DA D1 receptor expression was significantly reduced in CP-M (22%) and CP-L by (23%) ($p < 0.05$). No significant differences in D1 receptors were observed in cortex or NAc of BE vs. control rats. DA D2 receptors were significantly reduced in NAc (by 31%), CP-M (by 33%) and CP-L (by 35%) of BE vs control rats (all $p < 0.05$).

**Conclusions:** These results provide the first evidence that the expression of GABA biomarkers (GAD65 and GAD67 mRNA) are reduced in select brain regions of BE rats. Furthermore when assayed at a single concentration of radioligand, BE was also associated with a reduction of DA receptor levels in the CP and NAc. These findings suggest that alterations in GABA and DA neurotransmission in the frontal cortex, NAc and CP may contribute to the impulsive behaviors observed in BED patients.

**Keywords:** Binge-eating disorder, Dopmaine Receptors, GAD65/67 mRNA

**Disclosures:** Study partially funded by Shire Pharmaceuticals. FT received grant support from Shire, Lundbeck and Allergan. JG and DH are employees of RenaSci Ltd. PH is an employee of Shire Pharmaceuticals

### M49. Women Remitted from Anorexia Nervosa have Aberrant Baseline Cerebral Blood Flow in Gustatory and Homeostatic Neural Circuitry in Response to Hunger

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**Background:** The motivation to eat in humans is a complex process influenced by intrinsic mechanisms relating to hunger and satiety, and extrinsic mechanisms based on the appetitive incentive value of food. Eating disorders defy homeostatic drives, suggesting that pathological eating may result from a disruption in these mechanisms. Anorexia nervosa (AN) is characterized by severe food restriction leading to significantly low body weight along with an intense fear of gaining weight and distorted body image. Recent evidence suggests decreased sensitivity to the motivational drive of hunger may explain the ability of individuals with AN to restrict food when emaciated, and may implicate dysfunction of the homeostatic control system in AN. Cerebral blood flow (CBF) is tightly coupled with metabolism underlying cognition by increasing local delivery of oxygen and glucose to support neural function, and is an indirect marker of neuronal function. The purpose of this study was to explore whether women remitted from restricting-type AN (RAN) have altered CBF in response to hunger or satiety that may indicate homeostatic dysregulation contributing to appetite disruption and food restriction.

**Methods:** We compared resting CBF in 21 RAN (13 pure restricting subtype, 8 restricting-purging subtype) and 16 demographically matched healthy women (CW) when hungry (after a 16 hour fast) and when satiated (after being fed 30% of daily caloric needs). Participants completed a resting-state whole-brain pulsed arterial spin labeling (ASL) MR scan on 2 visits 24 hours apart. We examined remitted subjects to avoid the confounding effects of malnutrition on neural function. To determine whether CBF differed between the groups based on homeostatic state, we employed a Group (RAN, CW) × Visit (Hungry, Satiated) LME analysis in R. Regions of interest (ROIs) associated with homeostatic regulation were based upon prior findings and included the anterior cingulate cortex (ACC) (covering both subgenual, rostral and dorsal subregions of the ACC), limbic circuitry (including amygdala, ventral striatum, dorsal anterior caudate) and insula. Each ROI was treated as a search region. Small volume correction was determined with Monte-Carlo simulations, giving an a posteriori ROI-wise of $p < 0.05$ for all comparisons.

**Results:** The right ventral striatum, left caudate, right subgenual ACC, left rostral ACC, and left posterior insula demonstrated a significant Group x Visit interaction. Post-hoc within-group analyses demonstrated that, as expected, CW showed greater CBF when hungry relative to when satiated in the right ventral striatum and subgenual ACC. In contrast, RAN showed decreased CBF when hungry relative to when satiated in the left caudate, left rostral ACC, and left posterior insula. In terms of between group findings, RAN demonstrated greater CBF than CW when satiated in the left rostral ACC. A main effect of group was also detected. Within the right ventral striatum, RAN had decreased CBF relative to CW. There was a main effect of visit for the limbic and anterior cingulate ROIs, with elevated CBF when hungry relative to when satiated.

**Conclusions:** This represents the first study to show that women remitted from AN have aberrant baseline neural function in gustatory and homeostatic neural circuitry in response to hunger. Using pulsed ASL, we reproduced previous findings that found that hunger in CW was associated with increased CBF in regions involved in appetite regulation, including the right ventral striatum, bilateral subgenual ACC, and left posterior insula. In

contrast, RAN showed reduced CBF when hungry relative to when satiated in similar regions, including the vmPFC (bilateral subgenual ACC, left rostral ACC) and left posterior insula. Simply put, regions involved in homeostatic regulation were less active when hungry in RAN suggesting an insensitivity to the influence of hunger. Altered CBF response to hunger in RAN may reflect a neuronal metabolic support deficit associated with homeostatic regulation and contributing to appetite dysregulation that may explain disordered eating in AN. A better understanding of physiological changes in AN in response to hunger and satiety could provide a brain-specific marker relevant to treatment and outcome.

**Keywords:** anorexia nervosa, Cerebral Blood Flow, hunger, energy homeostasis

**Disclosures:** Nothing to disclose.

### M50. Structural and Functional Brain Development in Adolescents with Bulimia Nervosa

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**Background:** Our cross-sectional imaging findings suggest that self-regulatory capacities are impaired individuals Bulimia Nervosa (BN) due to functional and anatomical disturbances in inferior frontal cortices that arise early in the course of the illness and seem to persist into adulthood. Herein, we used longitudinal MRI data to determine if these circuit-based abnormalities indeed contribute to the development and persistence of BN symptoms over adolescence.

**Methods:** Structural and functional MRI data were acquired at baseline from 34 adolescents with BN (16.41 +/- 1.5 years of age) and 32 age- and BMI-matched healthy adolescents (16.13 +/- 2 years). Follow-up data were then acquired from both groups within 2-year intervals over adolescence. After quality control, structural data at two time points (T1 and T2) were available for 23 BN and 18 healthy adolescents; fMRI data meeting quality control at T1 and T2 were available from 21 BN and 16 healthy adolescents during their performance of the Simon Spatial Incompatibility Task, a measure of cognitive control and conflict resolution. Structural data were processed in Freesurfer and fMRI data were processed and analyzed in SPM8. General linear models were used to assess change in cortical thickness and patterns of brain activation in response to conflict stimuli (Simon task) across groups, over time.

**Results:** The frequency of binge-eating episodes and vomiting episodes (or other compensatory behaviors to avoid weight gain) decreased over two years in the BN group, but not all adolescents achieved clinical remission. A whole-brain analysis revealed that thinning of right prefrontal regions (caudal mid frontal, pre and post central gyri) increased at a faster rate over adolescence in the BN compared to HC groups ($p = 0.01$, uncorrected). In addition, whereas activation of frontal cortices during correct responses to conflict stimuli on the Simon task increased over time in the healthy adolescents, activation of fronto-parietal regions decreased in the BN group, regardless of changes in symptom severity.

**Conclusions:** These new longitudinal findings from adolescents with BN suggest that faster frontal cortical thinning during adolescence coincides with deficient activation of fronto-parietal circuits in the service of regulatory control. Thus, abnormal trajectories of structural and functional development within frontal cortices may contribute to the persistent inability of adolescents with BN to engage control over eating behaviors. We are currently acquiring T3 follow-up data from our adolescent participants and conducting linear mixed effect models on these structural and fMRI datasets to further elucidate how changes in brain structure and function over adolescents contribute to BN psychopathology.

**Keywords:** bulimia nervosa, Human Neuroimaging, longitudinal, Brain development

**Disclosures:** Nothing to disclose.

### M51. A Double-Blind, Placebo-Controlled Trial of N-Acetyl Cysteine in the Treatment of Skin Picking Disorder

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**Background:** Skin picking disorder is characterized by repetitive picking that results in skin lesions. Data on the pharmacological treatment of skin picking disorder are limited to conflicting studies of serotonergic medications. N-acetyl cysteine (NAC), an amino acid, appears to restore extracellular glutamate concentration in the nucleus accumbens and therefore offers promise in reducing compulsive behavior.

**Methods:** Sixty-six individuals (57 women; mean age = 34.7 ± 11.0) with skin picking disorder were randomized to NAC (dosing ranging from 1200mg/day to 3000mg/day) or placebo in a 10-week, double-blind, placebo-controlled trial. Participants were assessed with measures of skin picking severity and selected cognitive tasks. Outcomes were examined using a linear mixed-effects model without any ad hoc imputation.

**Results:** Participants assigned to NAC had significantly greater reductions in skin picking symptoms as measured by the clinician-administered Yale Brown Obsessive Compulsive Scale Modified for Neurotic Excoriation ($p = .008$) and the self-report Skin Picking Symptom Assessment Scale ($p = .029$). At study endpoint, 47.1% of participants were "much or very much improved" on NAC compared to 19.2% on placebo ($p = .031$). There were no significant differences, however, in terms of psychosocial functioning. In addition, those taking NAC demonstrated significant improvement on a cognitive task of motor impulsivity at study endpoint.

**Conclusions:** This study found that NAC demonstrated statistically significant reductions in skin picking symptoms and was well tolerated. The glutamate system may prove a beneficial target in treating compulsive behaviors.

**Keywords:** glutamate, compulsivity, impulsivity

**Disclosures:** Nothing to disclose.

## M52. Internet Addiction: A Meaningful Disorder? Associations with Impulsivity and Compulsivity in a Large-Scale International Study

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**Background:** The last decade has seen an explosive growth in internet use globally, including for potentially addictive behaviors such as gambling, gaming, use of social media, and viewing pornography. Internet addiction, first proposed as a discrete entity in 1995, is associated with functional impairment, with an average of 39h/week being spent on computers; several deaths have been reported due to cardiopulmonary collapse resulting from inactivity. In DSM-5, Internet Gaming Disorder was highlighted as condition warranting more research before being considered for inclusion. Research into the validity of Internet Addiction (and its analogues), including relationships with other impulsive and compulsive behaviours and syndromes, is lacking.
**Methods:** Adult individuals were recruited using media advertisements at three sites (USA, South Africa, UK), and completed an online questionnaire to quantify (i) problematic internet use (Internet Addiction Test, IAT); (ii) presence of certain mainstream putatively related psychiatric disorders (obsessive-compulsive disorder, OCD; attention-deficit hyperactivity disorder, ADHD); and (iii) questionnaire based measures of impulsivity and compulsivity (Barratt Impulsiveness Scale, BIS-11; Adult ADHD Rating Scale, ASRS; Padua Inventory Revised). Prevalence of problematic internet use was quantified, and its relationship with recognised impulsive/compulsive psychiatric disorders was explored using chi-square. Binary logistic regression and machine learning (Random Forrest approach) were used to identify strength of associations between problematic internet use and questionnaire based measures of impulsivity/compulsivity.
**Results:** 2503 subjects were recruited, of whom 500 (19.5%) had moderate maladaptive internet use and 35 (1.4%) had severe maladaptive internet use. Maladaptive internet use was associated with significantly elevated risk of OCD ($p < 0.001$; Odds Ratio 2.7 [95% confidence interval 2.1-3.6]), and ADHD ($p < 0.001$; OR 3.0 [2.4-3.8]). In logistic regression, maladaptive internet use was most strongly associated (in descending order of statistical significance) with age ($Z = 5.026$), ADHD symptom severity (ASRS, $Z = 4.562$), Padua 'impulses to harm self/others' ($Z = 4.214$), Padua 'checking compulsions' ($Z = 3.544$), and Barratt Motor Impulsiveness ($Z = 3.373$) (all $p < 0.0001$). These findings were supported by machine learning. Area under the curves for the regression and machine learning models were 0.82 and 0.84 respectively.
**Conclusions:** Problematic internet use appears to be relatively common in moderate forms at the population level, and is associated with elevated impulsive and compulsive formal psychiatric disorders (ADHD, OCD) and questionnaire-based measures. Internet use disorder clearly merits further clinical and research scrutiny, to clarify how best it should be categorized, and treated.
**Keywords:** impulsivity, compulsivity, internet
**Disclosures:** Nothing to disclose.

## M53. Low Coverage Whole Genome Sequencing of a Native American Community Sample Reveals Single Nucleotide Polymorphisms near CTNNA2 Associated with Impulsivity

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**Background:** Impulsivity is a personality trait characterized by acting suddenly in an unplanned manner without consideration for the consequences of such behavior. Several psychiatric disorders include the term impulsivity as a criterion and it has been suggested that impulsivity may be a phenotype that links a number of different behavioral disorders, including substance abuse. Native Americans experience some of the highest rates of substance abuse of all US ethnic groups. The present set of analyses used data from a low coverage whole genome scan to conduct a genome-wide association study of an impulsivity phenotype in an American Indian community sample.
**Methods:** Demographic and clinical information were obtained using a semi-structured interview ($n = 658$). Impulsivity was assessed using a scale derived from the Maudsley personality inventory. Blood samples were collected for DNA. Genotypes were imputed from low pass sequencing data, and were demonstrated to produce reliable genotypes compared to typed polymorphisms. The impulsivity score was tested for association with each variant adjusted for demographic variables, and corrected for ancestry and kinship, using EMMAX. Simulations were conducted to calculate empirical p-values.
**Results:** Genomewide significant findings were observed for a variant 50 kb upstream from catenin cadherin-associated protein, alpha 2 (CTNNA2), a neuronal specific catenin, in the REG gene cluster. CTNNA2 encodes for a cell-adhesion protein (alpha N-catenin) which has been shown to regulate synaptic plasticity, and is involved in the binding of cadherins and the actin cytoskeleton and as such is important for maintaining the stability of dendritic spines A meta-analysis of genome-wide association studies had previously identified common variants in CTNNA2 as being associated with excitement seeking.
**Conclusions:** The association between sequence variants in this area on chromosome 2 and impulsivity suggests a potential role in the genetic regulation of this phenotype in this population.
**Keywords:** catenin cadherin-associated protein, alpha 2, whole genome sequencing, Native Americans, impulsivity
**Disclosures:** Nothing to disclose.

## M54. In Vivo Evaluation in Monkey Brain of the COX-1 and COX-2 Selective Positron Emission Tomographic Radioligands [11C]PS13 and [11C]MC1

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**Background:** Positron emission tomographic (PET) imaging of neuroinflammation has largely been restricted to studies

of translocator protein (TSPO), which transports cholesterol across the mitochondrial membrane and is highly expressed in activated microglia and reactive astrocytes. However, use of TSPO as a biomarker of neuroinflammation is limited because TSPO is highly expressed both in microglia (the major source of inflammatory mediators) and astrocytes (which can form scars and merely mark sites of former damage and inflammation). For this reason, we sought to develop a PET radioligand for cyclooxygenase-1 (COX-1), which is almost exclusively localized in microglia and whose expression is increased by neuroinflammation. In parallel with this effort, we sought to develop a radioligand selective for COX-2, which is expressed in both neurons and glia. Although COX-2 is known to play a much larger role than COX-1 in peripheral inflammation, the relative role of these two enzymes in brain is controversial.

**Methods:** We synthesized and screened > 50 compounds for inhibitory potency using in vitro enzymatic assays in whole blood from monkey and human. The leading candidates for COX-1 (PS13) and COX-2 (MC1) were radiolabeled with 11C. About 180 MBq of each radioligand was injected intravenously in rhesus monkeys. Dynamic PET scans of brain were acquired for two hours, concurrent with frequent sampling of arterial blood to measure the concentration of parent radioligand separated from radiometabolites. The density of the enzyme was measured as distribution volume (VT), which corrects for differences in cerebral blood flow and peripheral metabolism that may occur between scans or between animals.

**Results:** In vitro enzymatic assays in monkey and human blood showed that PS13 was potent and selective for COX-1 (IC50 = 1 nM) compared to COX-2 (IC50 > 1,000 nM). Conversely, MC1 was potent and selective for COX-2 (IC50 = 3 nM) compared to COX-1 (IC50 >1000 nM). Both [11C] PS13 and [11C] MC1 showed good uptake in monkey brain (peak concentrations of 3 – 5 SUV) and washed out relatively quickly (demonstrating that the binding was reversible, as expected). The measure of enzyme density, VT, was well identified from serial brain scans and from the concentrations of parent radioligand in arterial plasma. In addition, VT values were stable after about 70 min, suggesting that brain uptake was not contaminated by radiometabolites. To determine the percentage of brain uptake specifically bound to each enzyme, we intravenously injected pharmacological doses of drugs selective for COX-1 (either non-radioactive PS13 or ketoprofen as its methyl ester prodrug) and for COX-2 (non-radioactive MC1). From these studies, we estimated that specific binding as a percentage of total uptake was ~80% for [11C] PS13 and ~40% for [11C] MC1.

**Conclusions:** These two PET radioligands showed excellent imaging properties for selectively measuring COX-1 or COX-2 in monkey. The percentage of total brain uptake specifically bound to each target (~40 – 80%) was moderate to good at baseline but would be expected to markedly increase in inflammatory conditions. The COX-1 radioligand, [11C] PS13, should provide a selective measure of microglial activation and, thus, of active inflammation. The COX-2 radioligand, [11C] MC1, will help identify the relative roles of both COX enzymes in neuroinflammation. Used together, these two radioligands can assess the relative in vivo selectivity and brain entry of non-steroidal anti-inflammatory drugs, two areas for which relatively little information is available. Finally, both COX-1 and COX-2 are obvious therapeutic targets. Thus, in addition to being used as biomarkers of disease, these two PET radioligands can also measure target engagement of anti-inflammatory drugs and potentially monitor therapeutic response.

**Keywords:** Positron emission tomography, Inflammatory Markers, COX-1

**Disclosures:** Nothing to disclose.

## M55. Efficacy of Lurasidone in Bipolar Depression: Population Exposure-Response Relationships in Patients with Bipolar Depression

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**Background:** Bipolar disorder is a chronic illness characterized by recurrent episodes of depression and mania, and is associated with significant impairment in social and occupational functioning, and an increased risk of suicide. Depressive episodes typically predominate as the illness progresses. Optimizing treatment response may benefit from an understanding of dose-response relationships, which may be difficult to determine due to various confounds such as use of flexible dose designs, and cross-study differences in attrition rates, use of concomitant medications, and placebo response. The aim of this population exposure-response analysis was to characterize the dose-response profile for lurasidone in patients with bipolar depression.

**Methods:** The exposure-response analyses utilized statistical modeling and simulation based on data derived from two randomized, double-blind, placebo-controlled, flexible-dose, 6-week studies of monotherapy treatment with lurasidone (20-60 mg/d or 80-120 mg/d) or adjunctive therapy with lurasidone (20-120 mg/d) and lithium or valproate in patients meeting DSM-IV-TR criteria for bipolar I depression. The pooled data set (lurasidone and placebo treatment groups, combined) consisted of 825 patients who provided a total of 5,245 Montgomery-Åsberg Depression Rating Scale (MADRS) observations. Exposure measures evaluated as predictors of lurasidone and placebo effects (assessed as mean change in MADRS score) included lurasidone dose, area under the concentration-time-curve for the 24 hours postdose (AUC0-24); concentration at the time of dose prior to MADRS measurement (C0); and concentration at two hours after dosing (C2), which served as a surrogate for maximum plasma concentration (Cmax). Prespecified covariates that might influence response to lurasidone independent of drug exposure (ie, body weight, age, race, sex, smoking status, psychiatric history, US vs non-US residency, use of background or concomitant medications) were accounted for in the models. The dropout pattern may not have been missing completely at random and was likely to depend on the MADRS score; therefore, a model describing the probability of patient dropout over time also was developed.

**Results:** The time course of placebo effect on MADRS total score was adequately described by an exponential asymptotic placebo model. A linear dose-response model best described the effect of lurasidone across the therapeutic dose range of 20-120 mg/d. The mean net reduction in MADRS total score at the Week 6 (after adjusting for placebo) was estimated to be -3.5 (for lurasidone 20 mg/d), -3.8 (40 mg/d), -4.5 (60 mg/d), -5.1 (80 mg/d), -6.0 (100 mg/d), and -6.5 (120 mg/d). The dose-response relationship was consistent for lurasidone when administered as monotherapy, or as adjunctive therapy with lithium or valproate. Covariate effects were significant only for placebo effect parameters, therefore no adjustment was necessary based on demographic covariates, background, or concomitant medications.
**Conclusions:** This population exposure-response modeling analysis of lurasidone in patients with bipolar depression indicates that higher doses are likely to produce greater therapeutic effects. The linear dose response was consistent for both monotherapy and adjunctive therapy.
**Keywords:** lurasidone, atypical antipsychotics, Bipolar Disorder, Dose-response Analyses, Major Depressive Disorder
**Disclosures:** Sunny Chapel was working under a contract from Sunovion Pharmaceuticals Inc. for the performance of these analyses. All other authors are employees of Sunovion Pharmaceuticals Inc.

### M56. Efficacy of Lurasidone in Major Depression with Mixed Features: Pattern of Improvement in Depressive and Manic Symptoms

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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). 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 secondary analysis, we evaluated the effect of lurasidone on specific depressive and manic symptoms, based on Montgomery Asberg Depression Rating Scale (MADRS) and Young Mania Rating Scale (YMRS) items, in patients with MDD and subthreshold hypomania (mixed features).
**Methods:** Patients meeting DSM-IV-TR criteria for major depressive disorder, who presented with 2 or 3 protocol-specified manic symptoms, were randomized to 6 weeks of double-blind treatment with flexible doses of either lurasidone 20-60 mg/d ($N = 109$) or placebo ($N = 100$). Changes from baseline in the MADRS total, MADRS-6 core depression subscale, individual MADRS items, and total and individual items of the YMRS were analyzed using a mixed model for repeated measures analysis. Cohen's d effect sizes for lurasidone vs placebo were calculated for Week 6 change scores.
**Results:** Mean scores were similar at Baseline for lurasidone vs placebo on the MADRS total score (33.2 vs 33.3) and the MADRS-6 subscale score (21.6 vs 21.5). Lurasidone significantly improved depressive symptoms at Week 6 in the MADRS total score (LS mean change, - 20.5 vs -13.0; $P < 0.0001$; effect size, 0.80) and the MADRS-6 core depression subscale score (-13.0 vs -8.5; $P < 0.0001$; effect size, 0.72). Significant improvement on lurasidone was observed at Week 6 on all ten MADRS items. Effect sizes for the MADRS-6 core depression subscale items ranged from 0.40 to 0.78 at week 6 endpoint. The proportion of patients at Baseline who reported core manic symptoms were as follows: flight of ideas/racing thoughts (66.8%), pressured speech (61.1%), decreased need for sleep (40.8%), increased energy/activity (28.0%), elevated/expansive mood (18.0%), increased/excessive involvement in pleasurable activities (15.6%), and inflated self-esteem/ grandiosity (6.6%). "Nonspecific" symptoms of irritability, distractibility, and psychomotor agitation were reported at Baseline by 57.3%, 59.2% and 36.5% of patients, respectively. YMRS total scores at Baseline for lurasidone and placebo were 11.1 and 10.3, respectively. Treatment with lurasidone was associated with significantly greater LS mean change at Week 6 compared with placebo on the YMRS total score (-7.0 vs -4.9; $P < 0.0001$, effect size, 0.61). Effect sizes for the five YMRS items with a mean item severity score $> 1$ at Baseline ranged from 0.32 to 0.48 at week 6 endpoint (the remaining YMRS items had mean scores $< 1$ at Baseline).
**Conclusions:** In this study involving patients with MDD associated with mixed features, lurasidone was effective in treating a wide range of depressive and manic symptoms, as assessed by standardized scales.
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Sponsored by Sunovion Pharmaceuticals Inc.
**Keywords:** mood disorder, Major depression, atypical antipsychotic drug
**Disclosures:** (1) The study was funded by Sunovion Pharmaceuticals Inc.; (2) Drs. Tsai, Mao, Pikalov, and Suppes are employees of Sunovion Pharmaceuticals Inc.; (3) Dr. Nierenberg has received grant support or honoraria from or served as a consultant to Agency for Healthcare Research and Quality, American Foundation for Suicide Prevention, AstraZeneca, Belvoir Publications, Brain and Behavior Research Foundation, Brain Cells, Bristol-Myers Squibb, Corcept, Eli Lilly, Forest, Genentech, GlaxoSmithKline, Hoffman LaRoche, Marriott Foundation, MedAvante, Merck, Methylation Sciences, Naurex, NIMH, Pamlab, Patient-Centered Outcomes Research Institute, Pfizer, Ridge Diagnostics, Slack Publications, Sunovion, Takeda/Lundbeck, and Teva; he owns stock options in Appliance Computing (Mindsite), Brain Cells, and MedAvante; income is possible from Infomedic.com, but no revenue has been received to date; through Massachusetts General Hospital (MGH), he is named for copyrights to the Clinical Positive Affect Scale and the MGH Structured Clinical Interview for the Montgomery-Åsberg Depression Rating Scale, exclusively licensed to the MGH Clinical Trials Network and Institute; (4) Dr. Suppes has received funding, medications for clinical grants, consulting fees and/or travel expenses from: AstraZeneca, Elan Pharma International, H. Lundbeck A/S, Merck, NIMH, VA Cooperative Studies Program, Sunovion, and royalties from UpToDate and Jones and Bartlett (formerly Compact Clinicals).

### M57. Witnessing Social Defeat Stress Induces a Depression-Like Phenotype in Female c57BL/6 Mice

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**Background:** Stress exposure is a prevailing risk factor for the development of mood-related illnesses, wherein women represent the majority of those afflicted with depression-, anxiety-, and posttraumatic stress disorder. Despite the growing literature suggesting that affective disorders can arise after a traumatic event is vicariously experienced, this relationship remains to be thoroughly examined in females at the preclinical level. Thus, the objective of the current investigation is to assess whether the witness defeat stress behavioral paradigm (Biol Psychiatry, 73[1], 7-14; 2013) – a model that dissociates emotional versus physical stress – induces a depression-like phenotype in female mice.
**Methods:** Adult (8 week old) female c57BL/6 mice witnessed the social defeat bout of a male conspecific, by a larger CD1 aggressor, for 10 consecutive days. Twenty-four hr. after the last stress exposure, mice were tested in the social interaction, sucrose preference, and tail suspension tests. Additionally, we examined body weight-gain across days of witness defeat exposure, and levels of blood serum corticosterone 40 min after the last episode of stress.
**Results:** We show that when compared to non-stressed controls, female mice exposed to the witness defeat stress paradigm exhibit a depressive-like phenotype, as inferred from decreases in social behavior, decreased sucrose preference, and increased immobility in the tail suspension test. Furthermore, female mice witnessing social defeat stress displayed lower body weights across days of defeat, along with increased blood serum corticosterone levels.
**Conclusions:** Collectively, our data suggests that the witness defeat stress paradigm may be used to examine the etiology of vicarious stress-induced mood-related disorders in the female population.
**Keywords:** Female, Depression, Animal Model, Social defeat stress, Emotional stress
**Disclosures:** Nothing to disclose.

### M58. Low Serotonin 1B Receptor Binding Potential in the Anterior Cingulate Cortex in Drug-Free Patients with Recurrent Major Depressive Disorder

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**Background:** Globally, mood disorders, particularly major depressive disorder (MDD), represent the 4th major cause of disability and will become the 2nd by 2020. Current treatment options are not very effective: 30-40% of MDD patients fail to remit despite trying at least two different classes of antidepressant drugs. The development of new treatments is hampered by the fact that the pathophysiology of MDD is still not fully understood. Accumulating evidences imply that the 5-HT1B receptor is involved in the pathophysiology of MDD. In animal studies low 5-HT1B receptor mRNA in the Dorsal Raphe Nucleus in rats has been reported to predispose to learned helplessness, and 5-HT1B receptor binding is low in the Hippocampus of maternally-separated rats. Furthermore, in a recent Positron Emission Tomography (PET) study with the 5-HT1B receptor selective radioligand [11C] AZ10419369 an upregulation of 5-HT1B receptor binding in the Nucleus Accumbens and Ventral Pallidum has been reported in non-human primates after administration of ketamine. In humans, lower 5-HT1B mRNA expression in the Frontal Cortex and higher in the paraventricular nucleus of Hypothalamus has been demonstrated in suicide victims compared with controls. Finally, in a recent PET-study the [11C]P943 binding potential (BPND) in the Ventral Striatum was lower in depressed subjects when compared with controls.
In conclusion, there is need for a more detailed mapping of 5-HT1B receptors in the brain of patients with MDD. The aim of the study was to use PET to examine 5-HT1B receptor binding patients with MDD.
**Methods:** A case-control design was used. A volunteer sample of ten drug-free patients (washout period $>1$ month. For patients with a history of antidepressant medication, the medication-free period was $5.2 \pm 2.9$ years) with recurrent MDD, (MADRS mean 25.5, range 20-35), and control subjects pairwise matched for age (mean 48, range 25-70) and sex (6F, 4M) were recruited. At the Karolinska PET Center, a ECAT HRRT (Siemens Molecular Imaging) and the radioligand [11C] AZ10419369 was used for the PET experiments. Data were corrected for head motion using a frame-by-frame realignment algorithm. Coregistration with MRI data was performed using SPM5. Partial volume effects were corrected for according to the method developed by Melzer et al. The main outcome measure was [11C] AZ10419369 binding (BPND) calculated using the SRTM with the cerebellum as reference region. Based on reported relevance in the pathophysiology of MDD the following brain regions were hypothesized to be of interest: the Orobitofrontal Cortex, the Anterior Cingulate Cortex, the Subgenual Prefrontal Cortex, the Hippocampus, the Amygdala, the ventral Striatum, the Pallidum and the Dorsal Brainstem.
**Results:** In MDD the [11C] AZ10419369 binding potential was 25% lower in the Anterior Cingulate Cortex (ACC; $p = 0.003$), 20 % lower in the Subgenual Prefrontal Cortex (SGPFC; $p = 0.019$), and 45 % lower in the Hippocampus ($p = 0.029$) when compared with controls. For other regions in the brain, including the Ventral Striatum and Pallidum, there were no significant differences in BPND between patients and controls. The finding in ACC survived correction for multiple comparisons according to Bonferroni.
**Conclusions:** Evidences based on different methodologies point to the importance of the Anterior Cingulate Cortex in the pathophysiology of MDD. In physiological conditions, the rostral ACC is involved in emotional regulation, related to motivation and assignment of emotional valence to stimuli. In clinical fMRI studies abnormal activity in the ACC in subjects with MDD has been a common finding. These findings have contributed to the rationale for

reversible lesioning with deep brain stimulation, a treatment which has shown antidepressive effects. Thus, the present study adds to a line of evidences implicating a role for the anterior cingulate cortex in MDD.
[11C]AZ10419369 binding has been shown to be sensitive to endogenous ligand concentrations. The lower [11C] AZ10419369 BPND in the ACC of depressed patients compared with controls might thus reflect either higher serotonin concentration or lower 5-HT1B receptor density. The interpretation that MDD subjects have higher serotonin concentration than controls is not supported by post mortem data. In two studies of serotonin concentration in brain of depressed suicide victims and controls there was no elevation of the serotonin concentration in cortical regions or hippocampus. The hypothesis that the result reflects lower 5-HT1B receptor density in MDD subjects is however in line with the low mRNA concentration of the functionally related protein p11 reported for the ACC in patients suffering from MDD. The view that 5-HT1B receptor expression in the cell membrane is dependent on p11 has received consistent support from studies using brain tissue from p11 KO mice as well as immunohistochemistry and in situ hybridization, and co-expression of 5-HT1B receptors has been demonstrated in p11 containing cells in the Cingulate Cortex. Finally, significant correlations between 5-HT1B receptor and p11 mRNA have been found post-mortem in Frontopolar Cortex, Orbitofrontal Cortex and Hippocampus in depressed as well as control subjects. In conclusion, the results position 5-HT1B receptor binding as a putative biomarker for MDD and corroborate a role of the anterior cingulate cortex and associated areas in the pathophysiology of MDD.
**Keywords:** Serotonin 1b receptor, Major Depressive Disorder, PET, Biomarker
**Disclosures:** Nothing to disclose.

## M59. CYP2C19 Predicts Response to Tricyclic Antidepressants in Affective Disorders

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**Background:** There is strong evidence for the role of cytochrome enzymes CYP2D6 and CYP2C19 in the metabolism of tricyclic antidepressants (TCAs), and some prior evidence of association with clinical response and adverse drug reactions.
**Methods:** Subjects (N = 41) had major depressive disorder (N = 37) or bipolar disorder (N = 4) treated with TCAs (the most frequently prescribed being amitriptyline) in a tertiary referral center. Venous blood was taken for genetic analysis and for levels of the TCAs and their primary metabolites, and was phenotyped for CYP2D6 activity using debrisoquine. Subjects were assessed at baseline and at six weeks for severity of depression using the Hamilton Depression Rating Scale (HDRS), and were also rated for side effects at six weeks.
**Results:** There was no association between clinical response or adverse drug reactions and CYP2D6 phenotype or genotype. However, an association between clinical response to TCAs and CYP2C19 genotype was found ($p = 0.005$). The direction of effect was such that it implies that the parent TCA may be more potent than its demethylated metabolite. This is consistent with the dual (serotonin-norepinephrine) reuptake inhibitory effect of TCAs such as amitriptyline. Moreover, the sample of subjects had a severe illness (mean pre-treatment HDRS score 25.9). The level of demethylated TCA was associated with anticholinergic side effects.
**Conclusions:** This study indicates that in subjects with affective disorders, clinical response to TCAs may be predicted by CYP2C19 genotype. The direction of effect was as hypothesized, with better response occurring in those who carried a non-functional allele of CYP2C19. Improved clinical response associated with fewer active CYP2C19 alleles suggests that the parent TCA in this sample is more important in terms of generating clinical response than the N-demethylated metabolite. The reasons for this are interesting to speculate about, and may include the fact that the most commonly prescribed TCA in this sample (amitriptyline) has serotonergic as well as noradrenergic activity. Another contributing factor for the failure to find an association between CYP2D6 genotypic category and the level of side effects may be due to the limited genotyping undertaken. Although the most common polymorphism resulting in non-functional alleles of CYP2D6 was genotyped in this study, there is still incomplete knowledge about the genotypes responsible for intermediate metabolizers and ultra-rapid metabolizers.
**Keywords:** Pharmacogenetics, CYP2C19, Antidepressants
**Disclosures:** Nothing to disclose.
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## M60. Effects of Serotonin-Transporter-Linked Polymorphic Region and Familial Depression Risks on DMN Connectivity

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**Background:** 5-HTTLPR (serotonin-transporter-linked polymorphic region), a degenerate repeat polymorphic

region in SLC6A4, is a potential genetic risk factor for depression, particularly in individuals experiencing stressful life events (Pezawas et al., 2005). Previous studies investigating underlying biological mechanisms have focused on the amygdala-prefrontal circuit. However, whether 5-HTTLPR affects other circuits implicated in depression has yet to be reported. Given mounting evidence for the role of the default mode network (DMN) in depression, we hypothesize that 5-HTTLPR influences DMN connectivity. We tested this hypothesis by comparing participants with or without a history of familial depression. This unique study design allowed us to investigate the impact of genetic and familial depression risk factors on the DMN.

**Methods:** The familial depression study began in 1982; complete details of the study have been previously reported (Weissman et al., 2005). Here we studied 92 participants (50 females, 42 males), mean age 32 years (range 12–59 years, s.d. 13.9). Familial risk status was defined based on the first generation (G1); offspring were labeled high-risk if a history of MDD was reported in G1; otherwise, they were labeled low-risk. In this study, 42 had low familial risk for depression, and 50 had high risk for depression. Regarding 5-HTTLPR, there were 31 La/La carriers and 61 S' or La/Lg carriers. Diffusion magnetic resonance images (dMRI) were acquired with a 3T scanner (voxel size = 0.94 x 0.94 x 2.5 mm, # of gradients = 25, b = 1000). We used FSL Diffusion Toolbox for preprocessing and probabilistic tractography. Effects of the 5-HTTLPR on white matter connectivity of the DMN were examined using non-parametric permutation testing. Regressors included 5-HTTLPR, familial risk for depression, 5-HTTLPR-by-familial risk interaction; covariates included age, gender, normalized depression and anxiety symptom scales, kinship information, and two in-scanner head motion parameters.

**Results:** Reconstruction of DMN White Matter Pathways:
Our probabilistic tractography successfully reconstructed the white matter pathway of the DMN. Posterior distribution of the tracts connected the precuneus and the frontal lobes primarily via the cingulum bundle. A secondary pathway was also observed connecting the precuneus and the frontal lobes via the inferior longitudinal fasciculus.
Influence of 5-HTTLPR and High Familial Risk for Depression on DMN Connectivity:
Probabilistic tractography measures were derived, representing structural connectivity between the precuneus and the dorsal medial prefrontal cortex (PFC). We detected a significant effect of 5-HTTLPR and high familial risk for depression on precuneus-dorsal medial PFC connectivity. S' or La/Lg carriers showed a significant decrease compared with La/La homozygotes ($p = 0.028$). Individuals with high familial risk for depression showed a significant decrease in the precuneus-dorsal medial PFC connectivity ($p = 0.033$). Interaction between 5-HTTLPR and high familial risk for depression was non-significant ($p = 0.89$). These findings controlled for covariates (see Methods).

**Conclusions:** In this study, we demonstrate novel evidence for decreased DMN structural connectivity in individuals with: (i) a well-known genetic risk factor for depression (5-HTTLPR) and (ii) a familial depression history. Abnormal DMN connectivity has been previously implicated in depression. A recent diffusion tractography study reported that decreased DMN connectivity is implicated in MDD (Korgaonkar et al., 2014). On the other hand, resting-state functional connectivity studies document abnormally increased resting-state functional connectivity of DMN in MDD (Greicius et al., 2007) and normalization by antidepressant therapy (Posner et al., 2013). This increased resting-state functional connectivity in MDD is thought to represent an impaired down-regulation of DMN in various cognitive processes. In line with this idea, we speculate that decreased structural connectivity of the DMN may represent a structural substrate of impaired DMN down-regulation in depression. Future studies may investigate structural-functional relationships of the DMN connectivity.
Comparable effects of 5-HTTLRP and high familial risk for depression on DMN structural connectivity are worth noting. Our group has documented that high familial risk for depression plays an important role in development and maintenance of MDD (Fendrich et al., 1990; Warner et al., 1999; Weissman et al., 2005). Therefore, the decreased DMN structural connectivity associated with high familial risk or with high genetic risk for depression may offer validation of the findings.

**Keywords:** Serotonin Transporter, Depression, default mode network, tractography, Diffusion Weighted Imaging
**Disclosures:** Nothing to disclose.
**Acknowledgements:** Clinical data for the study were collected as part of 2 R01 MH36197, (Weissman, P.I); DNA was collected as part of 1P50MH090966, (Gingrich, PI).

## M61. Ketamine as a Prophylactic Against Stress-Induced Depressive-Like Behavior

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**Background:** Stress exposure is one of the greatest risk factors for psychiatric illnesses like major depressive disorder and posttraumatic stress disorder. However, not all individuals exposed to stress develop affective disorders. Stress resilience, the ability to experience stress without developing persistent psychopathology, varies from individual to individual. Enhancing stress resilience in at-risk populations could potentially protect against stress-induced psychiatric disorders. Despite this fact, no resilience-enhancing pharmaceuticals have been identified.

**Methods:** Using a chronic social defeat (SD) stress model, learned helplessness (LH), and a chronic corticosterone (CORT) model in mice, we tested if ketamine could protect against depressive-like behavior. Mice were administered a single dose of saline or ketamine and then 1 week later were subjected to 2 weeks of SD, LH training, or 3 weeks of CORT.

**Results:** SD robustly and reliably induced depressive-like behavior in control mice. Mice treated with prophylactic ketamine were protected against the deleterious effects of SD in the forced swim test and in the dominant interaction test. We confirmed these effects in LH and the CORT model. In the LH model, latency to escape was increased following training, and this effect was prevented by ketamine. In the

CORT model, a single dose of ketamine blocked stress-induced behavior in the forced swim test, novelty suppressed feeding paradigm, and the sucrose splash test.
**Conclusions:** These data show that ketamine can induce persistent stress resilience and, therefore, may be useful in protecting against stress-induced disorders.
**Keywords:** Ketamine, Social defeat stress, Posttraumatic stress disorder, Depression, corticosterone
**Disclosures:** Denis David serves as a consultant for Lundbeck, Roche, and Servier. René Hen receives compensation as a consultant for Lundbeck, Roche, and Servier.

### M62. R-Ketamine: A Rapid Onset and Sustained Antidepressant Without Psychotomimetic Side Effects

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**Background:** The N-methyl-D-aspartate (NMDA) receptor antagonist ketamine shows rapid and sustained antidepressant effects in treatment-resistant patients with major depression and bipolar disorder. Ketamine (or RS-ketamine) is a racemic mixture containing equal parts of R-ketamine and S-ketamine (or esketamine). S-ketamine has an approximately 4-fold greater affinity for the NMDA receptor than the R-stereoisomer. Furthermore, S-ketamine shows an approximately 3-4 fold greater anesthetic potency and greater undesirable psychotomimetic side effects, compared with the R-stereoisomer. Recently, we reported that, compared with S-ketamine, R-ketamine produced rapid and long-lasting antidepressant effects in juvenile mice exposed neonatally to dexamethasone (Zhang et al., 2014). In the present study, we examined the effects of R- and S-ketamine in the social defeat stress and learned helplessness (LH) models of depression.
**Methods:** Behavioral tests, including the tail suspension test (TST), forced swimming test (FST), and 1% sucrose preference test, were performed. Furthermore, we examined the effects of these stereoisomers in the behavioral tests (locomotion, prepulse inhibition (PPI) and conditioned place preference) for side effects. To elucidate their potential therapeutic mechanisms, we examined the effects of these stereoisomers on brain-derived neurotrophic factor (BDNF)-TrkB signaling, and synaptogenesis in selected brain regions.
**Results:** In the social defeat stress and LH models of depression, R-ketamine showed a greater potency and longer-lasting antidepressant effect than S-ketamine. Furthermore, R-ketamine induced a more potent beneficial effect on decreased dendritic spine density, BDNF-TrkB signaling and synaptogenesis in the prefrontal cortex (PFC), CA3 and dentate gyrus (DG) of the hippocampus from depressed mice compared with S-ketamine. However, neither stereoisomer affected these alterations in the nucleus accumbens of depressed mice. In behavioral tests for side effects, S-ketamine, but not R-ketamine, precipitated behavioral abnormalities, such as hyperlocomotion, PPI deficits and rewarding effects. Additionally, a single dose of S-ketamine, but not R-ketamine, caused a loss of parvalbumin (PV)-positive cells in the prelimbic region of the medial PFC and DG.
**Conclusions:** These findings suggest that, unlike S-ketamine, R-ketamine can elicit a sustained antidepressant effect, mediated by increased BDNF-TrkB signaling and synaptogenesis in the PFC, DG and CA3. Therefore, R-ketamine appears to be a potent, long-lasting and safe antidepressant, relative to S-ketamine, since R-ketamine appears to be free of psychotomimetic side effects and abuse liability.
**Keywords:** Ketamine, Esketamine, Antidepressant, NMDA Receptor, BDNF
**Disclosures:** Dr. Hashimoto is an inventor of the patent application on the use of R-ketamine in the treatment of psychiatric diseases. Dr. Hashimoto has served as a scientific consultant to Astellas and Taisho, and he also received the research grant support from Abbvie, Dainippon Sumitomo, Mochida, Otsuka, and Taisho. Other authors report no biomedical financial interests or potential conflicts of interest.

### M63. Developing a Model to Predict the ACTH Response to a Social Stressor Using Clinical Variables and Genotype

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**Background:** The ability to predict an individual's stress response is essential to understanding disorders that are often precipitated by stress, including mood disorders. This understanding will also aid in the identification of individuals as stress resilient or stress vulnerable. This work is especially important for military populations where the incidence of stress-related disorders is quite high.
**Methods:** We performed a social stressor on controls, as well as individuals with major depressive disorder some of whom also had an anxiety disorder or post-traumatic stress disorder. Specifically, we measured the ACTH response to the Trier Social Stress Test (TSST) as an endophenotype. We also collected demographic data and a range of clinical rating scales. We then performed genotyping using Illumina's HumanOmniExpress BeadChips. We pre-specified 62 candidate genes based on the neuroendocrine literature and on previous work in human postmortem brains. The panel consisted of circadian, immune, stress and fibroblast growth factor genes. After filtering the SNPs in PLINK, we combined our entire genotyped dataset of 209 subjects with HapMap (release 23a) and determined that the first two principal components of variation in our dataset were likely to represent the effects of population stratification. After removing SNPs with linkage disequilibrium greater than 0.5, we had 448 SNPs to interrogate. We then performed linear regression on the 63 subjects that had neuroendocrine data controlling for demographic and clinical variables to identify informative SNPs.
**Results:** Models integrating clinical variables and genetic variants were able to explain approximately half of the

variance in the ACTH response to the TSST. Both clinical information and SNPs made significant contributions. A full model, explaining a significant proportion of neuroendocrine variability in response to a standardized psychosocial stressor, will be presented.
**Conclusions:** Extension and replication in larger groups is needed, but the data suggest that the endocrine response to a laboratory social stressor may represent a useful endophenotype for studying both genetic and environmental determinants of stress reactivity, which may well have relevance to vulnerability to mood disorders and PTSD. These results could provide useful biomarkers and help identify individuals who may be more vulnerable to stress-related disorders.
**Keywords:** Major depression, Stress, Neuroendocrine
**Disclosures:** Nothing to disclose.

### M64. Long-Lasting Alterations in Microglial HMGB1 Expression Correlates with Increased Vulnerability to Depressive-Like Behaviors after Chronic Unpredictable Stress

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**Background:** Major Depressive Disorder (MDD) is a recurrent mental health illness with more than half of affected patients relapsing after their initial episode. High levels of psychological or environmental stressors are associated with the initial development of MDD and may play a role in recurrent episodes. To better understand these processes it is important to study stress-associated mechanisms that promote depressive-like behavior. Various stress paradigms have been used to model the molecular, cellular and behavioral effects of depression. For instance, several reports support the hypothesis that repeated stress exposure increases neuroinflammation, which contributes to the development and persistence of depressive-like behaviors.
**Methods:** In this study, we identified long-lasting microglial changes that corresponded with development and recurrence of MDD using a chronic unpredictable stress (CUS) model in rodents. Microglial morphology was assessed by immunofluorescence. RNA was extracted from Percoll gradient isolated microglia to synthesize cDNA and perform gene expression quantification.
Results Following CUS exposure, microglia in the prefrontal cortex and hippocampus displayed robust morphological changes indicative of an activated phenotype. Gene expression analyses of enriched hippocampal microglia showed elevated levels of several inflammation-associated genes, including HMGB1 mRNA levels after CUS, and increased HMGB1 expression in microglia overlapped with the development of depressive-like behaviors during stress exposure. To determine if stress-induced microglia alterations persisted after CUS, additional studies were performed in which rats were exposed to CUS then allowed to recover for 4 weeks. Here we show that microglia morphological alterations along with elevated HMGB1 expression are present 4 weeks after CUS cessation. Furthermore, changes in microglia were associated with recurrence of depressive-like behaviors during the recovery period. Specifically, exposure to short-term unpredictable stress during the recovery period caused recurrence of anhedonia in rats previously exposed to CUS. Importantly, short-term unpredictable stress did not cause significant behavioral changes in naïve rats.
**Conclusions:** Taken together, these data suggest that initial CUS exposure can evoke alterations in microglia HMGB1 signaling that may contribute to increased vulnerability to depressive-like behaviors following subsequent stress exposure. Studies are being conducted to directly test this hypothesis.
**Keywords:** microglia, inflammation, stress, hippocampus, HMGB1
**Disclosures:** Nothing to disclose.

### M65. Ziprasidone Augmentation of Escitalopram for Major Depressive Disorder: Safety and Tolerability

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**Background:** To examine the safety and tolerability of a regimen of adjunctive ziprasidone in adults with unipolar major depressive disorder (MDD) with prior nonresponse to 8 weeks of open-label escitalopram (ESC).
**Methods:** A multi-center, parallel, randomized, double-blind, placebo-controlled trial was conducted at three United States academic medical centers. We recruited 139 outpatients with persistent symptoms of MDD following an 8-week open label trial of ESC (phase 1). Subjects were then randomized (1:1, $N = 139$) to adjunctive ziprasidone (ESC + ZIP, $n = 71$) or adjunctive placebo (ESC + PBO, $n = 68$), for 8 additional weeks. Treatment outcomes have been reported elsewhere. Main cardiac, endocrine, and metabolic measures were obtained at each treatment visit. Barnes Akathisia Scale (BAS) and Abnormal Involuntary Movement Scale (AIMS) scores were also assessed throughout the study. Changes in outcome measures for each treatment group were compared using the independent samples t-test.
**Results:** Subjects receiving ziprasidone were significantly more likely to discontinue treatment compared to those taking placebo. A significant difference in QTc increase was observed for the ziprasidone group. Ziprasidone also resulted in a significant weight increase (7.7 lbs) compared to placebo (2.2 lbs). Ziprasidone patients had a modest increase in akathesia scores, while placebo patients had a small decrease. No significant changes in AIMS scores were observed for either treatment group.
**Conclusions:** Adjunctive ziprasidone, when added to escitalopram, demonstrated a modest difference in cardiovascular effects, weight gain, and akathisia compared to placebo. These results suggest that while ziprasidone augmentation is safe, precautions should be taken in practice, specifically regular monitoring of ECG, weight, and akathisia.
**Keywords:** augmentation, Treatment Resistant Depression, Atypical antipsychotics

**Disclosures:** This study was supported by the National Institute for Mental Health (NIMH R01MH081235), Pfizer Inc. (providing free blinded ziprasidone/placebo pills), and Forest Laboratories, Inc. (providing free escitalopram).
George I. Papakostas financial disclosure: Consultant: Abbott Laboratories, AstraZeneca PLC, Avanir Pharmaceuticals, Brainsway Ltd, Bristol-Myers Squibb Company, Cephalon Inc., Dey Pharma, L.P., Eli Lilly Co., Genentech, Inc., GlaxoSmithKline, Evotec AG, H. Lundbeck A/S, Inflabloc Pharmaceuticals, Janssen Global Services LLC, Jazz Pharmaceuticals, Johnson & Johnson Companies, Novartis Pharma AG, One Carbon Therapeutics, Inc, Otsuka Pharmaceuticals, PAMLAB LLC, Pfizer Inc., Pierre Fabre Laboratories, Ridge Diagnostics (formerly known as Precision Human Biolaboratories), Shire Pharmaceuticals, Sunovion Pharmaceuticals, Takeda Pharmaceutical Company LTD, Theracos, Inc., Wyeth, Inc. Honoraria: Abbott Laboratories, Astra Zeneca PLC, Avanir Pharmaceuticals, Bristol-Myers Squibb Company, Brainsway Ltd, Cephalon Inc., Dey Pharma, L.P., Eli Lilly Co., Evotec AG, GlaxoSmithKline, Inflabloc Pharmaceuticals, Jazz Pharmaceuticals, H. Lundbeck A/S, Novartis Pharma AG, Otsuka Pharmaceuticals, PAMLAB LLC, Pfizer, Pierre Fabre Laboratories, Ridge Diagnostics, Shire Pharmaceuticals, Sunovion Pharmaceuticals, Takeda Pharmaceutical Company LTD, Theracos, Inc., Titan Pharmaceuticals, Wyeth Inc. Research support: AstraZeneca PLC, Bristol-Myers Squibb Company, Forest Pharmaceuticals, the National Institute of Mental Health, PAMLAB LLC, Pfizer Inc., Ridge Diagnostics (formerly known as Precision Human Biolaboratories), Sunovion Pharmaceuticals, Theracos, Inc. Speaker's bureau (past): Bristol-Myers Squibb Co. and Pfizer, Inc.

## M66. Regulation of Mood and Emotion Processing by GSK3 and FXR1P

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**Background:** Inhibition of glycogen synthase kinase 3 (GSK3) is a shared action believed to be involved in the regulation of behavioral by psychoactive drugs such as antipsychotics and mood stabilizers. Furthermore, polymorphisms in genes encoding proteins that are regulated by GSK3 have also been associated to schizophrenia and/or bipolar disorder. However, little is known about the identity of the substrates through which GSK3β affects behavior.
**Methods:** Pharmacological treatments leading to inhibition of brain GSK3 with different behavioral outcomes in mice were used to identify putative new substrates relevant to behavioral modulation. Substrates were validated using in vitro biochemical characterization along with pharmacological treatments, gene therapy and behavioral characterization in mice. Genetic, brain imaging and behavioral studies in humans were also undertaken to confirm interaction between GSK3 and new putative substrates.
**Results:** We identified FXR1P, a RNA binding protein associated to genetic risk for schizophrenia, as a substrate for GSK3β. In contrast behaviorally effective chronic mood stabilizer treatments in mice, inhibit GSK3β and increase FXR1P levels. In line with this, over-expression of FXR1P in the mouse pre-frontal cortex also leads to comparable mood related responses. Furthermore, functional genetic polymorphisms affecting either FXR1P or GSK3β gene expression interact to regulate emotional brain responsiveness and stability in humans.
**Conclusions:** These observations uncovered a GSK3β/FXR1P signaling pathway that contributes to regulating mood and emotionality. Regulation of FXR1P by GSK3β may provide a mechanistic framework that can explain how inhibition of GSK3β can contribute to the regulation of mood by psychoactive drugs. Furthermore, this pathway may also potentially be involved other biological functions such as inflammation and cell proliferation in which FXR1P and GSK3 are known to play a role.
**Keywords:** GSK3, FXR1P, Mood, Emotion processing
**Disclosures:** Nothing to disclose.

## M67. Lipid Peroxidation and Executive Function in Adolescent Bipolar Disorder: The Role of BDNF

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**Background:** Numerous studies have identified oxidative stress and brain-derived neurotrophic factor (BDNF) as putative biomarkers in bipolar disorder (BD). Similarly, cognitive dysfunction, particularly in frontal-executive tasks, is a highly replicated finding in BD. Because cognitive dysfunction is evident within and between mood episodes in BD, it contributes significantly to symptomatic burden and functional impairment. Prior studies in schizophrenia have shown that oxidative stress is associated with BDNF and with cognition. Examining these factors together in youth with BD may optimize signal detection as youth have shorter duration of illness, less exposure to psychotropic medication, and better physical health compared to adults with BD.
**Methods:** Serum levels of OS markers lipid hydroperoxides (LPH) and 4-hydroxy-2-nonenal (4-HNE), and BDNF levels were measured in 30 BD and 25 control adolescents. The intra-extra-dimensional (IED) set-shifting task assessed executive function. The Kiddie-Schedule for Affective Disorders and Schizophrenia for School Age Children, Present and Lifetime Version (KSADS-PL) was used to establish diagnoses. Analyses examined age- and sex-adjusted Z-scores. Lower IED scores indicated better performance. Between-group differences were analyzed using independent-samples t-tests and Mann-Whitney U-tests. OS-IED associations were analyzed using Spearman correlations and General Linear Models (GLM). High and low BDNF subgroups were defined by median split.
**Results:** There were no significant differences in biomarker levels between patients and controls. Several IED Z-scores, but not raw scores, were significantly different between groups. In patients, LPH was significantly correlated with IED completed stage trials ($\rho = 0.462$, $p = 0.012$). In high BDNF patients, LPH was significantly correlated with IED completed stage trials ($\rho = 0.755$, $p = 0.001$) and pre-extra-

dimensional shift errors ($\rho = 0.588$, $p = 0.017$). In GLM analysis, LPH, BDNF, and the interaction term significantly explained variance of IED total trials (adjusted) ($r2 = 0.203$, $F = 2.446$, $p = 0.047$), after adjusting for covariates.
**Conclusions:** There is a negative association between LPH and executive function in BD adolescents, which is modulated by BDNF. LPH and BDNF may be useful as biomarkers of executive function and antioxidant interventions for executive dysfunction may be warranted, especially when guided by BDNF levels.
**Keywords:** Bipolar Disorder, executive function, Adolescent, oxidative stress, BDNF
**Disclosures:** Nothing to disclose.

### M68. Comparing the Effects of an Index Course of Magnetic Seizure Therapy and Electroconvulsive Therapy on Quality of Life

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**Background:** Severe treatment-resistant depression is a neuropsychiatric illness that is chronic, debilitating, and results in decreased quality-of-life (QOL). Research has found that patients with depression treated with electroconvulsive therapy (ECT) show improved QOL. A newer convulsive therapy, magnetic seizure therapy (MST), uses magnetic pulses to induce a relatively focal seizure for the treatment of depression. MST has been found to have antidepressant effects similar to ECT. Unlike ECT however, MST produces no known adverse cognitive effects. To date, no study has examined the effects of MST on QOL. Thus, the purpose of this study was to compare the effects of an acute course of high-dose MST and right unilateral (RUL) ultrabrief-pulse width ECT on QOL in patients with a current major depressive episode.
**Methods:** This was a three-center, between-subject, randomized, double-masked controlled clinical trial that compared the effects of high dose MST and RUL ultrabrief-pulse ECT on QOL. All participants provided written informed consent for this IRB approved investigation before completing study procedures. The study was conducted under a US FDA IDE. Adults with a major depressive episode in the context of unipolar or bipolar depression, based on the SCID-I, were randomly assigned to treatment with MST or ECT. For MST, a Magstim Theta (Magstim Co., Whtland, Wales, UK) device with a round coil positioned on the vertex was used to administer the stimulus. Seizure threshold was titrated at the first session by increasing the train duration, and subsequent treatments were provided at maximal device output (100% stimulator output (252 J), 100 Hz pulse train frequency, 10 second train duration). For ECT, treatments were provided via standard RUL electrode configuration. ECT stimulus was delivered using a Thymatron System IV (pulse amplitude = 900 mA, pulse width = 0.25 ms; Somatics LLC, Lake Bluff, IL) or a MECTA Spectrum 5000Q (pulse amplitude = 800 mA, pulse width = 0.3 ms; MECTA Corp., Tualatin, OR). ECT seizure threshold was titrated at the first session by increasing the train duration and frequency. Subsequent treatments were provided at 6× the seizure threshold. Patients were treated until they achieved remission (<8 on the 24-item Hamilton Rating Scale for Depression) or received a maximum of 14 MST or ECT sessions. To assess QOL, we used the Medical Outcomes Study 36-item Short Form Health Survey (SF-36). The participants, who were masked to treatment condition, completed the SF-36 before and after the acute treatment course. The SF-36 produced 8 domain scores including Physical Function, Role-Physical, Role-Emotional, Energy, Emotional Well Being, Social Function, Pain, and General Overall Health Perception. ANCOVAs were computed for each SF-36 domain score with treatment condition (MST, ECT) as the between-subject factor. We explored effects of covariates including study site, number of treatments, race, and sex. However, the covariates had no effects and were excluded from the statistical analyses. Post-hoc t-tests were used to assess significant main effects. Statistical significance was defined as a two-sided p-value of less than 0.05.
**Results:** In terms of change from baseline to end for both ECT and MST, there was a significant effect of time for seven SF-36 domain scores including Physical Function ($F(3,63) = 10.5$, $p < 0.0001$), Role-Emotional ($F(3,63) = 5.6$, $p = 0.0002$), Energy ($F(3,63) = 16.0$, $p < 0.0001$), Emotional Well Being ($F(3,63) = 20.5$, $< 0.0001$), Social Function ($F(3,63) = 17.5$, $p < 0.0001$), Pain ($F(3,63) = 5.5$, $p = 0.002$), and General Overall Health Perception ($F(3,63) = 3.0$, $p = 0.04$). Only the domain of Role-Physical showed no change across time ($F\ (3,63) = 0.9$, $p = 0.4$). Patients who received MST relative to those who received ECT showed better outcome in Energy ($t63 = -2.56$, $p = 0.01$) and Social Function ($t63 = -2.03$, $p = 0.005$) QOL domains.
**Conclusions:** This is the first study to show that both RUL ultrabrief-pulse ECT, and MST improve quality-of-life in patients with treatment resistant depression. Further, the study showed that patients treated with MST, relative to ECT, showed greater improvement in energy and social function. The latter may be related to the cognitive safety of MST as it is associated with little change in psychomotor processing speed and improved executive function. These findings are consistent with prior research that found ECT to be associated with improved QOL. As those studies provided ECT with either bitemporal or right unilateral placement, and brief pulse width, this study adds new evidence for the advantage of RUL ultrabrief-pulse parameters. Future research is warranted to confirm the improvement in QOL observed with RUL ultrabrief-pulse ECT and MST, and to determine the association with clinical, neurocognitive, and functional outcome.
**Keywords:** electroconvulsive therapy, Magnetic seizure therapy, Major Depressive Disorder
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### M69. The Relationship Between Plasma Omega-3 Polyunsaturated Fatty Acid Levels and Depressive Symptom Severity

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**Background:** A diet rich in omega-3 polyunsaturated fatty acids (PUFAs) has been implicated in the prevention of numerous illnesses including depression. A limitation of the existing literature is that studies have generally examined PUFA consumption by self-report which is only moderately associated with serum PUFA levels. Minimal data are available on the relationship between serum PUFA levels and depression. The current study examines relationships between serum omega-3 PUFA levels and depressive symptoms in a large patient sample.
**Methods:** Adults (n = 9306) seen at the Cooper Clinic in Dallas, Texas for preventive medical examinations that included an extensive medical history, as well as physician and laboratory examination. Participants with diabetes, heart attack, stroke or cancer were excluded for the current analysis. Depressive symptoms were assessed with the Center for Epidemiologic Studies Depression scale (CES-D). Serum PUFAs levels were assessed from red blood cell membranes using chromatographic procedures, and expressed as the weight percentage of total fatty acids. A generalized linear model controlling for demographics was used to determine the relationship between CES-D scores and PUFA levels. A two-step regression approach was used to examine a gender difference in omega fatty acid level correlations with depressive symptoms. First, a generalized estimating equation (GEE) with gender interaction terms was conducted for all four models and multiple GEEs stratified by gender were conducted only when statistically significant gender interaction terms were found.
**Results:** The total sample of N = 9306 included N = 6492 (69.76%) men, N = 8528 (91.64%) Caucasians, and N = 877 (9.42%) smokers. The mean age of the sample was 51.57 ± 10.90 years; mean education 15.94 ± 2.45 years; mean CES-D score 3.76 ± 4.02 (range 0-30); mean BMI 26.73 ± 4.35; mean hs-CRP 1.94 ± 5.4 (mg/L); and mean drinks per week were 7.3 ± 6.5. The mean total omega-3 index was 6.07 ± 1.92 mg/L. The omega-3 index showed a trend ($p = .0872$) toward a significant association with CES-D score. A significant sex by omega-3 index interaction was observed ($p = .0116$) so men and women were examined separately. A highly significant association between omega-3 index and CES-D score was observed in women ($p < .0001$) but not in men ($p = .1080$).
**Conclusions:** To our knowledge this is the first large study using plasma levels of PUFAs and depressive symptom severity assessed by a validated questionnaire. A significant relationship between serum omega-3 PUFA levels and depressive symptoms in was observed in women but not in than in men. The findings suggest that the relationship between PUFAs and depression may differ based on sex.
**Keywords:** Omega-3 Fatty Acid, Depression, sex difference
**Disclosures:** Dr. Brown reports research grants from Alkermes, Forest Laboratories, Inc. and Sunovion Pharmaceuticals, Inc. Dr. DeFina, Dr. Sunderajan, Dr. Jeon-Slaughter and Mr. Ly report no financial relationships with commercial interests.

### M70. Ketamine-Induced Changes in [11C]ABP688 Binding in Healthy and Depressed Human Subjects

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**Background:** Ketamine is an NMDA glutamate receptor antagonist with anesthetic and analgesic properties that has been studied as a probe of NMDA receptor dysregulation in psychiatric disorders. Most recently, it has received intensive study in light of its rapidly emerging antidepressant effects. The present study was intended to evaluate ketamine's short- (1 hour) and long-term (24 hours) effects at metabotropic glutamate receptors, subtype 5 (mGluR5) in vivo in individuals with MDD. We previously showed that ketamine administration leads to a reduction in [11C] ABP688 (negative allosteric modulator of mGluR5) binding in healthy controls (HC)1. The present study examined whether 1) this ketamine-induced change is different in individuals with major depressive disorder (MDD); 2) the changes in binding are related to changes in mood in MDD; and 3) the observed changes in [11C]ABP688 are prolonged in both MDD and HC individuals.
**Methods:** Thirteen healthy (33.1 ± 13.1 years) and 10 MDD (32.4 ± 12.6 years) nonsmokers participated in two 60-min bolus [11C]ABP688 PET scans on the same day – before (baseline) and during i.v. ketamine administration (0.23mg/kg over 1min, then 0.58mg/kg over 1h; beginning 1 min after tracer injection) and a third 60 min bolus [11C]ABP688 PET scan 1 day after ketamine administration. Due to variability in outcomes of test-retest studies of [11C]ABP688, an additional 11 subjects (37.1 ± 11.4 years) participated in test-retest study (2 [11C]ABP688 scans on the same day)2, 3. Input functions for both studies were obtained through arterial blood sampling with metabolite

analysis. Time-activity curves (TACs) were generated and an unconstrained two-tissue compartment model was used to fit TACs and estimate distribution volume, VT. Subjects participating in the ketamine study were assessed for mood and cognitive functioning at baseline, immediately after ketamine administration, and 24 hours after ketamine administration.

**Results:** We did not observe a significant difference in baseline VT (mGluR5 availability) between control ($4.1 \pm 1.1$ mL/cm3) and MDD ($3.3 \pm 0.5$ mL/cm3) groups ($p > 0.1$). We observed a significant reduction from baseline VT during administration of ketamine in both MDD and control subjects (average of $16 \pm 8\%$ and $22 \pm 22\%$, respectively, in the relevant grey matter regions). A significant reduction ($12 \pm 13\%$ and $33 \pm 31\%$ in MDD and HC, respectively) in VT remained 24 h after ketamine administration. Ketamine-induced VT reductions were not significantly different between diagnostic groups at either time point. There was a significant reduction in depressive symptoms following ketamine administration (decrease of 57% following ketamine administration and decrease of 70% 24 h after ketamine) in the MDD group. Importantly, we observed a significant positive association between change in binding and change in mood symptoms immediately after ketamine administration in the MDD group ($p < 0.05$), but not at the 24-h assessment. In the test-retest portion of the protocol, we replicated our previous findings and determine that [11C]ABP688 binding is increased at retest by $33 \pm 41\%$.

**Conclusions:** We found ketamine-induced decreases in radioligand binding in both MDD and control subjects, and the degree of change may be underestimated given our test-retest findings of increased radioligand binding during the second scan, on average. Data from the ketamine paradigm suggest mGluR5 internalization due to the rapid ketamine-induced glutamate release. Interestingly, 24 hours after ketamine administration, there appears to remain a decrease in radioligand binding in both HC and MDD groups. This may be explained by long-term receptor internalization due to the rapid increase in glutamate, or other effects of ketamine that may cause downstream effects at mGluR5. Importantly, in the MDD sample, greater changes in ligand binding were associated with greater decrease in depressive symptomatology after ketamine administration, implicating mGluR5 in the anti-depressant response to ketamine. Although the variability in response is large, especially 24 h post ketamine, this study may provide insight into ketamine's mechanism of action and potentially imaging-based prediction of response.

**Keywords:** metabotropic glutamate receptor, PET, MDD

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## M71. Impact of Vortioxetine on Functional Capacity in MDD Patients with Subjective Cognitive Dysfunction: Performance on the University of California San Diego Performance-Based Skills Assessment (UPSA)

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**Background:** Vortioxetine is an antidepressant approved for the treatment of patients with major depressive disorder (MDD). Vortioxetine has multimodal activity that combines direct modulation of multiple serotonin receptor activities with inhibition of the serotonin transporter. Recent evidence from two clinical studies (NCT01422213, NCT01564862) demonstrated that vortioxetine improves cognitive function in adults with MDD. The primary objective of this post-hoc analysis of study NCT01564862 was to evaluate the effect of flexible-dose vortioxetine (10-20 mg) on functional capacity in adults with MDD with subjective cognitive dysfunction after 8 weeks of treatment through the evaluation of performance on the UCSD Performance-Based Skills Assessment (UPSA). Duloxetine 60 mg was included as an active reference for treatment-related changes in the Montgomery-Åsberg Depression Rating Scale (MADRS).

**Methods:** Adults (aged 18-65 yrs.) with moderate to severe MDD (MADRS $\geq 26$) who self-reported symptoms of cognitive dysfunction (e.g., difficulty concentrating, slow thinking, and difficulty in learning or remembering things) were enrolled in a double-blind, placebo and active-reference study. The UPSA composite score (comprising the full UPSA and UPSA-B in US and non-US patients, respectively) was evaluated at baseline and Week 8, MADRS at Baseline, Week 1, 4, and 8, with both outcome measures compared to placebo with an analysis of covariance

(ANCOVA) using the Full Analysis Set (FAS). Exploratory analyses were performed in patient subgroups based on the severity of functional impairment (baseline UPSA $\leq 75$, $\leq 70$). Additionally, clinically relevant improvements in functional capacity at Week 8 were evaluated using pre-defined cutoffs for the change from baseline in UPSA ($\geq 5$, $\geq 7$, $\geq 10$). Path analysis considering changes in the MADRS was conducted to determine the proportion of direct versus indirect effects of vortioxetine on functional capacity. An exploratory analysis of remission from depressive symptoms and functional improvement by week 8 (MADRS total score $\leq 10$ and UPSA $\geq 75$) was also conducted.

**Results:** A total of 602 patients were randomly assigned to treatment (vortioxetine, n = 198; placebo, n = 194; duloxetine, n = 210). Baseline demographics and clinical characteristics for patients entering the trial, including duration of the current depressive episode and overall depressive severity, were similar across treatment groups. Vortioxetine demonstrated a statistically significant increase in functional capacity compared to placebo, as measured by change from baseline in the UPSA composite score after 8 weeks of treatment in all patients (LS mean change: vortioxetine, n = 175, $\Delta + 8.0$; placebo, n = 166, $\Delta + 5.1$: $p < 0.001$ versus placebo, FAS), with a statistically significant improvement in patients with baseline UPSA $\leq 75$ (n = 62, $\Delta + 14.9$; n = 73, $\Delta + 9.9$, $p = 0.003$ vs. placebo, FAS) as well as UPSA $\leq 70$ (n = 41, $\Delta + 16.7$; n = 46, $\Delta + 10.8$: $p = 0.010$ vs. placebo, FAS). Patients with a baseline UPSA $> 75$ demonstrated a non-significant improvement in functional capacity after 8 weeks (n = 113, $\Delta + 3.5$; n = 93, $\Delta + 1.9$: $p = 0.060$ vs. placebo, FAS). Patients treated with duloxetine did not demonstrate a significant improvement in functional capacity compared to placebo ($p = 0.637$), with no effect of baseline UPSA scores.

A significantly higher percentage of vortioxetine patients were classified as a responder compared to placebo patients1, based on a change in the UPSA after 8 weeks of treatment of $\geq 5$ (vortioxetine: n = 114, 65.1%; placebo: n = 93, 56.0%: $p = 0.850$ vs. placebo), $\geq 7$ (n = 85, 48.6%; n = 59, 35.5%: $p = 0.015$ vs. placebo), and $\geq 10$ (n = 66, 37.7%; n = 46, 27.7%: $p = 0.049$ vs. placebo). There was no significant difference in UPSA response for duloxetine versus placebo using any of the pre-defined responder cutoffs.

Vortioxetine and duloxetine both significantly improved depressive symptoms compared to placebo, as measured by the change from baseline to Week 8 in MADRS ($p < 0.05$; $p < 0.001$, respectively), validating the study. Path analysis of the UPSA revealed that 96.9% of the effect of vortioxetine on performance-based functional capacity was direct, and not due to improvement in depressive symptoms. When considering remission rates for depressive symptoms (MADRS total score $\leq 10$) plus functional improvement (UPSA $\geq 75$), vortioxetine was significantly different from placebo (22.3% vs 10.2%, $p = 0.005$ vs placebo), but not duloxetine (16.0%, $p = 0.124$ vs placebo).

**Conclusions:** In addition to its effect on cognitive dysfunction and depressive symptoms, vortioxetine significantly improved functional capacity, as assessed by the UPSA. Vortioxetine, but not duloxetine, significantly separated from placebo in the improvement on the UPSA at Week 8, with significant effects in patients with baseline UPSA $\leq 70$ or $\leq 75$. Duloxetine provided no significant benefits on the UPSA. This is the first study to demonstrate a beneficial effect of antidepressant treatment on functional capacity using the UPSA. These results emphasize the distinct profile of vortioxetine in MDD patients with cognitive dysfunction, with clinical utility observed in a wide population of patients.

**Keywords:** Major Depressive Disorder (MDD), UCSD Performance-Based Skills Assessment (UPSA), Vortioxetine

**Disclosures:** William Jacobson owns stock in Takeda, Pfizer and United Health and is a full time employee of Takeda Development Center, Americas. 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. Elizabeth Merikle, Wei Zhong and George Nomikos are full time employees of Takeda Development Center, Americas. Christina Kurre Olsen is a full time employee and stock owner in H. Lundbeck A/S. Michael Cronquist Christensen is a full time employee of H. Lundbeck A/S. Commercial support: This study was funded by H. Lundbeck A/S and Takeda Pharmaceutical Company, Ltd.

**References:** 1. Harvey PD et al. CNS Summit 2015 Annual Meeting. Boca Raton, Fl (submitted).

## M72. Test-Retest Reliability and Effects of Repeated Testing and Satiety on Performance of an Emotional Test Battery

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**Background:** The P1vital® Oxford Emotional Test Battery (ETB) has been extensively used to examine drug-induced changes in emotional processing and cognition in both healthy volunteers and depressed patients using between-subjects experimental designs. There are potential advantages to using a within-subjects design for assessing changes in cognitive performance over time, particularly for repeated assessment of depressed patients in clinical trials, but whether practice effects occur for the ETB is unknown. In addition, although satiety state has been reported to affect performance on cognitive and emotional tasks, it is unknown whether this influences performance on the ETB. Therefore, test-retest reliability of the ETB and the potential influence of practice effects and of satiety state on ETB performance were examined in two experimental studies with healthy volunteers.

**Methods:** In Study 1, 30 female volunteers (mean age 19 years, SEM = 0.2; mean Body Mass Index (BMI) 21.5 kg/m2, SEM = 0.4) completed testing on the ETB in a within-subjects design. All test sessions took place on the same day of the week and at the same time, on 4 separate occasions (each one week apart). In Study 2, 30 female volunteers (mean age 21 years, SEM = 0.3; mean BMI 20.0 kg/m2, SEM = 0.4) were randomised to either a satiated or hungry condition (15 participants in each condition). Participants were provided with a lunch of cheese sandwiches (approximately 1500 calories) and asked to eat until satiated either before (satiated group) or after (hungry group) completing

the ETB. The ETB comprised the following tasks: The Facial Expression Recognition Task (FERT) displayed faces that participants were asked to categorize into one of six emotional categories (happiness, fear, anger, disgust, sadness and surprise). The Faces Dot Probe Task (FDOT) presented faces, some of which displayed an emotional expression, that were replaced by a pair of dots and participants were asked to report the orientation of the pair of dots. The Emotional Categorization Task (ECAT) displayed positive and negative self-referent personality descriptors (e.g. "cheerful" versus "hostile") and participants were asked to indicate whether they would like or dislike to be referred to by this descriptor. In the Emotional Recall Task (EREC) participants were asked to recall as many words as they could from the ECAT. In the Emotional Recognition Memory Task (EMEM) words were re-presented from the ECAT together with new distractor words and participants were asked to report if they had previously seen the word.

**Results:** Study 1 demonstrated acceptable test-retest reliability for the ETB. Of the 39 ETB measures analysed, 31 displayed sufficient test-retest reliability across all four test sessions. In particular, response bias measures for the FERT showed good stability across sessions. Thus, there was no effect of test session, an effect of emotion and an interaction that approached significance. Breaking down the interaction by emotion, there was a main effect of session for anger and surprise, but not for disgust, fear, happy and sad. Examining the effect of session for anger and surprise, there was a significant increase in response bias to anger expressions from session 1 to session 2 but there were no other significant effects for subsequent test sessions or for any other emotions across sessions. In addition, the ECAT showed acceptable test-retest reliability and was free from practice effects. For the FDOT, EMEM and EREC tasks, practice effects were observed across initial test sessions, however, performance stabilized across the subsequent test sessions. In Study 2, participants ate a mean of 568.7 calories of cheese sandwiches either before or after ETB testing. Participants in the satiated condition showed a significant decrease in appetite, confirming that the satiety manipulation was successful. There were no effects of satiety on either cognitive or emotional processing on the FERT, FDOT, EMEM or EREC tasks. However, eating to satiety significantly decreased response bias to positive emotional words on the EMEM task.

**Conclusions:** The results of the test-retest analyses show acceptable test-retest reliability for the ETB using a within-subjects design. It is significant that response bias measures for the FERT showed good stability across test sessions as recent studies have suggested that repeated testing using this task may be valuable for early (within 7 days) prediction of the response of depressed patients to antidepressant drugs prior to changes in mood after 6 weeks (see Dawson et al., this meeting). The ECAT was free from practice effects, possibly due to the simplicity of the task but for the remaining tasks, practice effects were evident. This is consistent with extensive evidence that cognitive tasks, particularly memory tasks, are associated with practice effects. However, these effects stabilized after the first two sessions. Therefore, the results suggest that the ETB can be used reliably in repeated-measures experimental designs after two initial training sessions. The results of the second study suggest that a robust satiety manipulation has very limited effects on ETB performance and therefore satiety state is unlikely to be a significant confound in ETB studies. In conclusion, the ETB is suitable for repeated testing in assessing the efficacy of antidepressant therapy, has acceptable test-retest reliability and shows limited susceptibility to changes in hunger and satiety.

**Keywords:** Emotional Test Battery, Test Retest, Cognition, Satiety, Depression

**Disclosures:** Colin Dourish is an employee and shareholder of P1vital Limited. Jason Thomas is supported by the Steve Cooper PhD CASE studentship funded by the BBSRC and P1vital Limited. Suzanne Higgs is a member of P1vital Limited's Advisory Panel.

### M73. Ketamine is Antidepressant and Enhances Neuropeptide Y Expression in Hippocampus and Frontal Cortex of a Serotonin Transporter Knock-Out Rat Model

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**Background:** Subanesthetic ketamine exerts rapid antidepressant effect already after a single administration. However, the molecular mechanisms of action have not been fully elucidated. Based on our work with rodent models of depression, PTSD and anxiety and neuropeptides, particularly neuropeptide Y (NPY), we hypothesized that i. the NPYergic system is altered in a rat model with a deficient serotonergic system (SERTKO) that displays depression-like behavior; ii. ketamine will be antidepressant in that model; and iii. in similarity to other antidepressant treatment modalities, ketamine will also target the NPYergic system.

**Methods:** Experiments were approved by the Board for Animal Experimentation and conducted according to the Karolinska Institutes (KI) Regulations. SERT (SERT +/−) mutant (Slc6a41Hubr) rats were bred at the KI, ear punches collected after weaning on PND21 and genotyped by KBioscience (Hoddesdon, Hertfordshire, UK). 35-45 week old male rats were injected S-ketamine (ip, 15mg/kg in 0.9% NaCl) or placebo (7-8 rats/subgroup) 60 min preceding the 5 min open field test (OFT) in a 60 x 60 x 60 cm box. Ten min forced swim test (FST) using 50 cm high cylinders filled to 35cm with 24o C water was done 10 min after OFT. OFT was analyzed using an automated video tracking system (Noldus Ethovision XT 8, Wageningen, The Netherlands) and FST was video recorded and manually scored in Ethovision XT8 by an investigator blind to the experimental condition. Brains were harvested 30 min after the FST, snap frozen in isopentane and stored at -80o C. Coronal sections (14 μm thick) were collected at -18o C using a cryostat sectioner (Leica, Kista, Sweden), 20-50 mg of hippocampus and PFC punches were homogenized in microtubes with disposable pestles and total RNA isolated using RNeasy kit (Qiagen, Hilden, Germany). cDNA was prepared by reverse transcription of total RNA with SuperScript III (Invitrogen, Carlsbad, CA, USA) and amplified by gene specific primers in RT-PCR reactions performed on an Applied Biosystems

7300 instrument with SYBR green or TaqMan mix (Applied Biosystems, Foster City, CA). The mRNA levels were normalized to the reference genes for each sample to adjust for uncontrolled variability.
**Results:** Behavior. (1) OFT. Neither genotype nor treatment had a significant effect on any of the measured parameters in the OFT, that is SERT-/- mutants showed normal locomotor behavior and ketamine did not acutely affect it; (2). FST. Vehicle-treated SERT-/- rats showed statistically significant higher immobility compared to the SERT + / + confirming previous findings that SERT-/- rats display a depressive-like phenotype (Neumann et al 2011). Ketamine reduced significantly the immobility in the FST.Neuropeptide Y. (1). VEHICLE. NPY expression was significantly reduced while NPYY2R was significantly increased in the frontal cortex and hippocampus of the SERT-/- rats compared to the SERT + / + . NPYY1R expression did not differentiate between the mutant and wild type; (2). KETAMINE significantly increased NPY and a trend to decreased NPYY2R in the frontal cortex and hippocampus of SERT-/- rats was found.
**Conclusions:** Dysregulation of the monoaminergic systems is a sufficient but apparently not a necessary factor in etiology/pathologyphysiology of affective disorders. Moreover, ample evidence indicates that drugs targeting neuropeptides and the glutamatergic signaling are efficient in treatment of those disorders. We and others have demonstrated changes in the NPYergic system in depression, anxiety and PTSD both in patients and in corresponding animal models (Cohen et al 2012; Heilig et al 2004; Sah et al 2009; Wu et al 2011). Consequently, aims of this research were to explore if i. the NPYergic system is altered in a rat model of deficient serotonergic system, ii. ketamine will be an antidepressant in that model, and iii. in similarity to other efficient antidepressants, ketamine will affect the NPYergic system. We found lower NPY expression in SERT-/- compared to the SERT + / + animals which is in line with our hypothesis that one of the hallmarks of depression is reduced NPY in brain regions relevant for the disorder. Whether due to decreased NPYY1R or increased NPYY2R effects or alternative splicing of Npy (Caberlotto, Hurd 2001; Melas et al 2012) is the subject of further investigations. Further, our findings indicate that intact serotonergic system is not a prerequisite for the antidepressant effect of ketamine. Lastly, our data add to the consistent findings that all so far investigated antidepressants modify NPY, making it a possible candidate for a final common pathway for antidepressant effects.
**Keywords:** ketamine, neuropeptide Y, serotonin
**Disclosures:** Nothing to disclose.

### M74. Rapid Ultrasensitive High Precision LC-MS Assays Identify Unique Neuroactive Steroid and GABA Profiles in Women At-Risk for Postpartum Depression

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**Background:** Antepartum depression and anxiety is common and a risk factor for postpartum depression (PPD) which affects 1 in 8 women. Antenatal depressive/anxiety symptoms are a risk factor for PPD. Neuroactive steroids (NAS) are potent modulators of the GABAA receptor. In preclinical models, differential metabolism of NAS during the peripartum period is associated with depressive-like symptoms via an interaction with the GABAergic system. Gold-standard measurements of NAS by gas chromatography mass spectrometry (GC-MS) require time-consuming chemical derivatization. We developed rapid non-derivatizing, fast extraction liquid chromatography mass spectrometry (LC-MS) assays that maintain the level of sensitivity obtained with GC-MS approaches to measure plasma NAS and GABA during the peripartum period in healthy comparison subjects (HCS) and those at-risk of PPD (AR-PPD).
**Methods:** A prospective observational cohort study evaluated 56 (HCS, n = 24; AR-PPD, n = 32) medication-free subjects of 18-40 yrs. of age at four time points between 24-37 weeks gestation and up to 10 weeks postpartum. Depression and anxiety symptoms were measured using the Hamilton Depression Rating Scale (HAM-D17), Spielberger State-Trait Anxiety Inventory-State (STAI-S) and Hamilton Anxiety Rating Scale (HAM-A). Plasma NAS and GABA were quantified by LC-MS. Calibrants and benchmarks containing deuterated internal standards were prepared in charcoal-striped plasma, extracted by methyl tert-butyl ether, and reconstituted in mobile phase containing lithium acetate. NAS were separated using a Waters NanoAcquity UPLC configured to a Thermo Orbitrap Velos Pro mass spectrometer. NAS were ionized to (M + Li) +, subjected to in-source CID, and activated using HCD fragmentation. The ion-molecule reaction product (M + Li + H2O) + was used as the basis for the quantitative assay. For GABA, plasmas were added a deuterated GABA internal standard, extracted using an acidified acetonitrile protein precipitation, and analyzed on a Waters Acquity UPLC configured to a Waters Quattro Premier XE triple quadrupole mass spectrometer. For biomarker measurement, calibration in 200 μL of charcoal-stripped plasma over a working concentration of 0.031 to 160 ng/mL gave an overall method detection limit of 7.9 pg/mL for allopregnanolone and pregnenolone, 15.7 pg/mL for pregnanolone and 39 pg/mL for progesterone. Inter-assay precision (CV) and accuracy was $<10.5\%$ and $>90\%$ respectively using benchmarks at 4 concentrations over three days. GABA was assayed over a 5-100 nM range.
**Results:** Subjects were on average 32.7 years of age (± SD 4.8). HAM-D17 total score was 5.3 (± SE 0.7) points higher in AR-PPD women compared to HCS across the peripartum period ($p<.0001$). After adjusting for the peripartum timing of the biomarker measurement, plasma GABA concentration was 1.9 ± 0.7 ng/mL ($p = 0.005$) lower and plasma progesterone and pregnanolone were 16.0 ± 7.6 and 1.4 ± 0.7 ng/mL higher in AR-PPD women across time as compared to HCS, respectively ($p = 0.04$ for both). HAM-D17 was inversely associated with GABA concentration ($\beta = -0.16 \pm 0.06$, $p = 0.005$) and positively associated with pregnanolone ($\beta = 0.18 \pm 0.07$, $p = 0.01$) concentration. STAI-S was positively associated with pregnanolone ($\beta = 0.11 \pm 0.04$, $p = 0.004$), allopregnanolone ($\beta = 0.13 \pm 0.05$, $p = 0.006$) and pregnenolone ($\beta = 0.02 \pm 0.01$, $p = 0.04$) concentrations. HAM-A was inversely associated with GABA concentration ($\beta = -0.12 \pm 0.04$, $p = 0.004$) and

positively associated with pregnanolone ($\beta = 0.11 \pm 0.05$, $p = 0.05$).
**Conclusions:** Decreased peripartum plasma GABA and increased pregnanolone in women at-risk for PPD suggest that neurosteroids and their interaction with the GABAergic system may play an important role in the pathophysiology of peripartum depression and anxiety. Ion-molecule based assays enable selective and sensitive, non-derivatizing LC-MS assays for plasma NAS and GABA.
**Keywords:** pregnancy, postpartum depression, neuroactive steroid, GABA, liquid chromatography/mass spectrometry
**Disclosures:** Nothing to disclose.

### M75. Social Stress Impacts the Pathogenesis of SIV Infection and Treatment Response

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**Background:** People living with HIV (PLWH) and treated with antiretroviral therapy (ART) have nearly the same life expectancy as uninfected individuals; however, PLWH suffer from neuropsychiatric and cognitive disorders more frequently than uninfected individuals. Stress and poor medication adherence are commonly cited as the driving variables for co-morbidities among PLWH, but these variables have not systematically been assessed. The extent to which a history of chronic stress impacts HIV/SIV pathogenesis and the response to ART, as well as the interplay between stress, immune activation, and HIV-associated neuropsychiatric disease, remains a critical gap in our collective understanding of HIV treatment response. Rhesus macaques (RM) live in social groups governed by a matrilineal dominance hierarchy and it is well established that subordinate female RM have higher cumulative exposure to chronic social stress than more dominant females, thereby providing an ethologically relevant model to assess the contribution of stress to immunologic and virologic outcomes and treatment response in the macaque correlate of HIV, simian immunodeficiency virus (SIV).
**Methods:** All RMs originated from social groups housed at Yerkes National Primate Research Center with established rank systems prior to isolation for further studies involving SIV or SHIV infection. Within these hierarchies, subordinate animals consistently demonstrate elevated markers of chronic stress compared to dominant ones. In the first study we tested the hypothesis that stress history, as dictated by the subject's social rank prior to study assignment, predicts viral load during chronic SIV infection. Viral loads from five previous studies (four involving SIVmac and one involving SHIVsf162p3) were measured by RTPCR as copies of SIV RNA/ml of plasma, and the relative data were retrospectively compiled and analyzed. All animals' social and medical histories prior to study assignment were obtained from colony records. Retrospective individual animal meta-analysis was conducted on viral load data across the acute, early chronic, and late chronic phases of infection. Furthermore, to understand the impact of chronic stress on SIV pathogenesis and response to ART, we collected social rank information for females who had been previously infected with SIVmac239 or SIVmac251 i.v. and treated with standard ART (PMPA + FTC + RTG + DRV +/- ritonavir boost). Social rank was determined by review of colony records and assessment of matriarchal lineage. Groups were compared using the nonparametric Mann Whitney U test.
**Results:** A total of 62 RMs infected with SIV or SHIV were stratified based on the pre-infection social rank, and then evaluated longitudinally throughout infection for viral load in plasma. We found that, in the acute phase of SIV or SHIV infection, the level of virus replication was not predicted by social rank prior to study assignment ($p = 0.37$). Interestingly, in both early and late chronic phases of the infection, social rank prior to study assignment significantly influenced the level of virus replication ($p = 0.01$ and $p = 0.002$ for subordinate vs. dominant/mid). In the early chronic phases of infection copies of SIV RNA/ml of plasma in subordinate subjects (mean = 5.202 log10 (copies/ml) was elevated compared to subjects that originated from dominant/mid ranks (mean = 4.488 log10 (copies/ml). Also during late chronic infection, higher viral loads were observed in subordinate subjects (mean = 5.806 log10 (copies/ml) compared to subjects from dominant/mid social ranks (mean = 4.213 log10 (copies/ml). We found that SIV-infected RM of low social rank had higher peak viremia than SIV-infected RM of high social rank (median 3.5x107 vs. 1.15x106, $p = 0.0485$). In the second study, viral loads at the start of ART between 7-9 weeks after SIV infection did not differ between social rank groups; however, the time to first undetectable viral load on ART in high ranking RM was a median of 37.5 days compared to 147 days in low ranking RM. In addition, 2/3 low ranking RM never demonstrated consistent suppression of viremia on ART (treatment duration 126-425 days), whereas ART was effective in suppressing viremia in all high ranking RM (median time to consistent suppression was 120 days). CD4 recovery after initiation of ART did not differ between groups.
**Conclusions:** These data demonstrate that social history prior to SIV or SHIV infection can influence pathogenesis and treatment response. Furthermore, these data suggest that treatment variability among humans may be driven by more than poor compliance. A better understanding of the physiological mechanisms by which stress exposure can impact the host response to HIV/SIV may provide a framework for testing therapies aimed at reducing stress-related disorders and bolstering ART in HIV-infected patients.
**Keywords:** life stress, Depression, HIV, Nonhuman Primate Models, Psychoneuroimmunology
**Disclosures:** Nothing to disclose.

### M76. Diversity of Reporter Expression Patterns in Transgenic Mouse Lines Targeting Corticotropin Releasing Hormone-Expressing Neurons

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**Background:** Transgenic rodent models enabling gene-based access to specific cell populations provide potent tools for neuroscience research. The use of Cre-driver lines

in combination with Cre-dependent methods for the regulation of gene expression, visualization of reporters or optogenetic activation / inhibition has yielded a large body of innovative discoveries in brain connectivity and in the contributions of specific neuronal populations–and of molecules produced in specific regions–to crucial brain functions including feeding, reward and addiction, memory and depression. Mouse lines targeting corticotropin-releasing factor (CRF/CRH) has been extensively employed to study stress neurobiology and are poised to revolutionize our understanding of the localization and connectivity of CRH-expressing neurons, and the crucial roles of CRH in normal and pathological conditions. Accurate interpretation of all studies using cell type-specific transgenic mice vitally depends on congruence between expression of the endogenous molecule and reporter: If reporter expression does not faithfully reproduce native gene expression, then effects of manipulating unintentionally-targeted cells may be misattributed.
**Methods:** Here, we studied CRH and reporter expression patterns in three adult transgenic mice: Crh-IRES-Cre;Ai14 (tdTomato mouse); Crfp3.0CreGFP, and Crh-GFP BAC. As gold standard, we employed the CRH antiserum generated by Vale, validating its specificity using CRH-null mice. We focused the analyses on stress-salient regions including hypothalamus, amygdala, bed nucleus of the stria terminalis (BNST) and hippocampus.
**Results:** Expression patterns of endogenous CRH were consistent among wild-type (WT) and transgenic mice. In tdTomato mice, most CRH-expressing neurons co-expressed the reporter, yet the reporter identified a few non-CRH-expressing pyramidal-like cells in hippocampal CA3. In Crfp3.0CreGFP mice, co-expression of CRH and the reporter was found in central amygdala and, less commonly, in other evaluated regions. In Crh-GFP BAC mice, the large majority of neurons expressed either CRH or reporter, with little overlap.
**Conclusions:** These data highlight significant diversity in concordant expression of reporter and endogenous CRH among three available transgenic mice. These findings should be instrumental in interpreting important scientific findings emerging from the use of these potent neurobiological tools.
**Keywords:** Animal Models, transgenic mice, optogenetics, Corticotropin-Releasing Hormone, stress
**Disclosures:** Nothing to disclose.

### M77. Role of AMPA Receptor Stimulation in the mPFC and Subsequent Serotonin Neuron Activation in Antidepressant Effects of an mGlu2/3 Receptor Antagonist and Ketamine

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**Background:** Metabotropic glutamate (mGlu) 2/3 receptor antagonists have been demonstrated antidepressant effects in animal models, and some of the mechanisms underlying antidepressant effects may be shared by ketamine, which raise the possibility that mGlu2/3 receptor antagonists could be an alternative to ketamine. Both mGlu2/3 receptor antagonists and ketamine reportedly increased serotonin release in the medial prefrontal cortex (mPFC), through AMPA receptor stimulation. However, neural mechanisms to activate serotonergic system by both mGlu2/3 receptor antagonists and ketamine have not been fully understood.
**Methods:** Antidepressant effects of an mGlu2/3 receptor antagonist (LY341495) and ketamine were evaluated in the forced swimming test of mice. To investigate roles of the mPFC, LY341495, ketamine or an AMPA receptor antagonist (NBQX) was injected locally into the mPFC. Roles of serotonergic system were investigated by depletion of serotonin with para-chlorophenylalanine (PCPA) and by immunohistochemical methods.
**Results:** Intraperitoneal administration of LY341495 and ketamine as well as microinjection of these compounds into the mPFC exhibited antidepressant effects in the forced swimming test of mice, which lasted at least for 24 h. Both acute and sustained antidepressant effects following either systemic administration or microinjection of these compounds were attenuated by depletion of serotonin with PCPA treatment. In addition, antidepressant effects of LY341495 and ketamine were attenuated by microinjection of NBQX into the mPFC, indicating that both an mGlu2/3 receptor antagonist and ketamine exert the antidepressant effects through AMPA receptor stimulation in the mPFC. We also found that both compounds, following intraperitoneal administration and microinjection into the mPFC, increased the c-Fos expression in the serotonin neurons in the dorsal raphe nucleus (DRN). Increase in c-Fos expression in the serotonin neurons by LY341495 or ketamine was blocked by microinjection of NBQX into the mPFC, suggesting that both compounds may activate subsets of serotonin neurons in the DRN through AMPA receptor stimulation in the mPFC.
**Conclusions:** These studies revealed that both an mGlu2/3 receptor antagonist and ketamine exert antidepressant effects through the actions in the mPFC. Moreover, our results suggest that activation of serotonin neurons in the DRN regulated by stimulation of the AMPA receptor in the mPFC may be involved in the antidepressant effects of an mGlu2/3 receptor antagonist and ketamine. Therefore, mGlu2/3 receptor antagonists may share mechanisms underlying antidepressant effects with ketamine at neural levels, which strengthen hypothesis that mGlu2/3 receptor antagonists may be useful as an alternative approach to treating patients with TRD.
**Keywords:** mGlu2/3 receptor, Antidepressant, Ketamine, ampa receptor, Serotonin
**Disclosures:** Authors are employees of Taisho Pharmaceutical Co., Ltd.

### M78. Affective Neurodynamics Predict Depression Treatment Response

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**Background:** Depression is a debilitating illness that causes suffering world-wide. There are many empirically

supported treatments, but few biomarkers predict whether an individual will improve. Developing biological tests to predict treatment response across treatment approaches could facilitate improved outcomes. In parallel, abnormalities in affective processing are core to depression. The majority of neuroimaging work has examined the magnitude of neural responses to emotion in depression, yet other affective neurodynamics may help to characterize the illness and predict treatment response.
**Methods:** Thirty-nine unmedicated patients with major depressive disorder were scanned using event-related fMRI in an emotional regulation paradigm. Patients then received an eight-week trial of interpersonal psychotherapy (IPT), venlafaxine or fluoxetine. Hamilton Rating Scale of Depression (HAM-D) was collected pretreatment and at eight-weeks to assess symptom severity. To examine whether affective neurodynamics predicted treatment response, we examined the time-to-peak of fMRI BOLD activity in response to affective stimuli (IAPS slides).
**Results:** Across all three-treatment types, a more rapid neural response in the medial Prefrontal Cortex (mPFC) to both positive and negative emotional stimuli predicted better treatment response (a greater drop in HAM-D). Furthermore, a more rapid neural response in the amygdala to negative, but not positive stimuli predicted a poorer outcome across treatments. Effects were significant when controlling for pretreatment treatment HAM-D score so cannot be attributable to initial severity. These effects were also significant when examining more traditional neural markers such as the amplitude of fMRI BOLD activity indicating that time-to-peak may be a unique neural biomarker predicting treatment response.
**Conclusions:** Across three separate treatments for depression (both psychotherapy and pharmacotherapy), we found that a specific affective neurodynamic predicted treatment response over an eight-week trial. These findings extend research that has solely examined the magnitude of neural responding, in that rapid responses in structures important for emotional regulation, such as the mPFC predicted better treatment outcome. Conversely, rapid time-to-peak of subcortical areas, such as the amygdala appeared to be maladaptive. Taken together, these data suggest that regardless of treatment type, rapid engagement of regulatory circuits facilitate treatment response while rapid engagement of subcortical emotional processing areas may be disadvantageous.
**Keywords:** Major depression, treatment outcome prediction, emotion processing, Medial Prefrontal Cortex, Amygdala
**Disclosures:** Dr. Kalin has served on scientific advisory boards for Corcept Therapeutics, Neuronetics, CeNeRx BioPharma, and Skyland Trail; is a stockholder with equity options in Corcept Therapeutics and CeNeRx BioPharma; owned Promoter Neurosciences; and holds patents for promoter sequences for corticotropin-releasing factor CRF2alpha and a method of identifying agents that alter the activity of the promoter sequences, promoter sequences for urocortin II and the use thereof, and promoter sequences for corticotropin-releasing factor binding protein and the use thereof. All other authors report no conflicts of interest.

## M79. GPR139, an Orphan Receptor Highly Enriched in the Habenula and Septum, is Activated by the Essential Amino Acids L-Tryptophan and L-Phenylalanine

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**Background:** G-protein coupled receptors (GPCRs) are among the most pursued targets for drug development. More than 30% of the drugs currently on the market target GPCRs yet, to date, they only affect a small proportion of all known GPCRs. For this reason, orphan GPCRs represent an attractive source of new targets for drug discovery research. GPR139 (aka GPRg1 or GPCR12) was identified as a novel Rhodopsin GPCR having exclusive expression in the central nervous system. A pharmacophore model based on known surrogate GPR139 agonists was recently disclosed to propose L-tryptophan (L-Trp) and L-phenylalanine (L-Phe) as putative endogenous ligands for GPR139 (Isberg et al., J. Chem. Inf. Model, 2014, 54, 1553-1557). The goal of the present study was to identify the physiological ligand for GPR139 using an experimental approach.
**Methods:** GPR139 receptor activity from recombinant cells following treatment with amino acids, various orphan ligands as well as serum and tissue extracts was measured using a guanosine 5'-O-(3-[35S]thio)-triphosphate binding assay. Effects of the natural ligand on calcium mobilization and extracellular signal-regulated kinases phosphorylation in recombinant systems were also tested. High throughput screening was carried out to identify novel tool compounds to study GPR139 function. A high affinity agonist was identified then radiolabeled and used to develop a radioligand binding assay in membranes from cells transfected with GPR139. GPR139 orthologues from various species were compared. RNA sequencing was applied to study the expression of GPR139 in human and rat central nervous system. The distribution of GPR139 was examined in greater detail in the mouse brain using an antibody specific for GPR139 and by using beta -galactosidase as a marker for GPR139 expressing cells in brains from GPR139 null lacZ knock-in mice. Lastly, a selective small molecule agonist and its less active enantiomer were tested for their effects on spontaneous locomotor activity in rats.
**Results:** The amino acids, L-Trp and L-Phe were both found to activate GPR139, with EC50 values in the 30-300 uM range, consistent with the physiological concentrations of L-Trp and L-Phe. Chromatography of rat brain, rat serum, and human serum extracts revealed two peaks of GPR139 activity which corresponded to the elution peaks of L-Trp and L-Phe. A selective small molecule agonist (JNJ-63533054, (S)-3-chloro-N-(2-oxo-2-((1-phenylethyl)amino)ethyl) benzamide) with low nM affinity and potency was identified. The tritium labelled compound bound to GPR139 and could be specifically displaced by L-Trp and L-Phe. Sequence alignment revealed that GPR139 is highly conserved across species. RNA sequencing studies of rat

and human tissues indicated its exclusive expression in brain and pituitary gland. Immunohistochemical analysis showed specific expression of GPR139 in circumventricular regions of the habenula and septum in mice. The small molecule agonist but not its less active enantiomer decreased spontaneous locomotor activity in rats.
**Conclusions:** In susceptible humans and in some animal models, reduced Trp and Phe intake has been shown to be depressogenic, whereas anecdotal reports of Trp and Phe loading are reported to have mood elevating affects. The effects of altered Trp and Phe levels on behavior have been hypothesized to be mediated by their downstream conversion to serotonin or dopamine. The localization of GPR139 expressing neurons in circumventricular brain regions potentially places the receptor in the correct position for sensing L-Trp and L-Phe levels in circulating cerebrospinal fluid. Our findings suggest that L-Trp and L-Phe are likely physiological ligands for GPR139 which may underlie the biological and behavioral effects of these substances without relying on their conversion to biogenic amines. We hypothesize that this receptor may act as a sensor to detect dynamic changes of brain L-Trp and L-Phe under physiological conditions.
**Keywords:** GPCR, Serotonin, Dopamine, Lateral Habenula, septum
**Disclosures:** Janssen Research & Development, LLC

### M80. Central Modulation of Parasympathetic Response to Negative Affect is Disrupted in Major Depression: Impact of Sex

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**Background:** Reduced parasympathetic cardiac regulation is significantly associated with the comorbidity of major depression and cardiovascular disease, which has twice the risk in women vs. men. Several studies have evaluated the brain circuitry correlates of autonomic modulation during emotional tasks in healthy participants. However the neural mechanisms responsible for impaired vagal regulation in major depression remain unknown. In the present study, we tested the hypothesis that alterations in functional connectivity between areas of the central autonomic network (CAN) in subjects with major depression are associated with decreased parasympathetic response to negative affective stimuli. We further predicted sex differences in deficits in connectivity in CAN regions and vagal activity will be associated with dysregulation of the hypothalamic pituitary gonadal (HPG) axis in depressed women.
**Methods:** Twenty subjects with recurrent major depression in remission (47.5 ± 1.3 years; 10 females) and 16 healthy controls (46.9 ± 1.7 years; 9 females) were included in the study. Baseline serum levels of 17ß-estradiol were analyzed using commercial immunoassay kits. Functional MRI data were acquired on a Siemens Tim Trio 3T MRI scanner with a 12-channel head coil (TR = 2000 ms, TE = 40 ms, FOV = 200x200 mm, matrix 64x64, slice thickness 5 mm). The fMRI task consisted of presentation of blocks of negative valence/high arousal, neutral valence/low arousal, and fixation images adapted from the International Affective Picture System (IAPS), comprising a mild visual stress challenge. A photoplethysmography sensor was used during the task to collect heart rate data. fMRI data were preprocessed using SPM8 and included EPI unwarping, motion correction, spatial smoothing and artifact detection. A one-sample t-test was used to examine the BOLD response to negative > neutral, with a whole-brain, voxel-wise FWE-corrected threshold of $p < 0.05$. Next an intersection analysis was performed to identify clusters from this analysis conjointly located within anatomic boundaries of CAN regions (amygdala, hippocampus, hypothalamus, orbitofrontal cortex, anterior cingulate cortex and medial prefrontal cortex).
Task-related connectivity using generalized psychophysiological interaction (gPPI) with the time courses from clusters located in seed ROIs and two PPI regressors (interaction of the seed time course with the regressors for negative and neutral content) was performed. Connectivity was measured at the single-subject level by estimating the difference between the interaction of the seed timecourse with the regressor for negative compared with the neutral stimuli, conducted separately for each ROI. Results of single-subject analysis were then entered into second-level random effects analysis to probe group-level changes in connectivity during negative versus neutral condition. The time interval between two successive peaks of pulse pressure waves was used to estimate R-R intervals. An adaptive point-process algorithm was applied to these recordings to compute variations in instantaneous estimates of the High Frequency component of heart rate variability (HF, 0.15 to 0.4 Hz). Correlation analyses between changes in functional connectivity (beta weights of PPI regressors) and variations in HF power were performed, followed by interactions with case status and sex.
**Results:** A higher HF change in response to negative vs. neutral images was associated with increased BOLD response in left amygdala ($t(15) = 2.78$, $p = 0.01$, $R2 = 0.36$) and lower right amygdala-right hippocampus connectivity ($t(15) = -4.10$, $p = 0.001$, $R2 = -0.55$) in healthy controls. No such relationship was present among depressed subjects ($t(19) = 1.27$, $p = 0.22$), ($t(19) = -1.01$, $p = 0.29$). Further, baseline 17β-estradiol levels in healthy females were significantly associated with right amygdala-hippocampus connectivity ($p = 0.01$, $r = -0.76$) and HF changes ($t(8) = 3.20$, $p = 0.02$, $R2 = 0.59$), correlations that were non-significant in depressed women ($p = 0.38$, $r = -0.21$), ($t(8) = 0.69$, $p = 0.51$).
**Conclusions:** Our results are consistent with previous reports in healthy populations showing bilateral amygdala, right hippocampus, and left hypothalamus as primary parasympathetic regulatory centers in response to negative affective stimuli. We extend these findings to demonstrate the lack of significant associations between the functional connectivity of these areas and HF power variations in depressed subjects, suggesting a disruption in the central mechanisms regulating cardiac vagal activity and potential explanation for altered parasympathetic responses to

psychological stress observed in major depression. Further, connectivity deficits were primarily among female cases, a finding that was, in part, explained by disruption of HPG axis function, specifically 17β-estradiol. Thus, loss of estradiol's regulatory actions on areas of the central autonomic network, regions densely populated with sex steroid receptors, suggests potential physiologic mechanisms for understanding sex differences in the comorbidity of major depression and cardiovascular disease.
**Keywords:** Major depression, Central autonomic network, heart rate variability, fMRI Functional Connectivity, sex differences
**Disclosures:** Nothing to disclose.

### M81. Adjuvant Thiamine Improved Standard Treatment in Patients with Major Depressive Disorder: Results from a Randomized, Double Blind, and Placebo-Controlled Clinical Trial

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**Background:** Given that antidepressants (ADs) work slowly, efforts are made to accelerate the therapeutic effect and to reduce side effects. In this regard, thiamine (vitamin B1) has gained increased interest. Thiamine is an essential nutrient, and thiamine deficiency leads to a broad variety of diseases, including irritability and symptoms of depression. On the flip side, regular thiamine intake has the potential to increase mood and to reduce stress perception. Thus, in search of further pharmacological avenues to treat patients with MDD, we tested the hypothesis that adjuvant thiamine improves depression, compared to placebo.
**Methods:** A total of 51 inpatients (mean age: 35.2 years; 47% females) with MDD (HDRS at baseline: $> 24$) took part in the study. A standardized treatment with SSRI was introduced and kept at therapeutic levels throughout the study. Further, patients were randomly assigned either to the thiamine or the placebo condition. Experts rated (HDRS) symptoms of depression at baseline, and after 3, 6, and 12 weeks (end of the study).
**Results:** From baseline to the study end 12 weeks later, depression improved in both groups. Compared to placebo, adjuvant thiamine improved symptoms of depression after six week till the end of the study. No adverse side effects were reported in both groups. Remission rates did not differ between the thiamine and placebo condition, at 12 weeks.
**Conclusions:** Results from the present randomized, double blind and placebo-controlled trial suggest, that among a sample of younger patients with MDD, adjuvant thiamine improved symptoms of depression, compared to placebo. Importantly, improvements were observed already after six weeks of treatment start. We hold that accelerated improvements with thiamine are important, given that maximum adherence to AD-treatment is about 50%, which most probably is due to a slow antidepressant effect of ADs. Thus, thiamine seems to have the potential to counteract the time lag of antidepressant effects of ADs.
**Keywords:** Adjuvant thiamine, Major Depressive Disorder, placebo-controlled trial
**Disclosures:** Nothing to disclose.

### M82. First-Episode Bipolar Disorder is Associated with Erythrocyte Membrane Docosahexaenoic Acid Deficits: Dissociation from Clinical Response to Lithium or Quetiapine

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**Background:** A growing body of evidence suggests that low habitual dietary intake of long-chain omega-3 (LCn-3) fatty acids, including eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), may be associated with the pathophysiology of mood disorders including major depressive disorder and bipolar disorder. Erythrocyte (red blood cell) membrane EPA + DHA composition is highly correlated with habitual intake of fish or fish oil and represents a valid biomarker of LCn-3 fatty acid biostatus. Case-control studies have observed reduced erythrocyte EPA and/or DHA levels in adults and adolescents with depression and in medicated and medication-free bipolar subjects. While depression frequently precedes the initial onset of mania, it is not currently known whether LCn-3 fatty acid deficits coincide with the initial onset of mania or are a consequence of chronic illness. Moreover, the relationship between LCn-3 fatty acid status and symptom response to pharmacologic treatments has not been evaluated prospectively from a medication-free baseline. The present study compared erythrocyte fatty acid composition in medication-free first-episode bipolar manic patients and healthy comparison subjects, and prospectively investigated the effects of 8- or 52-week treatment with either lithium or quetiapine on erythrocyte fatty acid composition and mood symptom severity.
**Methods:** Erythrocyte membrane fatty acid composition was determined in medication-free first-episode bipolar manic or mixed patients ($n = 40$) and healthy subjects ($n = 40$) by gas chromatography. Mood symptom ratings were obtained with the Young Mania Rating Scale (YMRS) and the Hamilton Depression Rating Scale (HDRS). Erythrocyte fatty acid composition and clinical ratings were also determined within a sub-group of bipolar subjects following 8-week ($n = 19$) or 52-week ($n = 11$) open-label treatment with lithium or quetiapine.
**Results:** Baseline erythrocyte DHA composition was significantly lower in subjects with bipolar disorder than healthy controls (-23%, $p \leq 0.0001$), and there was a trend for lower EPA ($p = 0.08$) but not DPA (22:5n-3)($p = 0.64$). The sum of LCn-3 fatty acids (EPA + DPA + DHA, -15%, $p = 0.0002$) and EPA + DHA ('omega-3' index)(-20%, $p \leq 0.0001$) were significantly lower in the bipolar group. A significantly greater number of bipolar subjects (93%) exhibited an omega-3 index (EPA + DHA) of $\leq 4.0$ percent (range: 1.7-4.9%) compared with healthy subjects (67%, range: 2.4-7.5%)($p = 0.01$). Erythrocyte AA composition did

not differ between groups ($p = 0.89$), and the AA/DHA ($+22\%$, $p \leq 0.0001$) and AA/EPA + DHA ($+22\%$, $p \leq 0.0001$) ratios were significantly greater in bipolar subjects. Other major saturated and monounsaturated fatty acids did not differ between groups. Following 8- or 52-week treatment with lithium or quetiapine, YMRS and HDRS total scores decreased significantly whereas erythrocyte fatty acids including DHA did not change.

**Conclusions:** This study demonstrates that significant erythrocyte DHA deficits coincide with the initial onset of bipolar mania, and that reductions in mood symptom severity following lithium or quetiapine are not associated with alterations in erythrocyte fatty acid levels. Reductions in mood symptom severity following lithium or quetiapine may be mediated through interrelated mechanisms including a reduction in elevated pro-inflammatory signaling secondary to LCn-3 fatty acid deficits.

**Keywords:** Bipolar Disorder, Omega-3 Fatty Acid, Lithium response

**Disclosures:** Nothing to disclose.

### M83. Change in Incidence of Suicidal Behavior among Antidepressant Clinical Trial Participants: 1991-2013

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**Background:** There has been ongoing concern about increased suicide risk among depressed patients assigned to placebo in antidepressant clinical trials. A retrospective, post-hoc analysis of the US FDA database (Khan et al., 2000) revealed that the suicide risk among those assigned to antidepressants or placebo was similar in antidepressant clinical trials. However, further examination of this data (Healey et al., 2003) and evaluation of pediatric clinical trial data raised concerns that the opposite may be true. In other words, it was suggested that antidepressants may increase suicide risk and the regulators in the US inserted a boxed warning about such a possibility.

However, no further prospective research or an examination of the data from more recent antidepressant clinical trials has occurred. In this study, we examined the incidence of suicidal behavior as measured by suicides and suicide attempts in antidepressant clinical trials according to the year of the drug's FDA approval; we compared suicidal behavior in trials of drugs approved prior to 2000 and those approved after 2000.

**Methods:** Our evaluation consisted of a review of all monotherapy medications approved by the US FDA for treatment of depression as new drug approval (NDA) after the year 1991. We specifically chose the Integrated Safety Summary (ISS) reports that quantify all serious adverse outcomes. We re-reviewed data from the earlier reports: sertraline (1991), paroxetine (1992), venlafaxine (1993), nefazodone (1994), mirtazapine (1996), venlafaxine XR (1997) and citalopram (1998); comparing them to the data from NDA reports after the year 2000: escitalopram (2002), duloxetine (2004), desvenlafaxine (2008), trazodone ER (2010), vilazodone (2011), levomilnacipran (2013), and vortioxetine (2013). For each program we tabulated exposure data, number of suicide attempts and number of suicides. This was followed by a calculation of the suicide risk and suicide attempt prevalence per 100,000 patient exposure years (PEY) for each individual NDA program, and for the programs approved prior to 2000 as well as those approved after 2000. We conducted a chi square analysis to evaluate any difference in suicidal behavior during the two time periods.

**Results:** There were 47,472 patients enrolled in the 14 NDA programs (38,656 assigned to antidepressants and 8,816 assigned to placebo). The seven NDA programs approved prior to the year 2000 enrolled 27,020 (23,461 assigned to antidepressants and 3,559 assigned to placebo). The seven NDA programs approved after 2000 enrolled 20,452 (23,461 assigned to antidepressants and 5,257 assigned to placebo). The total exposure for all of the patients enrolled in the programs was 13,547 years. There were 7,512 exposure years (6,664 for antidepressants, 848 for placebo) in the NDA programs approved prior to 2000 and 6,035 exposure years (5,461 for antidepressants, 574 for placebo) in the NDA programs approved after the year 2000.

Among the 14 NDA programs, there were 52 suicides, with 48 (92%) occurring among the 7 NDA programs approved prior to 2000 compared to only 4 occurring in the NDA programs approved after 2000 ($\square 2 = 27.2$, $p < 0.0001$). A similar pattern was seen in the frequency of suicide attempts. Of the 258 depressed patients who attempted suicide, 231 (89.5%) occurred in the NDA programs approved prior to 2000 compared to 27 occurred in the NDA programs approved after 2000 ($\square 2 = 123.7$, $p < 0.0001$). Using the PEY model, the rate of suicide was 639/100K PEY in the NDA programs approved prior to 2000 and was 66.3/100K PEY in the NDA programs approved after 2000. The rate of suicide attempt was 3,075/100K PEY in the NDA programs approved prior to 2000 and was 445/100K PEY in the NDA programs approved after the year 2000.

**Conclusions:** Contrary to expectations, the suicidal behavior including suicides and attempted suicides was approximately ten fold lower among depressed patients participating in the NDA programs approved after 2000 compared to the NDA programs approved prior to 2000. Such a dramatic change was not expected and as such there is no easy explanation as to this large change.

The suicide risk for US adult population remains about the same. There are no significant changes in the inclusion/exclusion criteria of the more recent antidepressant trials versus the earlier ones regarding factors associated with suicide risk. The only quantifiable change in this regard is the relative stringency in more recent antidepressant trials about including patients who are either treatment resistant or refractory to one or more antidepressants being excluded.

Part of the explanation may be a Hawthorne effect that comes into play when a phenomenon comes under close scrutiny. Specifically, close scrutiny of antidepressant trials regarding suicide risk may have changed the nature of the conduct of trials themselves.

More importantly, these data illustrate the weaknesses of post-hoc analysis. Specifically, the weakness seems to lie in the concept that firm conclusions cannot be drawn about

the results of trials whose primary hypothesis was different from the question being asked.
In conclusion, the results of our study suggest that suicidal behavior is susceptible to large fluctuations in adult antidepressant clinical trials. Thus, data from antidepressant clinical trials may not accurately inform the practicing clinician about suicide risk with antidepressants, including such factors boxed warnings by regulators. Further research is needed.
**Keywords:** Antidepressant, suicide, clinical trials
**Disclosures:** Nothing to disclose.

### M84. Transglutaminase 2-Mediated Regulation of GABAA Receptor in Depression

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**Background:** Major depression is one of the most prevalent debilitating illnesses worldwide causing an enormous personal and economic burden. Although the therapeutic options for this disorder have improved over time, it is sobering that depression is still characterized by persistent functional impairments for a large percentage of patients. Therefore, a major unmet medical need exists for more efficacious treatment strategies for depression. Interestingly, there is a growing body of clinical and preclinical evidence suggesting that gamma-amino butyric acid (GABA), the major inhibitory neurotransmitter in the brain, may have an important role in depression and its treatment. Chronic stress and stress-induced release of glucocorticoids have been shown to alter the expression and function of GABAA receptors (GABAARs). Moreover, both human studies, where GABAARs were found to be altered in the postmortem brains of depressed subjects, and animal experiments, where GABAA agonists were found to improve depressive behaviors in rodent stress models, suggest an important role of GABAARs (and their regulation) as therapeutic targets in depression. However, the factors that link chronic stress to GABAAR dysregulation have not been elucidated. In the present study, experiments were conducted to begin the process of elucidating the role of transglutaminase 2 (TG2) in the regulation of GABAAR in depression. TG2 is a calcium dependent enzyme that plays an important role in posttranslational modification of proteins. We hypothesized that increased TG2-dependent reduction in GABAA$\alpha$2 induces depression-like behavior in mice.
**Methods:** Stress conditions were induced in adult male mice using chronic unpredictable stress (CUS), chronic corticosterone exposure, or repeated restraint. Protein levels were determined by western blot analysis. Behavior tests related to depressive behavior were performed. Postmortem prefrontal cortex samples from depressed suicide and control subjects were obtained from McGill Group for Suicide Studies and Douglas Research Institute.
**Results:** We found that chronic unpredictable stress, chronic corticosterone exposure, as well as repeated restraint stress in mice leads to decreases in GABAA$\alpha$2 protein levels in PFC. Significant increases in TG2 protein levels were found in the PFC of mice exposed to the same stress-related conditions. Mice with neuronal TG2 overexpression (TG2+/+) showed depression-like behavior with reduced spine density and GABAA$\alpha$2 levels (with no change in GABAA$\alpha$1, GABAA$\beta$3 and GABAA$\gamma$2) in the PFC. TG2 inhibition attenuated CUS-induced depression-like behavior, and reductions in GABAA$\alpha$2 levels in mice. Moreover, an increase in TG2, but a decrease in GABAA$\alpha$2 protein levels, was found in the PFC of depressed suicide subjects.
**Conclusions:** Collectively, these data suggest that TG2 is involved in the regulation of GABAA$\alpha$2 in depression. Given the important role of GABA in neuroplasticity, identifying novel regulatory mechanisms of GABAergic signaling may provide avenues to develop newer therapeutics for stress-related neuropsychiatric disorders.
**Keywords:** DEPRESSION, GABA, TRANSGLUTAMINASE 2
**Disclosures:** Nothing to disclose.

### M85. Telomere Length as a Predictor of Response to Pioglitazone in Patients with Unremitted Depression: A Preliminary Study

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**Background:** Leukocyte telomere length (LTL) and insulin resistance (IR) have both been shown to contribute to age-related diseases, such as cardiovascular disease and type 2 diabetes (DM2). Previous studies have described associations between shortened telomeres and IR and depression. Antidepressant responses to an insulin-sensitizing agent, metformin, and to the PPAR-□ agonists, rosiglitazone and pioglitazone, have been reported in depressed patients. In addition, patients with major depression have been found to exhibit all the cardinal features of inflammation. Because increased inflammatory markers at baseline predict an antidepressant response, reducing inflammation may also augment response to psychotropic medications. Here we aimed to assess LTL as a predictor of antidepressant response to pioglitazone in groups of IR and insulin sensitive (IS) patients with unremitted depression.
**Methods:** Forty-two medically stable men and women ages 23-71 with non-remitted depression participated in a double-blind placebo-controlled add-on of pioglitazone to treatment-as-usual. Among enrolled participants, glucose metabolism ranged across the insulin sensitivity spectrum, including IS, IR, and/or pre-diabetes. Oral glucose tolerance tests (OGTT) were administered at baseline and at 12 weeks. Blood samples were obtained to measure baseline glucose and insulin concentrations, followed by administration of 75mg of oral glucose, after which additional samples were obtained at +30, +60, +90, and +120 minutes. Diagnostic evaluation of psychiatric disorders was performed at baseline and mood severity was followed weekly. The psychiatric examination at screening included the Structured Clinical Interview for DSM-IV (SCID), the 17-item Hamilton Depression Rating Scale (HDRS-21), and the

MMSE. Administration of the HDRS-21 was repeated at each interim visit (Weeks 2, 4, 6, and 8) and at the end-of-treatment. Statistical analyses were conducted using Statistical Analysis System 9.4. T-tests were conducted in order to assess baseline differences between groups. Correlations and linear regression analysis were used to assess for the association between baseline telomere length and treatment response, including change in HDRS-21 and change in OGTT.

**Results:** The demographics and clinical characteristics for the forty-two participants are presented in Table 1. At baseline, no differences in LTL were detected by depression severity, duration or chronicity. LTL was also not significantly different between IR and IS subjects at baseline. Subjects with longer telomeres exhibited greater declines in depression severity in the active group, but not in a placebo group ($p = .005$, $r = -.63$, CI 95% = (-0.84, -0.21)). Linear regression analysis of the active group predicted that for every .1 T/S increase in LTL, HDRS-21 score decreased by an additional 1.5 points, $\beta = -14.98$, $t(22) = -3.24$, $p = .005$. In addition, LTL predicted improvement in insulin sensitivity in the group overall and did not differ between intervention arms ($p = .036$, $r = -.44$, CI 95% = (-0.74, 0.02) for the active arm, and $p = .026$, $r = -.50$, CI 95% = (-0.78,-0.03) for placebo arm). Linear regression analysis predicted within the placebo group that for every .1 T/S unit longer LTL, there is a 13.6 mg/dL decrease in OGTT values, $\beta = -137.38$, $t(20) = -2.44$, $p = .026$. Similarly, regression analyses revealed that within the active group that for every .1 T/S longer LTL, there is a 6.6 mg/dL decrease in OGTT values, $\beta = -66.96$, $t(22) = -2.25$, $p = .036$. Adjusting for age was non-significant and did not change outcome of models. While there were no differences in LTL between IR and IS subjects at baseline, LTL strongly predicted change in insulin sensitivity in both active and placebo arm. While the correlation between LTL and change in depression severity as well as correlation with baseline IR was significant, the strength of correlation was stronger between LTL and antidepressant response.

**Conclusions:** The conceptual framework for the parent study was based upon a model wherein treatment of underlying IR in patients with non-remitted depression will improve treatment outcome. In this report we assessed LTL as a predictor of treatment outcome. The main finding was a significant association between the LTL and antidepressant response in active arm, but not in placebo arm in persons with unremitted depression. Understanding the role of biomarkers in accelerated aging for prediction of response to treatment of depression is based on a fundamental biological premise of shortened life span as a result of environmental insults. Among the most common mediators of accelerated aging in depressive disorders are metabolic dysfunction, e.g. IR, inflammation and oxidative stress. Chronic exposure to inflammatory cytokines, oxidative stress and glucocorticoids may also accelerate telomere shortening (7). IR is a proinflammatory state and is in turn associated with oxidative stress. Our results suggest that both IR and inflammation may mediate an antidepressant response to PPAR-□ agonist, as both IR and IS at baseline subjects exhibited improvement in depression severity (4). We observed an inverse association between baseline LTL and change in HDRS score in the active (PPAR-□ agonist), but not in placebo group. Our results augment current understanding of the mechanisms of antidepressant response, and if replicated in larger samples, will aid in predicting treatment outcome in a depressive disorder.

**Keywords:** leukocyte telomere length, depressive disorders, insulin resistance, antidepressant response

**Disclosures:** Nothing to disclose.

## M86. A Randomized, Placebo-Controlled Adjunctive Trial of Riluzole in Treatment-Resistant Major Depressive Disorder

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**Background:** Preclinical studies have shown that riluzole, a FDA-approved drug for amyotrophic lateral sclerosis, modulates glutamate release and clearance, and has potent neuroprotective properties. Riluzole has shown antidepressant-like effects in rodent models used to screen for antidepressant activity. In addition, several small open-label clinical studies have suggested that riluzole has antidepressant and anxiolytic properties, even in patients resistant to conventional monoaminergic medications. The aim of this NIMH-sponsored collaborative study was to examine the antidepressant efficacy and safety of riluzole, by conducting the first double-blind, placebo-controlled trial of this agent in adults with major depressive disorder (MDD) who were inadequately responsive to antidepressant medication.

**Methods:** Patients were enrolled at three academic medical centers (Baylor College of Medicine, Massachusetts General Hospital, Yale University School of Medicine), with oversight by a NIMH Data Safety and Monitoring Board. Patients were between the ages of 18-65, met DSM-IV criteria for MDD, and had at least a moderate level of depressive severity, indexed by an Inventory of Depressive Symptomatology-Self Rated (IDS-SR) score of $>20$ and a Montgomery Asberg Depression Rating Scale (MADRS) score of 18 or higher. Exclusion criteria included patients with serious suicide risk, unstable medical illness, substance use disorders within the last 6 months, lifetime histories of bipolar disorder or psychotic disorders, and those who had failed to respond to 3 or more adequate antidepressant trials during the current major depressive episode. Patients meeting initial eligibility criteria were assigned to one of 2 groups (A or B), depending on whether they were receiving concurrent antidepressant treatment at Screening. MDD patients not taking an antidepressant (Group A) were given an 8-week prospective trial of open-label sertraline (flexibly dosed to 150 mg/day). Following the 8 week sertraline treatment period, Group A patients were eligible for a subsequent randomized, placebo-controlled double-blind phase if they continued to meet depressive severity thresholds and had $<$ 50% decrease in the IDS-SR total score. Group B participants were individuals receiving an adequate dose of a SSRI, SNRI, or bupropion for at least 8 weeks, and

were taking a stable dose for at least 4 weeks prior to randomization. A sequential parallel comparison design was used for the 56 day double-blind, randomized adjunctive, placebo-controlled trial, which comprised two phases of approximately 28 days each. Patients were randomized to adjunctive treatment with either riluzole (50 mg BID) or placebo, with a 2:3:3 ratio for random assignment to the treatment sequences drug/drug, placebo/placebo, and placebo/drug, respectively. Clinical assessments were performed by trained raters every 7 days during the double-blind treatment period, followed by a 7 day taper period. The primary outcome was the change in the MADRS from baseline to the end of the double-blind treatment period. Secondary outcomes include the response rate, defined as at least a 50% improvement in MADRS compared to baseline. Safety and tolerability was assessed with the Systematic Assessment for Treatment Emergent Events (SAFTEE-SI).

**Results:** Enrollment occurred between June 2011 and December 2014, with the final study visit completed in February 2015. Across the three sites, 104 patients were randomized, and 85 patients completed the 8 week double-blind placebo phase.

For the primary outcome measure, the reduction in baseline MADRS total score using the LOCF analysis, the overall test of treatment differences was not statistically significant [Chi-sq(1) = 2.76, p = 0.10]. Neither of the estimates from the two blocks were statistically significant. The site and baseline MADRS score effects were not statistically significant. These negative results were confirmed using a mixed model imputation analysis.

There were no significant differences in MADRS response rates between riluzole and placebo (z = -0.21, p = 0.83), and there were no significant differences in MADRS remission rates between riluzole and placebo [z = -0.03, p = 0.98].

For the Clinical Global Impression-Severity scale, the overall test of treatment differences was also not statistically significant [Chi-sq(1) = 0.74, p = 0.39]. Neither of the estimates from the two blocks were statistically significant. The site and baseline CGI-S score effects were also not statistically significant. Likewise, for the CGI-Improvement, the overall test of treatment differences was not statistically significant [Chi-sq(1) = 2.24, p = 0.13].

**Conclusions:** A fixed dose of riluzole failed to demonstrate adjunctive antidepressant efficacy compared to placebo. The sequential parallel comparison design resulted in an acceptably low placebo response rate, suggesting that the failure of riluzole to separate from placebo was not confounded by an excessively high placebo response in this patient population. Additional planned analyses of neurotrophic factor expression might shed light on the relationship between clinical outcomes and riluzole treatment.

**Keywords:** riluzole, glutamate, clinical trial, major depression

**Disclosures:** Dr. Sanacora holds shares in BioHaven Pharmaceuticals Holding Company and is a co-inventor on a US patent (#8,778,979) held by Yale University. Sanofi provided riluzole and matching placebo for this NIH funded study. Funded by NIMH collaborative R01 MH085054 (Drs. Mathew, Fava, Sanacora)

## M87. What Doesn't Kill You: Risk and Resilience for New Onset Depression During the Menopause Transition

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**Background:** Stress exposures may have a differential impact on risk and resilience for depression depending upon their timing across development. From puberty to post-menopause, women are twice as likely as men to experience depression and risk is further accentuated during the transition to menopause suggesting a role for ovarian hormone fluctuations. Preclinical studies emphasize the impact of early life stress (ELS) on neurotransmitter systems and brain structures that are themselves targets of reproductive hormones such as estradiol. We sought to test the hypothesis that adverse childhood experiences (ACEs) would 1) increase risk for new onset major depressive disorder (MDD) during the transition from pre- to post-menopausal states, and 2) that number and timing of onset of ACE(s) with respect to puberty would modify this risk.

**Methods:** Two-hundred-forty-three post-menopausal women from the Penn Ovarian Aging Study (POAS) completed the ACE Questionnaire (ACE-Q) at study end. The POAS is a longitudinal community cohort of women who were all regularly menstruating, pre-menopausal, healthy and with an intact uterus at study entry. Each underwent extensive behavioral, cognitive and endocrine evaluations approximately yearly for 16 years. Women kept menstrual diaries and MDD was assessed using standardized interviews and/or questionnaires. The ACE-Q is a 10-item questionnaire that addresses three categories of childhood adversity; abuse, neglect and household/family dysfunction. ACEs that first occurred 2 or more years before menarche were considered pre-pubertal. All other ACEs were considered to be post-pubertal in onset. Incident menopause MDD was defined as first onset of the disorder in the peri- to post-menopause transition. Menopause stages were defined by STAW criteria and incident menopause MDD occurred in stage 2 (at least one menstrual cycle in the previous year was long or short by at least 7 days) or beyond.

**Results:** In this sample, 39.5%, 22.2% and 38.3% of women reported having experienced 0, 1, or 2+ ACEs before the age of 18. The most commonly reported ACEs were emotional abuse (24%), divorce (22%), familial alcohol or substance abuse (22%) and physical abuse (21%). Incident menopause MDD occurred in 47% of the 102 of women who reported a lifetime history of MDD. Women reporting 2+ Total ACEs were at significantly greater risk for lifetime (aOR = 1.95, p = 0.034) and incident menopause MDD (aOR = 2.23, p = 0.03) compared to those reporting 0 ACEs. Women with 2+ Post-Pubertal ACEs were 3.4 times more likely to experience incidence menopause MDD (p = 0.039) after controlling for race, smoking, body mass index and employment. Experiencing only one ACE in the pre-pubertal window, regardless of additional ACEs post-puberty, was associated with reduced risk for lifetime and incident menopause MDD.

**Conclusions:** ACEs are common and associated with considerable risk for MDD. However, these data suggest

that timing of early life adversity with respect to puberty impacts risk and resilience for MDD across the female lifespan and during the menopause transition in particular.
**Keywords:** menopause, early life stress, Depression, Risk, Resilience
**Disclosures:** Pfizer, J&J, Merck, Abbott & Abbvie (Personal/family investment)
Shire (Research grant support)

### M88. Adjunctive Brexpiprazole (OPC-34712) in Patients with MDD and Anxiety Symptoms: Results from Post-Hoc Analyses of Two Pivotal Studies

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**Background:** Symptoms of anxiety are prevalent in MDD and are associated with greater illness severity, suicidality, impaired functioning and poor response to antidepressant treatment (ADT). The presence of anxiety symptoms in MDD can be assessed using different definitions, e.g., anxious depression (score $\geq$7 on the HAM-D anxiety/somatization factor, as defined by the STAR*D investigators), or using the new DSM-5 specifier "anxious distress". Brexpiprazole was designed as a serotonin-dopamine activity modulator that is a partial agonist at 5-HT1A and dopamine D2 receptors at similar potency, and an antagonist at 5-HT2A and noradrenaline alpha1B/2C receptors. The efficacy, tolerability and safety of brexpiprazole as adjunctive to antidepressants in the treatment of patients with MDD were evaluated in two pivotal randomized, double-blind, placebo controlled studies. The objective of this post-hoc analysis of the pivotal studies was to assess the efficacy of adjunctive brexpiprazole when added to an ADT in patients with MDD and anxiety symptoms using two definitions 1) Anxious depression; 2) Anxious distress.
**Methods:** Patients with MDD and an inadequate response to 1–3 ADTs were enrolled and received single-blind ADT for 8 weeks. Patients with inadequate response throughout this prospective phase were randomized to ADT + brexpiprazole or ADT + placebo for 6 weeks. Both studies included fixed doses (2 mg [Study 1: NCT01360645]; 1 mg and 3 mg [Study 2: NCT01360632]). In these post-hoc analyses, scores on the specific items of the HAM-D anxiety/somatization factor at randomization (baseline) were used to identify patients with anxious depression. Proxies were used to categorize patients as having anxious distress if they had $\geq$2 symptoms of tension (MADRS item 3 score $\geq$3), restlessness (IDS tem 24 score $\geq$2), concentration (MADRS item 6 score $\geq$3), or apprehension (HAM-D item 10 score $\geq$3) at randomization. The efficacy endpoint was the change in MADRS total score from baseline to Week 6. The analyses were conducted using a Mixed Model Repeated Measure (MMRM) approach with pooled placebo groups.
**Results:** After 8-weeks of prospective antidepressant monotherapy, a total of 49.0% and 55.6% of the patients who had an inadequate response to ADT met the criteria for having anxious depression or anxious distress, respectively. The mean MADRS total score was 28.8 for patients with anxious depression and 29.1 for patients with anxious distress. Adjunctive brexpiprazole showed greater improvement than adjunctive placebo in the change from baseline to Week 6 in the MADRS total score in patients with anxious depression (least square mean differences to placebo + ADT [n = 187]: 1 mg + ADT [n = 97]: -1.42, p = 0.1531; 2 mg + ADT [n = 84]: -2.10, p = 0.0461; 3 mg + ADT [n = 112]: -2.05, p = 0.0324), as well as in patients with anxious distress (least square mean difference to placebo + ADT [n = 209]: 1 mg + ADT [n = 119]: -1.74, p = 0.0583; 2 mg + ADT [n = 103]: -2.95, p = 0.023; 3 mg + ADT [n = 112]: -2.81, p = 0.0027). The presence of anxiety symptoms was not associated with an increased incidence of activating adverse events (akathisia, restlessness, agitation, anxiety, and insomnia).
**Conclusions:** Results show that even after 8 weeks of treatment with an antidepressant monotherapy, about half of patients with inadequate response meet criteria for either anxious depression or anxious distress. The present data suggest that adjunctive brexpiprazole may be efficacious in reducing depressive symptoms in patients with anxious depression or anxious distress, which is an important finding as symptoms of anxiety with MDD suggests a more severe course of illness.
**Keywords:** Anxiety, MDD, Anxious distress, Brexpiprazole
**Disclosures:** Dusan Kostic, Catherine Weiss, Ross A. Baker and Peter Zhang are employees of Otsuka Pharmaceutical Development and Commercialization, Inc.
Emmanuelle Weiller is an employee of H. Lundbeck A/S
Anna Eramo is an employee of Lundbeck LLC
Ruth A. Duffy is an employee of Otsuka America Pharmaceuticals, Inc.

### M89. Using Pet Imaging of Translocator Protein (TSPO) to Investigate the Link between Inflammation and Depression

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**Background:** Neuroinflammation may be a predisposing factor for major depressive disorder (MDD). Translocator protein 18 kDa (TSPO) is a highly expressed protein in glial cells of the brain and, therefore, a potential biomarker of neuroinflammation. TSPO can be accurately quantified using positron emission tomography (PET) and [11C]PBR28, a TSPO tracer developed in our laboratory. Increased TSPO binding in multiple brain regions of unmedicated MDD patients currently experiencing a major depressive episode have been previously reported1. Our current study has three aims. The first aim is to replicate these findings. The second aim is to investigate antidepressant effects on TSPO binding in patients with MDD. The third aim is to determine the relationship of peripheral and central inflammatory markers to TSPO binding.
**Methods:** Unmedicated MDD (n = 13), medicated MDD (n = 10) and healthy control (n = 12) subjects underwent PET imaging using [11C]PBR28. We measured total distribution volume (VT, proportional to Bmax/Kd) using arterial input function and corrected for TSPO genotype. Based on previous post-mortem findings, we chose the

subgenual prefrontal cortex and anterior cingulum as regions of interest and compared VT values obtained in medicated and unmedicated MDD subjects and healthy controls. We also obtained peripheral blood samples and cerebrospinal fluid, for later analysis, to investigate the relationship between peripheral and central inflammatory markers and TSPO binding.

**Results:** The interim results of this ongoing study show no significant differences in TSPO binding in depressed patients compared to healthy subjects in any of the predetermined brain regions. In the anterior cingulate, VT was 12.5% higher in unmedicated MDD patients compared to healthy controls ($p = 0.25$, Cohen's $d = 0.49$) and 10.3 % higher in medicated patients compared to healthy controls ($p = 0.45$, Cohen's $d = 0.31$). In the subgenual cortex, VT was 11.1% higher in both the unmedicated ($p = 0.30$, Cohen's $d = 0.43$) and medicated patients ($p = 0.44$, Cohen's $d = 0.32$) compared to healthy controls. TSPO binding did not correlate to peripheral blood C-reactive protein levels.

**Conclusions:** With about 50% recruitment completed for this study, we have not replicated an earlier study showing increased TSPO binding in brain regions of depressed patients compared to healthy controls. However, based on the Cohen's d effect sizes reported, there is a moderate effect showing increased TSPO binding in the anterior cingulate and subgenual cortex of unmedicated patients. The moderate effect sizes noted indicate that increasing the sample size may result in significant differences, specifically with increased TSPO binding in unmedicated depressed patients. Effect size decreases when medicated patients are compared to healthy controls. These findings are important because they may help further elucidate pathways involved in the development of MDD as well as identify potential novel treatments and pharmacological targets.

**Keywords:** TSPO and [11C]PBR-28 PET, neuroinflammation, Major Depressive Disorder

**Disclosures:** Funding for this work was supported in part by the Intramural Research Program at the National Institute of Mental Health, National Institutes of Health. Giacomo Salvadore and Harmuth Kolb full-time employees and shareholder of Janssen Pharmaceuticals INC. These funding sources had no further role in study design; in the collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit the paper for publication. 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.

## M90. Behavioral and Neural Biomarkers of Improved Top-Down Control during Ventral Capsule/Ventral Striatum Deep Brain Stimulation in Major Depression

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**Background:** Deep brain stimulation (DBS) of the ventral capsule/ventral striatum (VC/VS) has open-label efficacy in obsessive-compulsive disorder (OCD) and major depressive disorder (MDD). The failure of a recent MDD controlled trial highlights how little is understood about this invasive therapy's mechanisms. Without that understanding, we are limited in our ability to select patients or optimally titrate stimulation parameters. In this work, we consider the hypothesis that VC/VS DBS increases patients' capacity for processing information, particularly conflicting emotional signals in the environment.

**Methods:** 12 patients with VC/VS DBS and severe MDD (two with comorbid OCD) performed the Multi Source Interference Task (MSIT) with DBS ON, then repeated the task after an hour with DBS OFF. We recorded 60-channel EEG during task performance, digitized electrode positions, and localized scalp EEG to cortical sources with the dSPM algorithm. We then analyzed task performance, event-related potentials (ERPs), and their correlations with clinical outcomes on the Montgomery-Asberg Depression Rating Scale (MADRS). 8 patients contributed usable EEG recordings and 12 contributed behavior data.

**Results:** Reaction times on MSIT were 7% faster with DBS ON ($t = -9.28$, $df = 3879$, $p < 2.8e-20$, Wald test on GLM coefficients), even after controlling for within-block learning, repeated-measures testing, and confounding trial factors. Testing ERPs in a non-parametric decoding framework against the same behavioral model showed a significant main effect of DBS on stimulus-locked currents in bilateral dorsolateral prefrontal cortex (dlPFC) and rostral anterior cingulate (rACC), $p < 0.035$ after temporal cluster-correction within structures and false discovery correction for number of cortical regions. We observed similar response-locked effects in bilateral dorsal anterior cingulate (dACC). These structures are implicated in depression and top-down control, although behavior changes did not directly correlate with MADRS effects.

**Conclusions:** VC/VS DBS improved processing of cognitive interference stimuli, reflected in both decision times and neural activation. DBS alters the magnitude of task-evoked cortical potentials in structures known to implement top-down control, with suggestions of temporal information flow between units. Improved executive functioning may be a mechanism of action for VC/VS DBS. These behavioral and electrophysiologic markers might in future be validated for patient-specific therapy titration.

**Keywords:** deep brain stimulation, executive function, Brain Based Markers for Depression, cognitive control

**Disclosures:** Nothing to disclose.

## M91. Neural Predictors of Treatment Response to Psychotherapy for Depression in Bipolar Disorder

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**Background:** Bipolar disorder (BD) is characterized by manic and most often also depressed mood that impairs functioning and quality of life. Pharmacotherapy often fails to bring patients with bipolar disorder to sustained clinical

and functional remission. Therefore, adjunctive psychosocial interventions have been developed and have shown some efficacy in preventing relapse and treating acute mood episodes adjunctive to medication. In order to better understand how bipolar patients different from normal controls in attention and cognitive control, we used functional magnetic resonance imaging (fMRI) to compare differences in brain activity between depressed patients with bipolar disorder and healthy controls. Patients were then randomized to either cognitive-behavior therapy (CBT) or supportive psychotherapy (SP). We used neural activity as measured by fMRI as a predictor of treatment response.
**Methods:** Study participants were 32 depressed participants (16 female, CBT = 17, SP = 15), 19 of whom completed fMRI (11 female) with DSM-IV Bipolar I disorder. Each patient received 21 sessions of CBT or SP. In addition, we collected fMRI data from 19 normal controls (11 females). Functional neuroanatomy of attention and cognitive control was assessed using the Multi-Source Interference Task (MSIT). In this task participants were shown three-digit numbers (e.g. 100, 020 or 003) overlaid on negative or neutral images from the International Affective Picture System (IAPS) during the fMRI scan and had to decide which number was different from the two other numbers (e.g. 1 0 0; correct answer = 1). A non-interference trial consists of the "different" number in its corresponding position (e.g. 1 0 0). An interference trial consists of the "different" number not in its corresponding position (e.g. 2 2 1). Trials were categorized according to interference and non-interference conditions, as well as whether the image shown had negative or neutral valence. MRI data were acquired using a 3.0-T whole-body scanner (Trio-System).
**Results:** At baseline, participants in both the CBT and SP condition exhibited moderate levels of depression. In both treatment groups, depression scores decreased significantly from baseline to the end of treatment ($p < .001$) but there was no difference between the two treatment groups mid-treatment ($p = .58$).
fMRI data: Bipolar patients showed increased anterior cingulate cortex (ACC) and dorsolateral prefrontal cortex (dlPFC) activation in interference vs noninterference conditions compared to normal controls. In negative vs neutral conditions, bipolar subjects had increased dorsomedial prefrontal cortex (dmPFC) and lateral PFC activity and both groups had greater amygdala activation. Bipolar patients demonstrated greater lateral PFC activity in negative, non-interference conditions than in the neutral non interference. Regression analyses showed positive correlations ($p < .05$) between decreases in depression scores from pre-to post treatment and bilateral activations in ACC, dmPFC, lateral prefrontal cortex (PFC), dlPFC, and the amygdala during negative vs neutral conditions. In interference vs non-interference conditions, decreases in depression scores were correlated with activity in posterior cingulate, dmPFC, and dlPFC.
**Conclusions:** Imaging data demonstrate differences between bipolar patients and normal controls in brain regions involved in cognitive control, attention and internal state modulation. Treatment response was predicted by activity in ACC, lateral PFC, dmPFC and dlPFC.
**Keywords:** Bipolar Disorder, fMRI, Depression
**Disclosures:** Nothing to disclose.

## M92. Inhibition of Phosphodiesterase 2 Ameliorates Stress-Induced Depression-Like Behaviors and Cognitive Deficits in Mice

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**Background:** Major depressive disorder (MDD) is chronic, recurring and potentially life threatening disorder, which is generally accompanied by lifetime anxiety and persistent cognitive deficits. Cognitive dysfunction is a critical determinant of functional outcome in major depression. Treatment for depression remains unsatisfied because the etiology of depression is diverse and complex. Recent evidence suggested that depression might be the consequence of a complex interplay between oxidative stress and the downstream second messages involving cyclic adenosine and/or guanosine 3', 5'-monophosphate (cAMP and/or cGMP)-BDNF signaling. The stress hormone glucocorticoid induces an increase in NADPH oxidase, which transfers an electron to molecular oxygen as an electron donor to produce superoxide, leading to alterations in cellular and molecular signaling in the brain, such as the cAMP and cGMP related signaling. Activation of cAMP/cGMP signaling by inhibition of phosphodiesterases (PDEs) appears to be a viable means of enhancing neuronal communication since these enzymes regulate the above second messengers. The high expression of PDE2 in the hippocampus and adrenal cortex makes it tractable in the treatment of stress-induced affective disorders and related cognitive symptoms. However, it is not clear how inhibition of PDE2 affects stress-induced depression-like behaviors and cognitive deficits, and subsequent structural remodeling in the hippocampus by regulation of NADPH oxidase and downstream series of molecular events.
**Methods:** Bay 60-7550, lentiviral vectors expressing shRNAs targeted to PDE2 were microinfused into the hippocampal CA1 bilaterally in the mice. Seven days after surgery, behavioral and biochemical tests including forced swimming and tail suspension tasks (depression), novelty suppressed feeding (anxiety), novel object recognition test (cognition), serum corticosterone levels, and morphological changes of hippocampal neurons were measured. In the subsequent study, the mice were administered with oxidizing and reducing agents (DTNB and DTT), as well as NADPH oxidase inhibitor (apocynin) 30 min before PDE2 inhibition every day, then they were subjected to chronic unpredictable stress for 10 days. Depression/anxiety-like behaviors, cognitive performance and morphological changes were also investigated. In vitro study, primary hippocampal neurons were exposed to different concentrations of corticosterone with various intervals to determine the correlation between NADPH oxidase-dependent ROS generation and PDE2A expression by the real-time PCR and immunoblot analysis. Pharmacological and siRNA-based antioxidant strategies, such as treatment cells with DTNB, DTT, apocynin, and lenti-gp91-miRNA were used for accessing the involvement of cAMP and/or cGMP-CREB-BDNF dependent pathway in the effect of PDE2 inhibition on oxidative stress.

**Results:** The in vivo study suggested inhibition of PDE2 by Bay 60-7550 and lenti-PDE2-miRNA reversed stress-induced depression/anxiety-like behaviors and cognitive deficits. Pretreatment with the oxidizing agent DTNB prevented, while the reducing agent DTT and NADPH oxidase inhibitor apocynin potentiated, the effects of Bay 60-7550 on behavior, indicating the role of PDE2 in the oxidative stress-induced depression/anxiety and cognitive disorders. Consistently, the increases in dendritic branching and length of hippocampal neurons after inhibition of PDE2 were suppressed by DTNB; whereas these were potentiated by DTT or apocynin. The subsequent in vitro study suggested that oxidative stress-induced ROS expression was positively related to PDE2 levels, which was consistent with the in vivo data. PDE2 inhibitor Bay 60-7550 and PDE2 silencing by lenti-PDE2-miRNA decreased stress hormone corticosterone-induced increases in NADPH oxidase subunits, such as gp91 phox, in the hippocampal neurons. These effects were significantly potentiated using pharmacological- and siRNA- based antioxidant strategies, providing clear evidence that the protective effects of PDE2 inhibition on all measured parameters against stress are positively related to downregulation of NADPH subunits, particularly gp91 phox, through activation of cAMP/cGMP-CREB-BDNA pathway.
**Conclusions:** The present results provide the evidence that chronic stress stimulates depression/anxiety-like behaviors, cognitive deficits and morphological changes through the upregulation of NADPH oxidase (gp91 phox) and the resultant metabolic oxidative stress, such as PDE2 expression. PDE2 inhibition may represent a novel therapeutic target for stress-related psychiatric disorders, such as depression, anxiety and related cognitive deficits.
**Keywords:** PDE2, Major depression, NADPH oxidase
**Disclosures:** Nothing to disclose.

### M93. A Genome-Wide Study of Suicidality in Bipolar Disorder

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**Background:** There are on average one million suicides each year worldwide. Having a diagnosis of mood disorder is an important risk factor for suicide. Between 25 and 50% of bipolar disorder patients have attempted suicide at least once. Family and twin studies support a genetic basis of suicide Thus, studying the genetics of suicidal behaviour in bipolar disorder is an important aim.
**Methods:** We recently reported a number of suggestive findings from a genome-wide association study (GWAS) of suicidal behaviour severity in bipolar disorder patients. Here, we report a similar study in an independent bipolar disorder patients from the STEP-BD (Systematic Treatment Enhancement Program for Bipolar Disorder) study using data from the Suicide Questionnaire (N = 412).
**Results:** Preliminary findings suggest chromosomal region 2q35 to be possibly associated with suicidality ($p < 0.00001$). We will be conducting whole-genome imputation to capture markers that were top findings in our previous GWAS.
**Conclusions:** We will be conducting whole-genome imputation to capture markers that were top findings in our previous GWAS. We will also incorporate additional information on suicidal behaviour from the study as well as medication data into our analysis.
**Keywords:** Bipolar Disorder, suicide behavior severity, GWAS
**Disclosures:** Patent applications not directly related to this poster.

### M94. Central and Peripheral Effects of Acute Isolation Stress on Transforming Growth Factor-B1 and Cortisol in Nonhuman Primates

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**Background:** Transforming growth factor-β1 (TGF-β1) is a multifunctional cytokine with anti-inflammatory, immunosuppressive and neuroprotective properties. The hypothalamic-pituitary-adrenal (HPA) axis and the immune system exert bidirectional influences in part through cortisol and TGF-β1, but the exact nature of their interaction is not well characterized. A functional linkage seen as strong positive correlation between the HPA axis and TGF-β1 has been observed in bonnet macaques exposed to moderate stress. In vitro assays have shown differential response of TGF-β1 to glucocorticoids in glial and T cells. Thus, any decrease of this cytokine centrally during acute stress would have potential therapeutic implications.
**Methods:** The current study examined the effects of mild stress (30 min confinement in an unfamiliar room), with and without 4 h pretreatment with dexamethasone (0.01 mg/kg IM), on contemporaneous levels of TGF-β1 and cortisol in the cerebrospinal fluid (CSF) and plasma of bonnet macaques (Macaca radiata). The subjects were nine male bonnet macaques, all born in Downstate Medical Center's Primate Behavior Laboratory. At the time of the present experiment, subjects were young adults, and groups were of comparable age (normally reared = 9.4 y [SE = 1.12], VFD-reared = 7.5 y [SE = 0.26]; t [7] = 1.49, p = .179).
**Results:** There was a significant increase in mean plasma cortisol level during stress (53.3 mg/dl) from a baseline value of 36.1 (mean difference = 19.2 mg/dl, p = .001). Reductions in plasma cortisol were observed during stress + dexamethasone condition, when compared to either baseline (mean difference = 11.0 mg/dl, p = .04) or stress only conditions (mean difference = 30.2 mg/dl, p = .00002). When CSF cortisol levels were measured, there was a significant increase in the mean CSF cortisol levels during stress (2.15 μg/dl) from baseline (mean difference = .54 μg/dl, p = .004). The values significantly decreased under stress + dexamethasone condition (1.19 μg/dl) when compared to baseline (mean difference = .43 μg/dl, p = .01) or stress alone (mean difference = .97 μg/dl, p = .00002).

Thus, cortisol, for both CSF and plasma, increased after stress and decreased below baseline levels under stress + dexamethasone condition. In contrast to the parallel changes of cortisol levels across the periphery and CSF, mean serum TGF-β1 values did not significantly differ in any condition ($F = 2.5$, $p = 0.12$). CSF levels of TGF-β1 decreased significantly to below baseline levels after exposure to stress (mean difference = 8.5 μg/dl, $p = .01$), and stress + dexamethasone conditions when compared to baseline (mean difference = 13.8 μg/dl, $p = .0003$). There was no significant difference observed in CSF TGF-β1 levels between the conditions of stress and stress + dexamethasone. The decrease in central TGF-β1 was significantly distinguishable from the peripheral TGF-β1 numeric increase in response to stress alone.

**Conclusions:** The results of the current study confirm the complex relationship between circulating levels of glucocorticoids and production of TGF-β1 in the CNS versus the periphery. These data suggest a reduced central anti-inflammatory and neuroprotective effect of TGF-Beta in the face of peripheral and central stress-induced cortisol increases. The current study supports the hypothesis of an intimate relationship between cortisol and TGF-β1 during stress, and suggests that this relationship may be expressed in different regulatory pathways in the periphery and in the CNS. Thus, TGF-β1 analogues may be proposed to be neuroprotective especially under conditions of stress. We therefore provide preliminary evidence for divergent compartmental effects for CSF (decrease) and serum TGF-β1 (no change) in response to stress although future studies with additional subjects is required.

**Keywords:** glucocorticoid, cytokines, CSF, stress
**Disclosures:** Nothing to disclose.

### M95. Cognitive Behavioral Therapy Increases Resting State Cognitive Control Network Connectivity Across MDD and PTSD

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**Background:** Major depression (MDD) and post-traumatic stress disorder (PTSD) are frequently co-morbid, and similar symptoms of depression and anxiety are often present in both disorders. Increasingly, both MDD and PTSD are conceptualized as a syndromes of dysconnectivity within specific brain circuits. Commonly implicated large-scale networks include cognitive control networks and the default mode network, often in concert with dysregulated amygdala connectivity. Notably, one of the most effective treatments for both disorders is cognitive behavioral therapy (CBT), suggesting a common mechanism for treatment response. Here we investigated whether similar neural profiles of amygdala dysconnectivity underlie both common symptomatology and treatment response to CBT in MDD and PTSD.

**Methods:** Participants included 62 un-medicated females age 18-55: 23 with MDD, 21 with PTSD, and 18 healthy comparison subjects. All subjects had been off all medication with CNS effects for at least 3 weeks (5 weeks for fluoxetine). Patients with PTSD or MDD enrolled in the study received 12 weekly individual outpatient CBT sessions. For MDD patients, the intervention was guided by a standard treatment manual, "Cognitive Therapy of Depression." PTSD patients received Cognitive Processing Therapy, shown to be an effective cognitive behavioral therapy that significantly reduces PTSD symptoms. At baseline and after finishing a course of CBT, all subjects completed resting-state fMRI at 3T (412 volumes over two concatenated runs, TR = 2.2s, 4mm isotropic voxels). Resting-state images were preprocessed with spatial smoothing (6mm FWHM), band-pass filtering (0.01-0.08 Hz), and confound regression using a 36-parameter model. The amygdala was defined on a subject-specific basis using an advanced multi-atlas label fusion (MALF) procedure; subject-level amygdala connectivity maps were co-registered to the T1 image using boundary-based registration and normalized to a study-specific template using ANTs. Longitudinal functional principal components analysis (LFPCA) was used to extract longitudinal multivariate patterns of connectivity change with treatment. Group differences in these connectivity patterns (scores of the eigenimages) were examined using linear regression while controlling for age and in-scanner motion.

**Results:** We identified the average pattern of connectivity for CBT-treated and HC separately using the first two eigen images and their treatment-specific average weights (principal scores) on the corresponding eigen-components. MDD and PTSD patients had diminished connectivity between the amygdala and cognitive control regions at baseline when compared to HC. By subtracting the 'null' HC pattern from the CBT pattern, we obtained regions with changes in connectivity after CBT. Both groups treated with CBT had a significant increase in amygdala connectivity with control regions, as described by the second eigenimage ($p = 0.02$). In contrast, there was no CBT-associated change in amygdala-DMN connectivity components.

**Conclusions:** CBT effects on dimensional depression-associated functional connectivity were examined in un-medicated patients with MDD or PTSD. We used a priori amygdala regions previously determined to have baseline resting state dysconnectivity with dorsolateral prefrontal cortex (DLPFC) and inferior frontal cortex (IFC), both regions important in cognitive control; at baseline the degree of amygdala dysconnectivity was correlated with depression severity. Here we found the network most implicated in CBT-associated improvement in depression was the cognitive control/executive network but not the default mode network (DMN). Cognitive control, the process by which executive processes allow information processing and behavior to vary adaptively from moment to moment depending on current goals, operates through regions in the prefrontal cortex (PFC) that represent and maintain context for responding and achieving goals. These, in turn, bias processing in posterior and premotor areas in order to support task appropriate responding. Depression-associated cognitive dysfunction can be conceptualized as reflecting deficits in these processes, their reduced efficiency also potentially underpinning reduced attention, concentration and executive function reproducibly reported in depression. Thus, the current imaging study, conducted

before and after CBT treatment, strongly implicates normalization of dysfunctional cognitive control network connectivity as the mechanism for CBT efficacy.
**Keywords:** cognitive control network, fMRI Functional Connectivity, cognitive behavioral therapy, major depression, post-traumatic stress disorder
**Disclosures:** Nothing to disclose.

### M96. Circuit-Wide Transcriptional Profiling Reveals Region Specific Gene Co-Expression Networks Regulating Depression Susceptibility

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**Background:** Depression is a complex and heterogeneous disorder reflecting dysfunction in multiple brain regions. Recent functional studies suggest that opposing alterations in prefrontal cortex (PFC) and ventral hippocampus (VHIP) neuronal activity regulate susceptibility to chronic social defeat stress (CSDS), a highly validated mouse model of depression (Bagot et al., 2015). The molecular mechanisms mediating depression-associated functional alterations in these brain circuits are largely unknown.
**Methods:** We performed RNA-sequencing on multiple brain regions, including PFC and VHIP, from control animals and mice susceptible or resilient to CSDS at multiple time points after defeat (total n = 132 libraries). We employed an intersectional bioinformatics approach combining differential expression analysis with weighted gene co-expression network analysis and key-driver analysis to identify novel transcriptional networks regulating depression susceptibility. We used viral-mediated over-expression of identified network hub-genes in mice exposed to CSDS and assessed effects on depression associated behavioral assays, electrophysiological assays of synaptic function and transcriptional regulation of gene networks.
**Results:** We identified two susceptible-specific gene co-expression networks that exhibited significant enrichment of oppositely regulated differentially expressed genes in PFC and VHIP. Both networks were significantly enriched for neuronal-specific genes and gene ontology analysis indicated relevant functions including synaptic transmission. Key-driver analysis identified susceptible-specific hub genes in both networks. Viral-mediated over-expression of these genes confirmed bioinformatic predictions, inducing increased stress susceptibility in VHIP and reduced stress susceptibility in PFC. Hub genes selected from each network, Sdk1 and Dkkl1, increased frequency of sEPSCs when over-expressed in VHIP, confirming the functional role of these networks in regulating synaptic transmission. Over-expression of Dkkl1 in VHIP induced expression of other genes enriched in its network, demonstrating functional connectivity of the co-expression network in vivo and providing molecular validation of our bioinformatics analyses.
**Conclusions:** These results demonstrate that opposing regulation of gene co-expression networks in PFC and VHIP mediates susceptibility to social defeat stress. Moreover, in vivo validation of bioinformatically predicted hub-genes validates the utility of a systems biology approach by identifying novel transcriptional mechanisms that control stress susceptibility and may offer new leads for antidepressant drugs.
**Keywords:** Depression, RNA-seq, ventral hippocampus, prefrontal cortex, chronic social defeat
**Disclosures:** Nothing to disclose.

### M97. Cholinergic-Adrenergic Interaction in the Amygdala Regulates Anxiety- and Depression-Like Phenotypes in Male and Female Mice: Effects of Guanfacine Following Cholinergic Dysregulation

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**Background:** Acetylcholine (ACh), like the nicotine in tobacco, acts at nicotinic acetylcholine receptors (nAChRs) to regulate behaviors related to depression. Several other neurotransmitters, including norepinephrine (NE) are also involved in regulation of mood and anxiety, and medications targeting the noradrenergic system are used to treat anxiety disorders and depression. Laboratory studies with human subjects have shown that guanfacine, an α2a-noradrenergic receptor agonist, can decrease stress-induced smoking in women and can aid in smoking cessation in both men and women. These data suggested that ACh and NE signaling might interact to modulate depression-like symptoms during nicotine withdrawal.
**Methods:** We investigated the effect of guanfacine in a hypercholinergic model of anxiety- and depression-like behavior in female and male C57BL/6J mice. Subsequently, we measured c-fos immunoreactivity in the brains of these animals to determine the effects of guanfacine on neuronal activity in brain regions related to anxiety- and depression-like behavior. Using local knockdown of α2a receptors in the amygdala, we next determined whether this brain region is a critical site for guanfacine's behavioral effects. We then determined whether the antidepressant-like effect of guanfacine was dependent on expression of β2 subunit-containing (β2*) nAChRs in amygdala. Finally, we investigated whether subthreshold blockade of nAChRs and subthreshold guanfacine administration can combine to produce antidepressant-like effects.
**Results:** Both male and female mice showed a dose-dependent, antidepressant-like response to guanfacine on its own. Depression-like phenotypes induced by increased ACh signaling were also reversed by guanfacine administration. Interestingly, there were significant sex differences in c-fos immunoreactivity following guanfacine administration in brain regions involved in anxiety- and depression-like behaviors, suggesting that activation of sex-specific neuronal networks could produce similar behavioral outputs following

modulation of the noradrenergic system. Knock-down of α2a receptors in the amygdala prevented its anxiolytic- and antidepressant-like effects and similar results were observed following knock-down of high affinity, β2* nAChRs in amygdala, suggesting that this might be an important brain area for NE-ACh interactions related to anxiety and depression. Finally, pharmacological experiments showed that guanfacine potentiated the antidepressant-like effect of the nicotinic antagonist mecamylamine only in female mice, providing a potential explanation for the sex-specific effects of guanfacine on stress-induced smoking in human studies.
**Conclusions:** Taken together, these results suggest that ACh and NE have opposing actions on behaviors related to anxiety and depression, and that both cholinergic signaling through β2-containing nAChRs and noradrenergic signaling through □2a receptors in neurons of the amygdala are critical for regulation of behaviors related to anxiety and depression. In addition, sex-specific networks are likely recruited to produce similar behavioral responses in male and female mice treated with guanfacine.
**Keywords:** guanfacine, nicotine, Amygdala, nicotinic acetylcholine receptors, sex difference
**Disclosures:** Nothing to disclose.

### M98. Brain Activation Correlates of Negative Attentional Bias in Depression

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**Background:** Altered emotion processing has been shown in major depressive disorder (MDD), as well as a cognitive bias toward negative stimuli. The dot probe task has been used to assess the effects of emotion on attentional parameters and to demonstrate attentional biases. In previous research, this task has elicited an attentional bias in MDD toward words and faces representing negative emotions. The majority of these studies have focused on sadness as the negative emotion, yet a bias toward anger and specifically angry faces may better relate to issues in interpersonal situations. The negative processing bias in MDD has been associated with differences in brain activation in limbic, frontal, and parietal regions. However, there has been limited work thus far using the dot probe task during functional magnetic resonance imaging (fMRI) in MDD.
**Methods:** We examined brain activation during attentional bias using fMRI during a dot probe task. In this task, two faces were presented side by side, one displaying an angry, happy, or neutral expression and the other neutral. Following the faces presentation, a blank screen appeared with a dot on one side, to which the participant responded with a button press to indicate whether the dot was on the left or right. Trials in which the dot replaced the emotional face were considered congruent. We tested 27 participants with MDD (unmedicated) and 26 healthy controls on this task while in the fMRI scanner. We then compared fMRI blood oxygen level dependent (BOLD) activation using a multivariate model with congruency, emotion, and diagnostic group as factors (with FWE-correction at a cluster level of $p < 0.05$).
**Results:** Our findings showed a main effect of congruency, in which there was greater activity during congruent than incongruent trials in bilateral parahippocampal gyrus and the left posterior cingulate. There was also a main effect of emotion, with greater activation in angry versus happy trials in bilateral putamen and the right fusiform gyrus. Additionally, we found a group-by-emotion interaction in bilateral middle temporal gyrus, the left precuneus, and the left middle frontal gyrus. This interaction was driven by greater activation to angry faces in depressed participants, coupled with greater activation to happy faces in healthy participants.
**Conclusions:** This dot probe task was shown to activate brain regions involved in emotion, memory, and cognitive processing. While some regions that have previously been associated with negativity bias, such as the amygdala, were not found to be differentially activated here, our findings are consistent with other literature and may be more specific to the type of task design used. The activation differences between participant groups supported the bias in depression toward negative and away from positive emotion, specifically in a task using stimuli representing angry faces. The MDD participants had greater activation during angry trials in the middle temporal gyrus, which could be associated with memory processing related to negative emotional experiences, especially in interactions with others. Healthy participants on the other hand, had greater activation in this region during happy trials, perhaps related to memory of positive interpersonal experiences. The differences in activation between participant groups in the precuneus and middle frontal gyrus could be associated with the dorsal attention network, as in greater attentional processing of the angry stimuli and lesser attention toward the happy stimuli in the depressed individuals. Overall, these findings show that this dot probe paradigm can be used to better understand the neurobiology of the negative attentional bias in depression. This could be valuable for assessing new treatments and their effects on brain function.
**Keywords:** depression, fMRI, attentional bias, brain based markers for depression
**Disclosures:** The NIMH has filed a use-patent for the use of scopolamine in the treatment of depression, and Dr. Furey is identified as a co-inventor on this pending patent application in the US and an existing patent in Europe. Dr. Furey is an employee of Janssen Pharmaceuticals of Johnson and Johnson Inc. Dr. Zarate is listed as a co-inventor on a patent application for the use of ketamine in depression. Dr. Zarate has assigned his rights in the patent to the US government but will share a percentage of any royalties that may be received by the government.

### M99. Ankyrin-3 Bipolar Disorder GWAS Gene Regulates Activity of Dentate Gyrus Granule Neurons and Adult Hippocampal Neurogenesis

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**Background:** Human genetic studies identified a significant association between the Ankyrin 3 gene (ANK3) and risk of

bipolar disorder (BD). Abnormalities in the hippocampus are consistently linked to BD, in particular, deficits in dentate gyrus function. Our recent work has shown that disruption of Ank3 expression in the hippocampal dentate gyrus of mice induces manic-like behaviors in a battery of behavioral tests to measure impulsivity. Proper development of neurons in the dentate gyrus is crucial for hippocampal function and plasticity in response to changing stimuli. We speculate that Ank3 is crucial for maintaining dentate gyrus neurogenesis and hippocampal function, and that defects in this process may contribute to the observed behavioral changes induced by Ank3 disruption.

**Methods:** Mice that are heterozygous for a targeted disruption of the brain-specific isoform of ankyrin-3 (Ank3 + /-) and their wild type littermates (Ank3 + / +) were used in this study. The heterozygous Ank3 + /- mice have a 50% reduction in ankyrin-3 expression in the brain. Mice were group housed and allowed ad libitum access to food and water. In experiments involving lithium, animals were either fed medicated chow with 0.4% lithium chloride or standard rodent chow. Using this mouse model, we investigated neuronal activation after exposure to the passive avoidance task, a task in which the Ank3 + /- mice exhibit impulsive-like behavior. We also investigated adult hippocampal neurogenesis in Ank3 + /- mice compared to wild-type Ank3 + / + littermates. Neuronal activity in mice exposed to the passive avoidance task was evaluated by immunohistochemical detection of c-fos in fixed brain sections. Adult hippocampal neurogenesis was examined using stage-specific cellular markers - bromodeoxyuridine (BrdU) to label proliferating cells and doublecortin to assess maturation of newborn neurons. Stereological counts were performed throughout the entire rostral/caudal extent of the dentate gyrus for comparison between each group. Statistical analysis was performed using a student's t-test or ANOVA (alpha = 0.05).

**Results:** We have detected a 50% decrease in c-fos expression in dentate gyrus granule cells in Ank3 + /- mice compared to Ank3 + / + mice following exposure to the passive avoidance task ($p < 0.01$). This suggests that neuronal activation is impaired while Ank3 + /- mice are exhibiting impulsive behavior, which may reflect perturbed hippocampal function. Altered proliferation of neural progenitor cells in the dentate subgranular zone ($p < 0.05$) in Ank3 + /- mice compared to Ank3 + / + mice suggest that Ank3 reduction is associated with impaired adult hippocampal neurogenesis. Defects in neuronal maturation ($p < 0.05$) were also found as the number and distribution of doublecortin-positive immature neurons was also altered in Ank3 + /- mice. Furthermore, we found that the changes to neurogenesis in Ank3 + /- mice are reversed by chronic treatment with the mood stabilizer lithium, which inhibits glycogen synthase kinase 3 (GSK3), suggesting that the mechanism of Ank3 regulation of adult neurogenesis may involve GSK-3 signaling.

**Conclusions:** Taken together, these new data suggest that Ank3 functions to regulate the development of adult-born hippocampal neurons and, in turn, hippocampal function and plasticity through a mechanism involving GSK3. Our results provide novel insight into how disruptions in neurodevelopment may contribute to impaired hippocampal function.

**Keywords:** ankyrin-3, adult neurogenesis, impulsivity, c-Fos
**Disclosures:** Nothing to disclose.

## M100. A Randomized Trial of Internet-Based Cognitive Behavioral Therapy for Major Depressive Disorder

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**Background:** Major depressive disorder (MDD) is a common and debilitating illness, and many affected individuals do not pursue or cannot access traditional psychotherapeutic or pharmacological treatments. Internet-based cognitive behavioral therapy (iCBT) is receiving increasing empirical support as an efficacious and accessible psychological treatment for MDD. Our research group has recently completed a clinical trial of an iCBT tool originally developed at St. Vincent's Hospital, University of New South Wales, Australia. In this study, we sought to determine this tool's efficacy in an American MDD sample, after modifying it for both cultural relevance and compliance with the Health Insurance Portability and Accountability Act.

**Methods:** MDD participants (ages 18-45 yrs.) were recruited from the local Boston metropolitan area and interviewed by doctoral-level psychologists using the Structured Clinical Interview for DSM-IV. Participants were excluded if they were currently taking psychotropic medications, or if they had a history of substance use disorder, neurologic illness, or psychotic disorder. Eligible participants were randomized to a ten-week period of iCBT (N = 29) or a monitored attention control group (MAC; N = 29). Participants in the iCBT group completed six online therapy lessons, and had access to content summaries and homework assignments. During the ten-week trial, participants in both the iCBT and MAC groups were required to log into the web-based system six times to complete self-report symptom scales, and they were contacted weekly by telephone by a non-clinician technician who provided encouragement and support. The primary outcome measure was the 17-item Hamilton Rating Scale for Depression (HRSD), and secondary outcome measures were the Patient Health Questionnaire (PHQ-9) and Kessler-10 (K10). For each outcome, pre- and post-treatment scores were entered as dependent variables in a repeated measures analysis of variance (ANOVA) with treatment group as the predictor. Note that all subjects also participated in brain MRI before and after treatment. The MRI results are not presented here.

**Results:** iCBT and MAC participants did not differ significantly at the pre-treatment timepoint on HRSD, PHQ-9, or K10 scores. iCBT participants showed significantly greater reduction of HRSD depression scores than MAC participants over the ten-week trial ($F(1,56) = 9.18$, $p = .004$; 54% vs 15% reduction), and there was a significantly higher remission rate (HRSD $\leq$ 7) in the iCBT than MAC group (62% vs 14%). Using the PHQ-9 measure, there was a greater reduction of depression in

iCBT versus MAC participants at a non-significant trend level ($F(1,56) = 2.99$, $p = .09$), while remission rates (PHQ-9 $< 6$) were significantly higher in iCBT participants (41% vs 14%). Finally, K10 distress scores showed a significantly greater reduction in iCBT than MAC participants ($F(1,56) = 4.18$, $p = .05$; 32% vs 18% reduction).
**Conclusions:** iCBT was associated with both statistically and clinically significant reductions in symptoms of MDD, and greater than an active symptom monitoring control. With its potential to be delivered in a readily scalable, cost-efficient manner, iCBT is a promising strategy for enhancing access to effective care for individuals with depression.
**Keywords:** Major Depressive Disorder, cognitive behavior therapy, internet, randomized clinical trial, non-pharmacological interventions
**Disclosures:** Dr. Rauch has received research funding from NIMH, US Army and royalties from APPI and Oxford University Press. He further receives honoraria for advisory board service from the Harvard Football Players Health Study. He is employed by, and receives salary from McLean Hospital/Partners Healthcare. Dr. Rauch also holds leadership roles with the SOBP, APA, NNDC and ADAA.

### M101. User-Centered Development and Field Testing of LiveWell: A Smart Phone Application for Bipolar Disorder

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**Background:** Bipolar disorder is a chronic mental illness with high levels of morbidity and mortality. Multiple acute episodes, long episode duration, and significant inter-episode sub-syndromal symptoms are common, even with current pharmacological treatment. Combining psychotherapeutic interventions with pharmacological treatment improves clinical outcomes. However, these psychotherapeutic interventions are resource intensive, and few patients receive treatment with evidence-based therapies for bipolar disorder. Behavioral intervention technologies (BITs), including mobile interventions, can increase access to psychosocial interventions with demonstrated efficacy and thus provide an important avenue for improving treatment. In addition, BITs can provide real-time assessment and feedback to patients and providers so that treatment may be more rapidly adjusted thereby increasing intervention impact. Here, we describe the iterative user-centered design process and initial field testing of LiveWell, a mobile phone-based BIT for bipolar disorder that aims to: (1) enhance patient self-management, (2) serve as a platform for obtaining feature-rich behavioral data, and (3) adapt intervention content, in order to improve the efficiency and timeliness of care delivery.
**Methods:** Patients were screened for bipolar disorder 1 or 2 using a structured phone interview (MINI), followed by a face-to-face clinician diagnostic interview (ADE) and medical record review. Initial development of content was based on review of current published psychosocial interventions for bipolar disorder. Patient input was first obtained from feedback on a coach training session. Because human support promotes adherence to application use, we explicitly examined the coach role in addition to application design. An initial field trial ($N = 4$) of the daily monitoring component was then carried out with exit interviews focused on acceptability, usefulness, and feasibility. Additional patient input ($N = 5$) on coach training and application design for the full intervention (lessons, skills, instructions, daily check-in and feedback, patient development of a wellness monitoring and action plan) was obtained using paper and phone based (Balsamiq) mock-ups. Subsequently, usability testing ($N = 5$) was carried out with the full application. A two month field trial using the full application is now being carried out. An iterative design process was utilized with review of sessions, session notes, and abridged review of audio and video tapes immediately after each interview resulting in application modifications. Subsequently, qualitative text-based analysis of all transcripts utilizing both deductive and inductive thematic analysis was used to identify important user issues and to redesign the application. In addition to patient input, psychiatrist input on the application and secure web-based clinical summaries was obtained using structured interviews with paper application mock-ups ($N = 6$) and an online survey ($N = 42$) of faculty from the Feinberg School of Medicine.
**Results:** During initial training, participants anchored 7-point scales for mood and thoughts based on their prior experiences with depression and mania. Participants often anchored the scales with overlapping content and included behavioral symptoms as anchors. As a result, mood and thought scales were replaced by a single wellness scale and training on symptoms, early warning signs, and personal anchors was expanded. The 7-point scale was also expanded to a 9-point scale as both participants and providers indicated that the 7-point scale lacked sufficient range. Feedback on modifications was positive, with users stating the application was easier to use and better captured their wellbeing. Feedback from patients and psychiatrists indicated that a time window of 4-7 days would be most appropriate for summarizing and updating provider alerts. 93% of providers were comfortable with the application directing patients to call them based on self-report and sensor data, but if and when, automated email alerts should be sent was less clear. Compared with depression, psychiatrists wanted patients to call earlier for changes suggestive of impending mania. During the testing of the simplified application one patient was recovering from depression and converted into a manic episode. Review of his sensor data suggests that daily activity levels preceded the transition into a full-blown manic episode and were present before the patient contacted his psychiatrist with concerns.
**Conclusions:** The user-centered development of LiveWell aims to improve system usability. By incorporating patient and psychiatrist feedback, we anticipate that the application will be used more frequently and over longer periods of time. This should lead to enhanced self-management and earlier, more responsive provider intervention. In addition, use of sensors to track behaviors, such as activity level, should enhance the ability to identify and predict clinical

status allowing more timely intervention. In this way, LiveWell will achieve its goal of reducing the occurrence and severity of symptoms in bipolar disorder.
**Keywords:** Bipolar disorder, Mobile phones, User-centered design, Field trial
**Disclosures:** Nothing to disclose.

### M102. miRNA Dysregulation in Prefrontal Cortex by Chronic Corticosterone Administration in Rats: Role in Depressive Disorder

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**Background:** Depression is one of the most prevalent psychiatric disorders worldwide and is a major public health concern. It has increasing been clear that depression arises from a combination of genetic and environmental risk factors. How these factors affect physiological processes leading to the development of depression, however, remain largely unknown. Stress represents one of the major environmental factors that can lead to precipitation of depression. There is a well-established connection of hyperactive hypothalamus–pituitary–adrenal axis and depression, which is primarily associated with altered expression and function of glucocorticoid receptors that may lead to feed-back inhibition, resulting into elevated levels of circulating glucocorticoids and protracted responses to stressors. Recently, the emergence of small non-coding RNAs as a mega controller of gene expression, and thus coordinately regulating gene expression has gained much attention in various disease pathophysiology, including depression. The present study examined the impact of corticosterone on miRNA expression and related gene networks in rat brain.
**Methods:** To dissect the role of miRNAs in depression pathophysiology, we examined miRNA expression in prefrontal cortex of rats given chronic administration of exogenous corticosterone (CORT; 50 mg/kg for 21 days) as a means to study the elevated CORT levels that would occur as a consequence to stress exposure. Animals were decapitated 24 h after the last CORT injection. The trunk blood was collected and serum CORT levels were measured. For miRNA assays, brains were removed quickly and were dissected on ice immediately frozen on dry ice before transferring to -80 °C for storage. miRNA expression was studied in frontal cortex using microarray. Group differences were analyzed using ANOVA. Statistical significance was calculated using both the non-parametric Wilcoxon paired sign-rank test, 2-tailed and the paired t-test, 2-tailed. To correct for multiple testing, SAM analysis (Significance Analysis of Microarrays, Stanford University) was carried out. Statistically significant miRNAs were analyzed for their mRNA targets and gene networks using Ingenuity Pathway Analysis Software and DAVID. Validation of a few key miRNAs and corresponding target genes were carried out using qPCR.
**Results:** Animals given chronic CORT administration showed key behavioral features that resembled phenotypic characteristics of clinical depression. miRNA microarray expression analysis revealed differential regulation of 26 miRNAs in frontal cortex of CORT-treated rats. Chromosomal coordinates, seed sequences and transcriptional units for these altered miRNA show very strong overlapping patterns indicating that the CORT response occurred in a coordinated manner. Eight significantly affected miRNAs were encoded at adjacent genomic positions, and presumably arose from the same primary miRNA gene transcript or even from the same pre-miR hairpin precursor. Further analysis examining interaction between altered miRNAs and target genes showed a very dense affected molecular network. To examine the phenotypes associated with miRNA changes, we performed mapping of genes that are predicated to be affected by CORT-induced altered miRNAs to known human diseases and disorders. The results revealed top 5 disorders including behavior, developmental disorder, inflammatory response, protein degradation, and psychological disorders. Analysis of the two most significantly affected miRNAs miR-124 and miR-218 showed target genes that have been reported to be associated with stress-related disorders. These include: CREB1, MECP2, GRIA2, GRIA4, SP1, PIK3C2A, NFATC1, GSK3β, and BDNF. Some of them showed overlapping pattern of regulation by these two miRNAs.
**Conclusions:** Overall, our study show that CORT-mediated miRNA dysregulation of key gene networks may be critical in stress-induced induction of depressive behavior.
**Keywords:** microRNA, Depression, stress, rat, prefrontal cortex
**Disclosures:** The study was funded by R01MH101890 and R01MH100616 to Dr. Yogesh Dwivedi.

### M103. Ziprasidone vs. Placebo Augmentation of Escitalopram for Patients with vs. Without Anxious Depression

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**Background:** Anxious depression is a common subtype of major depressive disorder (MDD) that is, often, difficult to treat. Recently, augmentation with the atypical antipsychotic ziprasidone exhibited superior anxiolytic efficacy in patients with MDD compared to placebo. Therefore, we hypothesized that ziprasidone augmentation to escitalopram would be equally efficacious in treating depression and anxiety in patients with versus without anxious depression.
**Methods:** 139 outpatients with escitalopram-resistant MDD received eight weeks of augmentation with ziprasidone or placebo in a randomized, double blind, three-site trial. A post-hoc intention-to-treat (ITT) ANCOVA analysis was done comparing treatment outcome using the Hamilton Depression Rating Scale (HDRS) and Hamilton Anxiety Rating Scale (HAMA) between patients with anxious ($n = 38$) versus patients without anxious ($n = 101$) depression. Analyses were conducted controlling for baseline scores. Anxious depression was defined as MDD plus $\geq 7$ on the HDRS Anxiety/Somatization Factor Score at baseline.

**Results:** HDRS total change scores from baseline and endpoint were not significantly different (overall $p = 0.91$) in patients with anxious depression on ziprasidone augmentation ($n = 19$; change scores -9.1 ± 4.9) or placebo ($n = 19$; change scores -6.1 ± 8.9) versus patients without anxious depression on ziprasidone ($n = 52$; change scores -5.5 ± 6.7) or placebo ($n = 49$; change scores -2.3 ± 4.5).
133 patients completed at least one HAMA rating after baseline. There was a trend towards statistical significance (overall $p = 0.1$) for a difference in HAMA total change scores from baseline to endpoint in patients with anxious depression on ziprasidone augmentation ($n = 19$; change scores -2.7 ± 5.3) or placebo ($n = 19$; change scores -3.3 ± 5.8) versus patients without anxious depression on ziprasidone ($n = 51$; change scores -3.9 ± 6.6) or placebo ($n = 44$; change scores -0.9 ± 4.7).
Statistical power for these tests was $> 0.8$ to detect medium effect sizes.
**Conclusions:** Ziprasidone augmentation was equally efficacious in patients with versus without anxious depression. However, there was a trend towards statistical significance for patients with anxious depression to demonstrate inferior anxiolytic efficacy with ziprasidone versus placebo, than those without. Further research into the treatment of anxious depression, as well as targeting treatments toward residual anxiety within depression, is necessary.
**Keywords:** Treatment Resistant Depression, Psychiatric Comorbidity, ziprasidone, mood and anxiety disorders
**Disclosures:** Pfizer provided blinded ziprasidone/placebo. Forest Labs provided Lexapro.

### M104. The Novel Short-Acting Kappa Opioid Receptor Antagonist LY2444296 Blocks Neuroendocrine and Behavioral Effects of Chronic but not Acute Mild Stress in Rats

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**Background:** Considerable evidence supports the hypothesis that psychiatric co-morbidity (including depression) in the context of addictive diseases triggers relapse, and is an underlying factor of failure of treatment-seeking patients to remain abstinent. Stress is one major trigger of relapse to specific addictive diseases, which results in upregulation of Kappa opioid receptor (KOPr) signaling tone, largely through increased mRNA transcription and activity of its endogenous agonists, the dynorphins, leading to depressive-like and relapse-like behaviors. Synthetic KOPr agonists induce dysphoria in humans, as well as depressant-like effects in preclinical models. Therefore, KOPr antagonism has received considerable attention as a novel antidepressant and anti-relapse pharmacotherapeutic approach. However, most of the current knowledge on the pharmacotherapeutic potential of KOPr antagonism is based on antagonists (e.g. nor-BNI, JDTic) with unusual pharmacokinetic and pharmacodynamic properties, including delayed onset of KOPr selectivity, very slow onset and extended durations of action. These features have limited experimental designs, interpretation and translation of results into the clinic. Therefore, novel compounds with typical medication-like durations of action (i.e. $< 24$ h) are needed to further the understanding of KOPr/dynorphin involvement in the neurobiology of stress and relapse. In the light of these premises, we characterized in vivo the pharmacotherapeutic-like effectiveness of systemic administration of LY 2444296 (also known as FP3FBZ; kind gift of Eli Lilly Co.), a novel shorter-acting KOPr antagonist, which is a close structural analog of LY 2456302 that has reached the clinical investigation stage.
**Methods:** First, we verified the specificity of the novel KOPr antagonist by testing the efficacy of systemic administration of LY2444296 (0, 1, 3 mg/kg; i.p.; pre-treatment time 30 minutes) in blocking KOPr-agonist induced behavioral and neuroendocrine effects, using U69,593-induced conditioned place aversion, U69,593-reduced locomotor activity and U69,593-induced rise in serum corticosterone.
Next, we tested the efficacy of systemic administration of LY2444296 (0, 1, 3 mg/kg; i.p.; pre-treatment time 30 minutes) in reducing signs of depressive-like behavior (measured as immobility time during a Porsolt's forced swim test, FST). We also tested stress levels (measuring the stress-responsive hormone corticosterone 30 minutes after the FST) in rats with three different stress exposure, including a) 2-day forced swimming (acute stress); b) repeated saline injections 3/day for 14 consecutive days (chronic stress), followed by a FST; protracted (8 weeks) social isolation (chronic stress), followed by a FST. A non-stressed group of rats was also included for comparison.
Data were analyzed with factorial or repeated measures two-way ANOVA, followed by the Student Neumann Keul's test for multiple comparisons. Significance was set at $p < 0.05$.
**Results:** Acute LY2444296 administration decreased KOPr agonist-induced conditioned place aversion and KOPr-induced hypolocomotion. LY2444296 alone did not alter locomotor activity, and did not induce place preference or aversion. Pretreatment with LY2444296 prevented the KOPr agonist-induced rise in serum corticosterone.
In rats never exposed to stress, acute LY2444296 administration did not alter basal serum corticosterone levels. In rats exposed to an acute stress, acute LY2444296 administration did not reduce immobility time during the FST or affect serum corticosterone level measured 30 min after the FST. However, acute pre-treatment with LY2444296 reduced both immobility time in the FST and the serum corticosterone level in animals exposed to repeated injections over 14 days. Preliminary results suggest that acute pre-treatment with LY2444296 also reduces immobility time in the FST for animals exposed to protracted social isolation.
**Conclusions:** Taken together, these results suggest that a KOPr antagonist with medication-like duration of action can acutely prevent KOPr-mediated behavioral and neuroendocrine effects. Also, the KOPr antagonist decreased behavioral and neuroendocrine effects occurring after chronic, but not acute stress conditions. Further studies are needed to determine its efficacy in preventing relapse-like behaviors in specific addictive diseases because this and similar compounds have potential in the translational investigation of stress responsiveness and depression-like co-morbidity in addictive states.

**Keywords:** acute stress, chronic stress, kappa opioid receptor, short-acting Kappa opioid receptor antagonist
**Disclosures:** Nothing to disclose.

### M105. Identification of a Hormone-Modulated Hypothalamic Reward Circuit in the Female Mouse

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**Background:** Gonadal steroids bias mammals toward reproductively relevant stimuli and influence activity in reward circuits to promote mating and ensure offspring survival. Such actions are adaptive for reproductive strategies, but can perturb motivational and reward related systems. Further, changes in gonadal steroids are linked to affective dysregulation in some but not all women. However, no studies to date have functionally identified a neural circuit for hormone regulated reward processing. The medial preoptic area (mPOA) of the hypothalamus is a steroid sensitive, sexually dimorphic neuroanatomical region essential for reproductive behavior across species and is well positioned to orchestrate motivational states through interactions with midbrain reward circuits. In the present study, we investigate the potential role of a genetically defined mPOA circuit in hormone modulated reward processing.
**Methods:** First, we optogenetically manipulate the activity of a genetically defined subset of mPOA neurons containing neurotensin (NTS), in freely behaving and naturally cycling intact female mice. Specifically, we examine reward related phenotypes in a real time place preference (RTPP) assay and during optical self-stimulation of NTS mPOA neurons, across the estrous cycle. Next, we optogenetically target NTS mPOA neurons in a second cohort of ovariectomized (OVX) female mice and measure reward related behavior before and after steroid replacement. Then, we test whether optical stimulation of these neurons promotes motivation for reproductive stimuli in a sexual preference test or consumption of a palatable food, to distinguish between general rewards and those with reproductive relevance. Additionally, in situ tissue processing reveals whether estradiol increases neurotensin mRNA within the mPOA of OVX females. Neuroanatomical tracing studies identify inputs to and projections from NTS mPOA neurons. Optical inhibition of NTS mPOA neurons in a separate cohort of OVX females will follow up from these studies to establish the necessity of NTS mPOA circuitry in motivated behavior, including sexual preference and food reward. Ongoing studies utilizing in vivo calcium imaging are also underway to monitor natural network dynamics of NTS mPOA populations under varying hormonal conditions and in response to reproductive stimuli. Physiological approaches will also examine how estradiol affects neuronal excitability within the NTS mPOA circuit. All procedures were approved by the Institutional Animal Care and Use Committee at University of North Carolina at Chapel Hill and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.
**Results:** We show that in vivo photostimulation of NTS mPOA neurons in intact naturally cycling female mice differentially regulates reward related behaviors across the estrous cycle. This was evident during RTPP ($F(3,30) = 12.83$, $p < 0.001$) and optical-self stimulation ($F(3,27) = 11.37$, $p < 0.001$). These reward related behaviors were most robust in proestrus compared to estrus. In OVX mice, optogenetic activation of NTS mPOA neurons in conjunction with steroid priming restores and enhances reward phenotypes in a long-lasting manner, in the RTPP assay ($F(12,99) = 7.630$ $p < 0.001$). Further, subsequent optical stimulation promotes investigation of an adult male rather than an adult female in a novel sexual preference assay ($F(2,13) = 7.363$ $p = 0.007$). However, stimulation does not alter consumption of a palatable food. Channelrhodopsin assisted circuit-mapping reveals a dense NTS containing projection from the mPOA to the ventral tegmental area (VTA). Optical stimulation of NTS mPOA-VTA projecting neurons promotes similar RTPP phenotypes ($F(1,18) = 15.35$ $p = 0.001$) and enhances male preference ($F(1,8) = 19.1$ $p = 0.002$). In addition, estradiol treatment enhances NTS mRNA in the mPOA of OVX female mice, compared to vehicle treated controls (t-test, $t(18) = 3.705$, $p = 0.014$). Rabies-assisted circuit tracing identifies a number of monosynaptic inputs to NTS mPOA neurons that border estrogen-receptor containing populations. (All statistical measures reported above are interactions derived from two-way ANOVAs unless otherwise noted).
**Conclusions:** Together, these findings are the first demonstration of a genetically defined, functional reward circuit that can be dynamically modulated by hormonal state. Such actions may be related to estradiol-enhanced production of NTS mRNA in the mPOA. Further, we reveal that activation of the NTS mPOA-VTA circuit is innately rewarding and may promote motivated behavior that is reproductively relevant. Elucidating the role of sex-specific neural circuits in motivational states will provide important insights that may aid in the development of sex- and- reproductive-status- based treatments for psychiatric disorders, such as reproductive mood disorders. This research was supported by an NIMH-funded Postdoctoral Training Program in Reproductive Mood Disorders, Department of Psychiatry, University of North Carolina at Chapel Hill (T32MH093315), the Foundation of Hope, and the National Institute on Drug Abuse (DA032750 and DA038168).
**Keywords:** Gonadal Hormones, hypothalamus, reward neural circuitry
**Disclosures:** Nothing to disclose.

### M106. Comparison of Diffusion Kurtosis Imaging and Diffusion Tensor Imaging Tractography Measures in Relation to Anhedonia in Young Adults

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**Background:** Diffusion tensor imaging (DTI) has been widely used to investigate microstructural changes in white

matter tracts associated with psychopathology. However, this method is limited in its ability to resolve intra-voxel crossing fibers and account for non-Gaussian diffusion. Recently, diffusion kurtosis imaging (DKI) has been developed to address these limitations and provide a more accurate model of diffusion, which may lead to more sensitive estimations of white matter changes. For example, the non-Gaussian distribution allows for a better estimate of signal-to-noise ratio (SNR) in brain regions that are susceptible to echo planar imaging (EPI) distortions such as the orbital frontal cortex.

To compare DKI and DTI-based tractography measures, we acquired DKI and DTI data in a subsample of participants (n = 29) recruited for the DIAMOND (Dimensions of Affect, Mood, and Neural circuitry underlying Distress) study, which is aimed at elucidating pathophysiologic processes in distressed young adults (18 to 25) to help identify predictors of future outcome.

**Methods:** Twenty-nine individuals (Mean age = 21.5, SD = 2.1; 16 females), 14 distressed (based on help-seeking) and 15 healthy, completed DKI, DTI and structural scans. Participants also completed the Mood and Anxiety Symptom Questionnaire-Anhedonic Depression subscale (Mean = 35.45, SD = 8.27, Range: 23 - 50).

Image acquisition: Participants were scanned on a 3T Siemens Trio at the Magnetic Resonance Research Center (MRRC) in the University of Pittsburgh Medical Center. DKI data were acquired with 197 diffusion-weighting directions (32 with b = 700, 65 with b = 1000, 100 with b = 2500) and 13 b0 images (repetition time (TR) = 3000 ms, echo time (TE) = 120 ms, flip angle = 90, field-of-view (FOV) = 256 × 256, 2 mm isotropic voxels, Multiband factor = 4). DTI data were acquired with 61 diffusion-weighting directions (b = 1000) and 7 b0 images (TR = 8100 ms, TE = 86 ms, flip angle = 90, FOV = 256 × 256, 2 mm isotropic voxels). In addition, we acquired anatomical images using an axial 3D-MPRAGE sequence with TR = 1500 ms, TE = 3.19 ms, flip angle = 8, FOV = 256 × 176 and 1 mm isotropic voxels.

Image analysis: Data were processed with explore DTI (Leemans et al., 2009), Freesurfer (http://freesurfer.net) and TRACULA (TRActs Constrained by UnderLying Anatomy; Yendiki et al., 2011). Diffusion–weighted images were corrected for eddy current, subject motion and EPI distortions with exploreDTI and used for white-matter tract reconstruction in TRACULA with anatomical priors from each subject's cortical parcellation and subcortical segmentation (Freesurfer) and the TRACULA tract atlas. We examined the relationship between anhedonia and DKI-based and DTI-based fractional anisotropy (FA) and radial diffusivity (RD).

**Results:** Anhedonia severity was negatively associated with DKI-based mean FA (r = -.414, p = .026) and positively associated with DKI-based mean RD (r = .39, p = .037) across all tracts. Examination of individual tracts, showed a negative association between anhedonia and DKI-based FA of the forceps minor (r = -.487, p = .007) and a trend towards a significant association between anhedonia and DKI-based FA of the cingulum-cingulate gyrus (r = -.357, p = .057). There were no significant associations between anhedonia and DTI-based measures.

**Conclusions:** DKI-based tractography measures, but not DTI-based measures, were predictive of anhedonia severity, providing support for a link between abnormalities in frontal and limbic pathways and anhedonia. These findings suggest that DKI is more sensitive for detecting changes in white matter tracts that may contribute to psychopathology in depression.

**Keywords:** diffusion, tractography, White Matter, Fractional-anisotropy, DKI

**Disclosures:** Mary Phillips is a consultant for Roche.

## M107. Adjunctive Brexpiprazole (OPC-34712) in Patients with Major Depressive Disorder and Irritability: A Post-Hoc Analysis on Symptoms of Anger

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**Background:** Irritability and anger attacks are common in major depressive disorder (MDD), affecting about one third to half of the patients. Brexpiprazole is a serotonin-dopamine activity modulator that is a partial agonist at serotonin 5-HT1A and dopamine D2 receptors with similar potency, and an antagonist at serotonin 5-HT2A and noradrenaline alpha1B/2C receptors. Brexpiprazole was recently approved by the FDA as adjunctive treatment for adults with major depressive disorder (MDD). In an exploratory, open-label study, irritability and depressive symptoms improved in patients with MDD and irritability treated with adjunctive brexpiprazole (NCT01942785). The hypothesis that brexpiprazole may be effective in treating irritability and anger is based on preclinical studies showing that serotonergic and dopaminergic antagonists or partial agonists could reduce aggression in rodents. In addition, the alpha 1 receptor antagonist prazosin is currently used to treat agitation and aggression in Alzheimer's, and for treatment of trauma nightmares and other sleep disturbances in post-traumatic stress disorder, recognizing the potential of noradrenergic antagonism in treatment of agitation/aggression and hyperarousal. The aim of the present post hoc analysis was to extend the findings of the previously reported exploratory study by evaluating the effects of brexpiprazole adjunctive to ADTs on symptoms of anger in patients with MDD and irritability using clusters of items related to anger on the SIS and KSQ anger/hostility subscale.

**Methods:** Patients with MDD and inadequate response to ADT were treated with their current ADT for a period of 2-weeks. Patients who still had an inadequate response, and were irritable (IDS-C30 item 6 $\geq$2), received 6-weeks open-label treatment with their current ADT at the same dose and adjunctive treatment with brexpiprazole. Brexpiprazole was discontinued at Week 6, and the patients continued with their current ADT during the follow-up period to Week 10.

**Results:** A total of 54 patients were treated with brexpiprazole + ADT, and of these 50 (92.6%) patients completed 6 weeks of treatment and 48 patients completed the follow-up period. At baseline, the patients frequently reported symptoms of anger, such as feeling angry (90.7%; KSQ item 20), furious (51.9%; KSQ item 37), infuriated (59.3%; KSQ item 56), or enraged (50.0%; KSQ item 69) and had high levels of anger symptoms as measured by the

Sheehan Irritability Scale (SIS) anger score (SIS items 5, 6, and 7) (mean [SD]: 17.3 [5.4]). The mean (SD) MADRS score at baseline was 28.5 (4.5). At Week 6, a lower percentage of the patients reported feeling angry (38.0%; KSQ item 20), furious (16.0%; KSQ item 37), infuriated (16.0%; KSQ item 56), or enraged (18.0%; KSQ item 69) than at baseline. In addition, improvements from baseline to Week 6 were observed in the SIS anger scores (-8.35, n = 50, 95% CI [-10.6;-6.1]). Depressive symptoms also improved at Week 6 as assessed by MADRS total score (-14.2, n = 50, 95% CI [-16.7;-11.6]). Adjunctive brexpiprazole was well tolerated, and no new safety concerns were observed.

**Conclusions:** Results from the current post-hoc analysis extend findings from the parent exploratory study that brexpiprazole adjunctive to ADTs may represent a novel strategy for reducing symptoms of anger in patients with MDD and inadequate response to ADT.

**Keywords:** Anger, MDD, Irritability, Brexpiprazole

**Disclosures:** François Menard, Charlotte Kampp Davidsen and Emmanuelle Weiller are employees of H. Lundbeck A/S. Ross A. Baker is an employee of Otsuka Pharmaceutical Development & Commercialization, Inc.

## M108. A Precision Medicine Approach to Antidepressant Treatment in Depression

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**Background:** Antidepressants were the third most common prescription drug taken by Americans of all ages in 2005–2008 and the most frequently used drug in those aged 18–44 years (1). Unfortunately, first line antidepressant treatment does not reliably lead to remission. For example, in the STAR-D study first line treatment with citalopram resulted in only 47% of patients achieving a response (mean time to response was 6 weeks) and 28-33% reached remission (mean time in treatment was 12 weeks). Compounding these problems, antidepressants have a slow clinical onset of action, meaning each treatment must be taken for 4 to 6 weeks before its efficacy in improving subjective mood can be assessed. It can therefore take many months before an effective antidepressant therapy is identified for individual patients during which their ability to work and function socially is severely impaired. Currently, no tests exist to guide clinicians as to whether their patient is responding to an antidepressant or not, contributing to a long delay before patients return to good mental health. The electronic Health Emotional Test Battery (eH-ETB) is a set of computer-based tasks which measure antidepressant-induced changes in the processing of emotional information. Such changes are apparent after brief periods of treatment and before improvement in subjective mood can be detected. The test-retest reliability of the emotional task used in the eH-ETB, the Facial Expression Recognition Task (FERT) was established in a separate study (see Dourish et al., this meeting). In the current study we assessed whether the eH-ETB could be used to predict, after 7-9 days of citalopram treatment, whether a depressed patient's mood would eventually improve after 6 weeks of antidepressant treatment.

**Methods:** From 10 primary care health centres around the UK we recruited 74 patients who were prescribed citalopram to treat their depression. Patients completed the eH-ETB and the Quick Inventory of Depressive Symptomatology Self-Report (QIDS-SR) before starting treatment and then again 7-9 days later. Response to treatment was assessed using the QIDS-SR which was completed again at week 6. Using the eH-ETB and QIDS-SR scores from the baseline and 7-9 day assessment a machine learning algorithm was derived to predict whether a patient would respond or not to treatment (treatment response was defined as >50% reduction in QIDS-SR score between baseline and week 6). Feature selection and algorithm validation was performed using a 'leave one out' validation procedure.

**Results:** 58 patients completed the full 6 weeks of the study. Of these 22 (37%) responded to treatment. The eH-ETB algorithm, derived from baseline and day 7-9 data, was able to predict with 76% accuracy a patient's response status after 6 weeks of treatment. The predictive value was better for patients who did not respond (negative predictive value 78%) than for patients who did respond (positive predictive value 72%).

**Conclusions:** These results demonstrate that in a primary care setting the eH-ETB can provide a sensitive early measure of the antidepressant efficacy of citalopram for individual patients. Thus, the eH-ETB system shows considerable promise as a tool to improve the treatment of depression by reducing the time taken for the majority of patients to return to good mental health.

**Keywords:** Precision Medicine for Depression, Machine learning, Biomarker risk assignment algorithm

**Disclosures:** Gerard Dawson and Colin Dourish are employees and shareholders in P1vital. Michael Browning is an employee of P1vital. Guy Goodwin, Catherine Harmer and Michael Brammer are consultants to P1vital.

## M109. Neural and Hormonal Responses to Negative Affective Stimuli: Impact of Sex and Depressed Mood

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**Background:** Dysregulated stress response and hypothalamic pituitary adrenal (HPA) axis function are implicated in numerous psychiatric disorders. Both are regulated by neural circuitry including periaqueductal gray (PAG), hypothalamus (HYPO), amygdala (AMYG), hippocampus (HIPP), and medial and orbital prefrontal cortices (mPFC, OFC). These regions include some of the most sexually dimorphic nuclei in the brain. Therefore it is not surprising previous work identified sex differences in prevalence of psychiatric disorders and neural and physiologic responses to stress. Here we investigated neural and physiologic functioning in major depressive disorder (MDD), psychoses (PSY: bipolar and schizophrenia) and healthy controls (HC)

during mild visual stress and studied the impact of sex and depressed mood in these deficits. Understanding hormonal physiology and stress response circuitry function is a critical step in the search for biosignatures.

**Methods:** Adults (n = 99) were recruited from a community population of individuals with recurrent MDD (14 F, 13 M), PSY [schizophrenia (SCZ) or bipolar psychoses (BP); 15 F, 16 M), and HC (19 F, 22 M). All participants underwent functional magnetic resonance imaging (fMRI) on a 3T Siemens scanner, with a mild visual stress task presenting negative, neutral, and fixation images adapted from International Affective Picture System. Bloods and saliva were collected at several time points throughout the fMRI protocol (time 0, 15, 30, 60, and 90 minutes after scanning) and standard immunoassay kits assessed 17β-estradiol and free androgens from blood and cortisol from saliva. Cortisol response was calculated as cortisol peak minus cortisol measured at time 0, all adjusted for cortisol measured at time 0. We also assessed mood and anxiety symptoms using the Profile of Mood States and State-Trait Anxiety Inventory and factor analyzed them, yielding a primary component reflecting depressed mood.

fMRI data were analyzed using SPM8. Anatomically-defined masks (PAG, HYPO, AMYG, HIPP, ACC, OFC, and mPFC) were overlaid on a supergroup (n = 99) mean of the negative > neutral contrast with a voxel-wise FWE-corrected ($p < 0.05$) threshold and showing that the mild visual stress task elicited significant (FWE $p < 0.05$) BOLD response in PAG, HYPO, AMYG, HIPP, OFC, and mPFC. Mean BOLD responses from these intersections were extracted for each participant and exported into SAS to test the impact of cortisol response and sex. Significant interactions with sex were further examined to assess the associations with 17β-estradiol (women) or free androgens (men). Finally, we assessed the possible modulatory role of depressed mood in these relationships. Similar analyses were conducted for task-related connectivity, analyzed using generalized psychophysiological interaction (gPPI), and with HYPO, the key relay station for HPA function and stress response, as a seed.

**Results:** Two-way ANOVA assessing the interaction between cortisol response and sex on BOLD signal in stress circuitry showed a main effect of cortisol response on BOLD in L OFC ($F(3,87) = 2.7$, $p = 0.05$), with higher cortisol response related to lower BOLD in L OFC, and cortisol response * sex interaction on BOLD in L mPFC ($F(3,88) = 3.14$, $p = 0.03$). Posthoc analyses revealed that higher cortisol response was related to lower BOLD in L mPFC in females ($t(43) = -2.07$, $p = 0.04$, $R2 = 0.09$) but not males ($t(48) = 1.62$, $p = 0.11$). In males, there was a cortisol response * free androgen interaction ($F(3,45) = 3.14$, $p = 0.03$). While males with high free androgens showed no relationship between cortisol response and BOLD in L mPFC ($t(23) = -0.24$, $p = 0.81$), males with low free androgens showed a positive relationship ($t(24) = 2.22$, $p = 0.04$, $R2 = 0.18$).

Task-related connectivity analyses showed a main negative effect of cortisol response on connectivity between HYPO–L AMYG ($z = 3.36$, FWE $p = 0.01$) and cortisol response * sex interaction on connectivity between HYPO–L HIPP ($z = 4.39$, FWE $p = 0.001$). Posthoc analyses revealed that higher cortisol response was associated with lower HYPO–L HIPP connectivity in females ($t(42) = -3.39$, $p = 0.0003$, $R2 = 0.27$) but not males ($p = 0.95$).

Follow-up analyses, asked whether these connectivities were modulated by depressed mood. In females but not males, there was an interaction of cortisol response and depressed mood on HYPO–L AMYG (Females: $F(3,31) = 7.97$, $p = 0.0004$; Males: $p = 0.46$) and HYPO–L HIPP connectivity (Females: $F(3,31) = 16.02$, $p < 0.0001$; Males: $p = 0.39$). Posthoc analyses using a median split showed that higher cortisol response was associated with less HYPO–L AMYG ($t(16) = -3.13$, $p = 0.007$, $R2 = 0.39$) and HYPO–L HIPP ($t(16) = -4.43$, $p = 0.0005$, $R2 = 0.57$) connectivity in females with depressed mood but not in females without depressed mood (HYPO–L AMYG: $t(17) = -0.66$, $p = 0.52$; HYPO–L HIPP: $t(17) = 1.51$, $p = 0.15$).

**Conclusions:** These findings contribute to the RDoC initiative to determine biosignatures associated with regulation of arousal and negative affect across psychiatric disorders and offer significant insight into the role of sex and depressed mood on hormonal physiology associated with response to negative affective stimuli. Individuals with high cortisol response were unable to recruit cortical regulatory centers (L OFC, L mPFC) and had decreased connectivities between HYPO–AMYG and HYPO–HIPP. These findings were particularly true for women with depressed mood compared with other women and men, possibly explaining why some women are more vulnerable to mood disorders than men.

**Keywords:** Research domain criteria (RDoC), stress response circuitry, mood disorders, sex difference, Cortisol

**Disclosures:** Nothing to disclose.

## M110. Zinc as a Mediator of Inflammation in the Brain: Implications for Mood in Bipolar Disorder?

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**Background:** Bipolar disorder (BD) is associated with altered functioning of neurotransmitter systems in the brain and neuroinflammation due to excitotoxicity of these systems, especially the dopaminergic system. Zinc (Zn) is necessary for certain dopaminergic neurotransmission in the brain, and plays a role in the inflammatory process. Excitotoxic insults can increase intracellular zinc levels in neurons, contributing to cell damage and apoptosis in conditions such as stroke and traumatic brain injury. However, the factors influencing zinc accumulation and dysregulation in the brain in relation to mood disorders are not understood. Low peripheral Zn has been associated with mood disorders in both human and rodent studies. Though the neurobiological role of Zn is still being elucidated, very little work has focused on BD. We hypothesized that 1) in humans, low Zn is a state marker of mania in BD, and 2) in rodents, inflammation found during mood episodes contributes to cortical atrophy via effects on intracellular zinc dysregulation.

**Methods:** Human studies: Subjects with BD were recruited while in episode and were assessed for depression and

mania using Hamilton's Depression Rating Scale (HDRS) and Young Mania Rating Scale (YMRS), respectively. All procedures were approved by the Penn State College of Medicine IRB #39364. The presence of manic and depressed symptoms defined a mixed-manic state (MM). Blood samples were taken on admittance to the study. Healthy control subjects (HC) were recruited using advertising and underwent the same procedure as BD subjects. Human serum samples were digested in nitric acid (0.1N) for 24 hours and Zn concentration was analyzed using flame atomic absorption spectroscopy. Rodent studies: All rodent studies were approved by the Penn State IACUC #46108. We injected male C57Bl/6 mice (N = 14) intraperitoneally with lipopolysaccharide (N = 7; LPS, 0.83 mg/kg) or saline (N = 7; .9%) and sacked the mice 24 hours later. Fresh brain tissue was stored in RNAlater at -20° C until use (N = 10). We then harvested right and left hippocampi for protein and mRNA analysis, respectively. Two brains from each group were fresh-frozen in isopentane over dry ice and cut with a cryostat at -20° C at a thickness of 12μm.

**Results:** Average serum Zn concentration was lower in subjects with BD when compared to healthy controls (HC). When the BD group was subdivided by mood state, we found a significantly lower serum Zn concentration in patients with BD with mixed mania (MM) group compared to HC. We observed no difference between the depressed BD group and either the MM or HC groups. Rodent studies: After 24 hours of LPS-induced inflammation, we observed a significant increase in expression of the zinc importer ZIP12 in the hippocampus. Imaging studies using the membrane-permeable zinc specific fluorophore, Zinpyr-1 (ZP1), revealed accumulation of intracellular zinc in the hippocampi of LPS-treated mice.

**Conclusions:** Here, we highlight a link between zinc and inflammation, and zinc and mood in BD. Lower peripheral zinc may be a marker of sequestration of zinc in a chronic inflammatory state. Rodent studies link altered zinc transportation to neuroinflammation, which may influence neurotransmission of dopamine and thus affect excitotoxicity. Elucidating the physiological underpinnings of these observations will form the basis of future work. We conclude that LPS drives intracellular zinc accumulation in the hippocampus, which may contribute to hippocampal atrophy.

**Keywords:** Bipolar Disorder, neuroinflammation, systemic inflammation

**Disclosures:** Nothing to disclose.

### M111. Variation Does Matter - Fast BDNF Serum Level Increase and Diurnal BDNF Oscillations are Associated with Therapeutic Response after Partial Sleep Deprivation

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**Background:** Preclinical and clinical studies support a role for brain-derived neurotrophic factor (BDNF) in the pathophysiology of stress-related mood disorders. It has been shown that serum BDNF levels were decreased in depressed patients compared to healthy controls. Furthermore, BDNF seems to be linked to classic antidepressant action. Available pharmacological treatments for depression are characterized by significant limitations with low efficacy and a major delay until treatment response. This demonstrates the urgent need for more efficient and fast-acting antidepressants. Besides ketamine, sleep deprivation (SD) as well as partial sleep deprivation (PSD) are effective and fast-acting antidepressant methods. However, the underlying molecular mechanisms of SD are not well understood; especially possible mechanisms explaining the rapid, but transient antidepressant effect of SD are unknown.

**Methods:** We evaluated serum BDNF from 28 patients (13 men, 15 women; age 45.1 + /-12.1 years) suffering from major depressive disorder (MDD), who were naïve to SD therapy at seven different time points within a 32 hour time window before and after PSD. Participants experienced PSD the second part of the night starting at 1:30 am. PSD-response was assessed by 6 items of the Hamilton Depression Rating Scale (HDRS) before (day 0), during, after PSD (day 1) and at follow-up after 2 weeks (FU2).

**Results:** PSD induced a very fast increase in BDNF serum levels the day after PSD which parallels clinical findings, since levels increased with decreasing depression scores in all participants. Notably, responders showed a significant diurnal BDNF serum variation the day before PSD as well as after PSD, while diurnal profile of serum BDNF from non-responders did not vary.

**Conclusions:** The elasticity in diurnal serum BDNF variation is associated with favourable treatment response to PSD in patients suffering from MDD. Therefore, a normalized BDNF serum profile which oscillates in a circadian fashion seems to precede, rather than follow a favourable treatment outcome in depressed patients. Accordingly, we suggest that diurnal profiling of BDNF should be monitored at baseline especially before therapeutic intervention begins for the purpose of early response prediction.
Furthermore the fast increase of BDNF is comparable to effects seen with ketamine infusion.

**Keywords:** BDNF, Major Depressive Disorder, Biomarker, Fast-acting Antidepressant, circadian rhythm

**Disclosures:** This project was supported by a grant from the Swiss National Science Foundation (SNF-Nr 320080-104022, E.H.).

### M112. Using the Cambridge Neuropsychological Test Automated Battery (CANTAB) to Find Cognitive Markers of Vulnerability to Mental Illness in Healthy Siblings of Bipolar Parents

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**Background:** Impairments in verbal memory, attention and executive functions have been consistently reported in

patients with bipolar disorder (BD). The cognitive profile of siblings of BD patients is however less clearly established. The aim of this study was to assess the cognitive function of siblings of patients with BD and compare it with that of patients with BD and healthy controls (HC).
**Methods:** We recruited 23 HC (33.52 ± 10.29, 8 males), 15 siblings of bipolar parents (37.47 ± 13.15, 4 males) and 27 individuals with BD (34.26 ± 10.19, 9 males, 25 BD I, 1BD II and 1 BD-NOS). Siblings had no current or lifetime history of mental disorders. Participants performed the Cambridge Neuropsychological Test Automated Battery (CANTAB) - a comprehensive and validated computerized cognitive battery - and completed questionnaires assessing mood (e.g. Young Mania Rating Scale, Montgomery-Asberg Depression Rating Scale) and global functioning (e.g. Functioning Assessment Short Test – FAST). Multivariate analyses were performed to compare the three groups in these measures.
**Results:** Siblings of BD parents were less accurate on a task of sustained attention (Rapid Visual Processing) when compared to HC. As expected BD displayed pronounced deficits in affective processing, sustained attention and visual memory compared to HC. Overall siblings responded faster than BD to visual memory items. Patients with BD, and to a lesser extent siblings of BD parents displayed more mood symptoms than HC but the severity of these symptoms did not reach clinical significance.
**Conclusions:** Decreased sustained attention may constitute a cognitive marker of vulnerability to mental illness in siblings of BD patients. The current findings will inform future prevention intervention program targeting cognitive vulnerability to mental illness. The use of both validated neuropsychological measures and functional neuroimaging techniques will give researchers a broader understanding of the neurocognitive differences between BD, healthy siblings of BD parents and HC.
**Keywords:** Bipolar Disorder, endophenotype, Cognition, prevention
**Disclosures:** Funding source: This work was supported by R01MH69774, the Dunn Foundation, and Pat Rutherford, Jr Chair in Psychiatry at UTHealth.

### M113. Influence of Reproductive Hormones and Nighttime Hot Flashes on Mood in Depressed Perimenopausal Women Reporting Stressful Life Events

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**Background:** The perimenopause is a period of increased risk for depression. Estradiol variability, hot flashes, and stressful life events each increase the risk for depressive symptoms during this time period. However, the relative contribution of these factors to depressive symptom severity in depressed perimenopausal women is not well understood. We hypothesized that estradiol variability and nighttime hot flashes would independently predict worse mood in depressed perimenopausal women.
**Methods:** Perimenopausal women with mild depression (Montgomery-Åsberg Depression Rating Scale [MADRS] score 10–24) completed assessments of mood, serum estradiol and progesterone weekly for 9 weeks, as well as a stressful life event survey and a daily hot flash diary. Repeated-measure regression was used to examine independent associations of mood with the coefficient of variability in estradiol, the number of distinct progesterone elevations exceeding 6 nl/dl, and hot flashes, while accounting for recent stressful life events.
**Results:** Among 51 perimenopausal participants with a mean age of 48.2yrs, menopause status was evenly divided between the early and late menopause transition. The mean baseline MADRS score was 15.4 reflecting mild-to-moderate depressive symptom levels, 84% of women reported experiencing hot flashes, and 88% reported recent stressful life events. During the study period, 90% had variable but detectable estradiol levels while 10% were persistently hypo-estrogenic; 61% never had a progesterone elevation, while the remaining 39% had at least one distinct elevation. Fewer progesterone elevations ($p < 0.001$), greater variability in estradiol ($p = 0.049$), and stressful life events ($p = 0.06$) were associated with higher depression scores in univariate models. In adjusted models, MADRS scores were lower in women who had episodic elevations of progesterone ($p < 0.001$), while greater variability in estradiol increased MADRS scores in the absence ($p < 0.001$), but not the presence ($p = 0.80$), of episodic progesterone elevation. Nighttime (but not daytime) hot flashes were associated with higher MADRS scores in women with persistent hypo-estrogenism ($p = 0.001$), but were not associated with depressive symptom severity in those with detectable estradiol levels ($p = 0.22$). Stressful life events were not associated with depression symptom severity in adjusted models.
**Conclusions:** In perimenopausal depressed women, increasing dysregulation of ovarian hormones with greater estradiol variability and loss of ovulation indicated by absence of progesterone production is associated with worse mood. In addition, nighttime hot flashes are associated with higher depression scores among those who are persistently hypo-estrogenic. These menopause-specific correlates of depression are strongly linked with worse mood after accounting for stressful life events.
**Keywords:** Depression, Estradiol, Progesterone, Perimenopause, Hot Flashes
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### M114. Exponential State Transition Dynamics in the Rest-Activity Architecture of Patients with Bipolar Disorder

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**Background:** Understanding sleep dynamics is important in patients with bipolar disorder (BD), a mood disorder characterized by transitions from depression to mania/hypomania. Sleep disruptions are characteristic of the acute phase of the illness; in those who are stable (euthymic), sleep disruption may signal a high risk of transition into depression or mania. Even in euthymia, however, BD patients have reduced sleep efficiency, longer sleep latencies and a tendency to sleep longer relative to controls.
Our goal was to model the temporal dynamics of sleep-wake transitions from actigraphic data in patients with bipolar disorder using a probabilistic state transition approach.
**Methods:** We collected actigraphic data for 14 days from 20 euthymic bipolar disorder patients, who had been characterized clinically, demographically and with respect to their circadian preferences ("chronotype"). We processed each activity record to generate a series of transitions in both directions between the states of rest (R) and activity (A) and plotted the estimated transition probabilities (pRA and pAR). Each 24-h period was also divided into a "rest phase" consisting of the 8 consecutive least active hours in each day and an "active phase" consisting of the 16 consecutive most active hours in each day. We then calculated separate transition probabilities for each of these phases for each participant. We subsequently modeled the rest phase data to find the best fit for sleep-wake transitions using maximum likelihood estimation. We also calculated the association of clinical and demographic variables with transition probabilities using parametric and nonparametric tests.
**Results:** The best fit model for sleep-wake transitions during the rest phase was a mixture (bimodal) of exponential functions. Of those patients with rapid cycling, 75% had an evening-type chronotype. Men with an evening chronotype had a higher probability of waking up during the rest phase ($kRA = 0.219 \pm 0$) compared to women who were either morning type ($kRA = 0.082 \pm 0.032$) or evening type ($kRA = 0.094 \pm 0.036$) [$F(1) = 13.73$, $p < 0.005$]. Furthermore, BD II patients on antidepressants had a lower probability of transitioning back to sleep after arousal during the rest phase than those not on antidepressants ($kARREST = 0.050 \pm 0.006$ vs $0.141 \pm 0.058$; $F(1, 15) = 3.40$, $p < 0.05$).
**Conclusions:** The dynamics of transitions between rest and activity in BD can be accounted for by a mixture (bimodal) of exponential functions. Patients taking antidepressants, those with BD II and those with an evening chronotype had a reduced probability of sustaining and returning to sleep.
**Keywords:** sleep, Bipolar Disorder, Actigraphy
**Disclosures:** Nothing to disclose.

### M115. Automatic Detection of Social Rhythms in Bipolar Disorder via Smartphone

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**Background:** Substantial evidence indicates that greater regularity of daily routines is associated with improved outcomes for individuals with bipolar disorder. Indeed, stability of social rhythms is central to several forms of empirically-supported psychosocial treatment for bipolar disorder, including interpersonal and social rhythm therapy (IPSRT), family-focused treatment (FFT) and cognitive behavioral approaches. Ironically, when information about rhythmicity would be of greatest value to the clinician, patients are often unable or unwilling to complete self-reports of such data. To evaluate the feasibility of automatically assessing the Social Rhythm Metric (SRM), a clinically-validated marker of the stability of daily routines, we assessed the relationship between self-reported SRM data and passively-sensed data from smartphones.
**Methods:** Seven patients with bipolar disorder used smartphones for 4 weeks, passively collecting sensor data including accelerometer, microphone, location, and communication information to infer behavioral and contextual patterns. Participants simultaneously completed SRM entries using a smartphone app.
**Results:** We found that automated sensing can be used to infer the SRM score. Using location, distance traveled, conversation frequency and non-stationary duration as inputs, our generalized model achieves root-mean-square-error (RMSE) of 1.40, a reasonable performance given the theoretical range of the SRM score (0 – 7). Personalized models further improve performance with mean RMSE of 0.92 across users. Classifiers using sensor streams can predict stable (SRM score $>= 3.5$) and unstable (SRM score $< 3.5$) states with high accuracy (precision: 0.85 and recall: 0.86).
**Conclusions:** Our results suggest automatic smartphone sensing is a feasible approach for inferring rhythmicity, a key marker of wellbeing for individuals with bipolar disorder and that automatically-sensed data provide an excellent proxy for self-reported data on daily routines, offering novel opportunities for clinical intervention when it is most needed.
**Keywords:** Bipolar Disorder, Technology, Social Rhythms
**Disclosures:** Equity interest in HealthRhythms.

### M116. Lymphoblast Cell Lines from Women with Premenstrual Dysphoric Disorder (PMDD) Differ in mRNA and Protein Expression Profiles of the ESC/E(Z) Pathway Compared with Asymptomatic Controls

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**Background:** Premenstrual dysphoric disorder (PMDD, in the DSM-V), a mood disorder linked to the luteal phase of

the menstrual cycle, affects 5-10% of women of reproductive age. PMDD is characterized by cyclic symptoms during the luteal phase that disappear after the onset of menstruation; symptoms include increased anxiety, irritability, and sadness. Diagnosis-related differences in reproductive hormone levels have not been consistently observed, suggesting symptoms are not due to an excess (or deficiency) of peripheral steroid hormone. In a clinical trial of ovarian steroid hormone suppression and add-back, we determined that symptoms remit during suppressed hormone secretion, and recur after re-exposure to physiologic levels of ovarian steroids only in women that had been diagnosed with PMDD – women with no history of PMDD (controls) did not exhibit symptoms. These findings suggest behavioral differences reflect abnormal cellular responsivity to ovarian steroids, rather than abnormal hormone levels.
**Methods:** To identify altered cellular pathways underpinning a differential hormonal sensitivity in PMDD, we created lymphoblastoid cell cultures from blood samples of women (with confirmed presence or absence of PMDD) from the ovarian steroid hormone suppression and add-back clinical trial. We confirmed our lymphoblast cell cultures express receptors for estradiol (ESR1, ESR2) and progesterone (PGRMC1, PGRMC2) with qRT-PCR and protein assays. Cell lines from women with and without PMDD (n = 10, 9, respectively) were grown in phenol red-free media for 5 days, exposed 24 hours to vehicle or ovarian steroids (estradiol or progesterone) and examined for differences in gene expression via whole transcriptome RNA analysis (RNA-seq). Two pathway analyses, DAVID and GSEA, were applied to the RNA-seq results. Among several significantly altered pathways, the ESC/E(Z) complex pathway was chosen for further study because of its role in gene regulation, reported modulation of/by steroid signaling, and small number of genes in the pathway. A larger number of lymphoblastoid cell cultures from PMDD ($n = 30$) or asymptomatic control subjects ($n = 30$) was used to examine baseline mRNA expression of the 13 genes in the ESC/E(Z) pathway via qRT-PCR and baseline protein expression via ProteinSimple (capillary electrophoretic size separation and chemiluminescent antibody quantification). Whole exome sequencing was then performed on DNA extractions from blood of women with and without PMDD ($n = 52$, $n = 27$, respectively) and compared against allele frequencies in ExAC (Exome Aggregation Consortium) to look for potential sequence variants correlated with PMDD.
**Results:** Whole transcriptome RNA analysis revealed many genome-wide RNA expression changes between cell lines from women with and without PMDD. DAVID and GSEA pathway analyses identified several altered molecular pathways, the most interesting being the ESC/E(Z) complex. Within this pathway RNA-seq showed an overall pattern of increased baseline mRNA expression in PMDD cell lines over controls, with significant effects in MTF2, PHF19, and SIRT1 ($p < 0.05$). This overall baseline pattern was confirmed with the larger sample cohort for qRT-PCR, with significant differences in MTF2, SIRT1, HDAC2, and RBBP7 ($p < 0.05$). Interestingly, protein analysis revealed an expression in the opposite direction, with a decreased baseline protein expression in PMDD cell lines over controls, with MTF2, PHF19, and SIRT1 reaching significance ($p < 0.05$). Further, we found a diagnosis x progesterone interaction effect in RNA expression (EED, EZH2, MTF2, $p < 0.05$) where expression increased after progesterone treatment in control but not PMDD cell cultures, and a diagnosis x estradiol interaction in RNA expression (JARID2, $p < 0.05$) where expression decreased after estradiol treatment in PMDD but not control cell cultures. Among the sequence variants discovered by exome sequencing, several of the 13 genes of the ESC/E(Z) complex pathway have differentially expressed alleles between groups that could potentially disrupt protein coding.
**Conclusions:** These data suggest that women with PMDD have a dysregulated ESC/E(Z) complex at both baseline and in response to ovarian steroid hormones, which could serve as the potential cellular basis for the difference in behavioral response to hormones observed in PMDD. Disruption of the ESC/E(Z) complex, which is modulated by steroid signaling and involved in gene regulation, has been associated with other mood disorders such as depression and anxiety, but has yet to be connected to hormone-related mood disorders such as PMDD, postpartum depression (PPD), or perimenopausal depression (PMD). In a search of the association network index GeneMANIA.org using the 13 genes of the ESC/E(Z) complex pathway and the 4 hormone receptor genes we examined in our lymphoblastoid cell lines, we found that HDAC2 is the only gene associated with the ESC/E(Z) complex, ESR1, and PGRMC2, making it a strong potential candidate for the link between hormone response and gene silencing. Additionally, the finding that components of the ESC/E(Z) complex have higher mRNA expression, but lower protein expression, in PMDD vs control samples is the first evidence of differences in cellular function in PMDD. These cellular mechanisms may underlie differential behavioral response to normal hormone levels and help explain their translation into pathological mood states in hormone-related mood disorders.
**Keywords:** mood disorder, ovarian steroids, Whole exome sequencing, RNA Sequencing, premenstrual dysphoric disorder
**Disclosures:** Nothing to disclose.

### M117. The Effect of IL-6 Neutralizing Agents on Depressed Mood and Anhedonia in Immunology and Oncology Clinical Trials

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**Background:** Cytokines have been implicated in neuronal plasticity, and stress coping. Clinical and preclinical studies have suggested a role for cytokines such as IL-6 in the pathophysiology of depression; however it is unclear whether blockage of IL-6 can directly alleviate depressive symptoms. Here we report changes in depressive symptoms measured in patients treated with two different IL-6 neutralizing antibodies in phase II clinical trials: sirukumab

for rheumatoid arthritis (RA) and siltuximab for multicentric Castleman's disease (MCD).

**Methods:** IL-6 data were from phase 2, multicenter, randomized double-blind, placebo-controlled studies evaluating sirukumab in RA patients (N = 176), and siltuximab in MCD patients (N = 79). Two core depressive symptoms (depressed mood and anhedonia) and two fatigue symptoms (worn out and tired) were documented on the SF-36 Health Survey, version 2.0. Patients were grouped by presence/absence of prevalent depressed mood and anhedonia (PDMA), meaning one of the depressive symptoms was present at least 'most of the time' and the other at least 'some of the time' for four weeks. Treatment groups were defined as receiving any treatment dose. Efficacy on depressive symptoms and fatigue were evaluated directly and with adjustment for symptom severity of the primary treated disease (DAS28-CRP for RA, and MCDOS for MCD) using a mixed-effects model for repeated measures. Fixed-effects included treatment group, visit, and interaction between treatment group and visit. Change in PDMA status was also investigated separately in responders and non-responders to the primary disease being treated (RA or MCD). The first follow up visit assessed was at week 12 for sirukumab, and week 6 for siltuximab. Cytokine biomarkers were evaluated for correlations with depressive symptom improvement where available.

**Results:** At baseline, 26% of RA patients and 20% of MCD patients were classified as having PDMA. This group also experienced significantly more fatigue than those without PDMA ($p < 0.001$). In the sirukumab study, presence of PDMA was associated with significantly greater RA symptoms, but no difference in baseline biomarker levels. By week 12, sirukumab treatment, compared to placebo, significantly improved depressive symptoms ($p = 0.04$) and fatigue ($p = 0.05$) among PDMA patients. The within-group mood effects remained significant after covarying for changes in RA severity (Depressive symptoms: sirukumab, $p = 0.0006$; placebo, $p = 0.23$. Fatigue symptoms: sirukumab, $p = 0.02$; placebo, $p = 0.5$). In patients designated as RA non-responders significant improvement in depressive symptoms, but not fatigue, were observed (Depressive symptoms: sirukumab, $p = 0.0024$; placebo, $p = 0.33$. Fatigue symptoms: sirukumab, $p = 0.07$; placebo, $p = 0.6$). In the siltuximab study, the presence of PDMA was not associated with a difference in MCD severity, however patients with PDMA had higher levels of baseline CRP ($p = 0.03$). By week 6, siltuximab treatment, compared to placebo, significantly improved depressive symptoms ($p = 0.04$) and fatigue ($p = 0.04$) among PDMA patients. This improvement over baseline persisted at week 15 for both depressive symptoms ($p = 0.06$) and fatigue ($p = 0.01$). After co-varying for changes in MCD symptom severity, depressive symptom reductions remained significant at week 6 and marginally significant at week 15 (Depressive symptoms: week 6, $p = 0.05$; week 15, $p = 0.07$). In patients designated as MCD non-responders significant improvement in depressive symptoms, but not fatigue, were observed in treated subjects (Depressive symptoms: siltuximab, $p = 0.04$ improvement; placebo, $p = 0.79$. Fatigue symptoms: siltuximab, $p = 0.004$; placebo, $p = 0.16$). In the sirukumab study, pre-treatment serum levels of sIL-6R correlated with the magnitude of improvement in depressive symptoms ($r = 0.44$, $p = 0.015$), and could be useful for enriching for patients whose mood improves on sirukumab treatment.

**Conclusions:** The current findings show that treatment with IL-6 neutralizing antibodies administered intravenously and subcutaneously is associated with improvement in depressed mood and anhedonia, as well as fatigue, in patients with RA and MCD, even when correcting for RA and MCD symptoms severity. Results are consistent for patients with two different disease backgrounds treated with two different antibodies, suggesting anti-IL-6 may afford a novel mechanism for producing antidepressant effects. A trial directly examining the antidepressant effects of the IL-6 neutralizing agent sirukumab in depressed patients is underway.

**Keywords:** neuroimmune, cytokines, Major Depressive Disorder (MDD)

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### M118. Phosphatidylcholines (PCs) in Major Depressive Disorder: A Plasma-Based Endophenotype Related to Inflammation

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**Background:** Evidence is mounting to suggest a key role for inflammation in the etiology of neuropsychiatric disease including major depressive disorder (MDD). An inflammation response is characterized by changes in the concentration of plasma proteins, including lipids. Consequently it is thought that certain lipids play a crucial role in regulation of the immune response. Thus, we investigate the differing genetic overlap between 23 lipid classes and a continuous scale of MDD, using the endophenotype ranking value (ERV), and follow-up on our top-ranked lipid class using polygenic, linkage and association analyses. Identifying risk genes for depression, via a focus on plasma-based endophenotypes, might enhance our understanding of the etiology of MDD, enabling earlier and more reliable detection as well as, potentially, the development of new and more effective therapies.

**Methods:** An ERV, which represents the standardized genetic covariance between the endophenotype and illness, was calculated for depression (derived using a factor model of all items from the past depressive episode section of the Mini International Neuropsychiatric Interview) and 23 lipid classes acquired from 10 μl of plasma. We then followed up on the top-ranked lipid class by, investigating possible sub-groupings of species within that class, using hierarchical cluster analysis. We followed up on those sub-groupings, and their genetic overlap with MDD, using univariate and

bivariate linkage and association analysis. All genetic analysis was conducted in SOLAR in a sample of Mexican-American randomly selected, extended pedigrees (N = 569, 40 families, average size 13.52 people, range = 2-80, and 29 singletons).
**Results:** The highest ranked plasma-based lipid endophenotype for MDD was the phosphatidylcholines (PCs; ERV = 0.14, h2MDD = 0.20, seMDD = 0.06, h2PC = 0.38, seMDD = 0.06, rhog = -0.53, p = 0.01). Five clusters emerged from hierarchical cluster analysis applied to the genetic correlations of all 26 species of PCs, of these one was significantly associated with MDD after correcting for multiple tests (h2 = 0.41, se = 0.06, rhog = -0.54, p = 0.011). This cluster was characterized by those species with an increased number of double bonds. Significant bivariate linkage was observed for this cluster of PCs and MDD on chromosome 14 at 112cM (LOD = 3.44). Two genes were found under the peak C14orf177 and BCL11B, the latter of which is involved in human T-cell function. Post-hoc bivariate association analysis, correcting for the LD-adjusted number of SNPs under the peak ($\alpha$ = 1.7x10-04), revealed a suggestively significant regulatory variant rs1257633 ($\chi 2$ = 13.562, p = 1.1x10-03) located in the intergenic region between the two genes.
**Conclusions:** PCs have been previously implicated in the etiology of MDD and anxiety (Demirkan et al, 2013). Moreover, PCs contain arachidonic acid, an omega-6 fatty acid that is abundant in the brain and which is a precursor to eicosanoid biosynthesis, where eicosanoids are inflammatory mediators (Lone et al, 2013). Interestingly, arachidonic acid levels in blood have previously been associated with symptoms of MDD (Adams et al, 1996; Lotrich et al, 2013). The results of the present study taken together with previous research suggest that the genes C14orf177 and BCL11B warrant further investigation as potential candidates for MDD, particularly when looking at the potential role of inflammation in the etiology of illness risk.
**Keywords:** inflammation, lipids, Depression, fatty acid, Linkage
**Disclosures:** Nothing to disclose.

### M119. Retrieval of Positive and Negative Associations Produces Opposite Responses in BLA Neurons Projecting to NAc and CeA

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**Background:** The valence of our emotions guide our daily life, allowing us to produce adaptive behaviors in order to ensure our survival and well-being. The amygdala, and more specifically the basolateral amygdala (BLA) is necessary for processing negative valence (fear conditioning) and has more recently be shown to be involved in positive valence conditioning (reward seeking). However, very few studies describe how BLA neurons encode valence and the downstream targets of these neurons remain unknown.
**Methods:** By combining optogenetic photo-identification of BLA neurons with a specific projection target and large scale electrophysiological recordings in behaving mice, we tested whether BLA neurons projecting to different downstream target encode valence differentially. After mice learned to associate an auditory cue with a rewarding outcome (sucrose delivery), and a second tone with an aversive outcome (quinine delivery), we recorded BLA neurons while the animals were performing the task.
**Results:** By combining optogenetic photo-identification of BLA neurons with a specific projection target and large scale electrophysiological recordings in behaving mice, we tested whether BLA neurons projecting to different downstream target encode valence differentially. After mice learned to associate an auditory cue with a rewarding outcome (sucrose delivery), and a second tone with an aversive outcome (quinine delivery), we recorded BLA neurons while the animals were performing the task. Amongst the 1570 single units we recorded in the BLA, 56% responded to cues of positive and/or negative valence. Units photo-identified as projecting to the nucleus accumbens (60 NAc projectors, n = 8 mice) were either 1) selectively excited by cues of positive valence, or 2) selectively inhibited by cues of negative valence, or 3) inhibited by both cues. Conversely, BLA neurons projecting to the central amygdala (66 CeA projectors, n = 6 mice) were either 1) selectively excited by cues of negative valence, or 2) selectively inhibited by cues of positive valence, or 3) excited by both cues. On the other hand, the population of BLA units photo-identified as projecting to the ventral hippocampus (31 vHPC projectors, n = 3 mice) showed a similar distribution of responses compared to the entire BLA population.
**Conclusions:** These results support a model of dynamic valence coding during expression of valence discrimination where NAc and CeA projectors provide valence specific excitation to their downstream target during cues of positive and negative valence, respectively, and are inhibited during cues of opposite valence. The mutually exclusive pattern of coding of the NAc and CeA projectors also suggests that these two populations are part of a microcircuit allowing reciprocal inhibition.
**Keywords:** neural circuits, basolateral amygdala, Memory Encoding and Retrieval, optogenetics
**Disclosures:** Nothing to disclose.

### M120. Inflammation and Memory: Associations among the CRP Gene, Serum CRP, and Memory Performance

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**Background:** C-reactive protein (CRP) is a biological marker of systemic inflammation that has been linked to multiple psychiatric and medical conditions, many of which involve compromised attention, working memory, and executive functioning. However, there is a lack of data on whether CRP levels affect cognition, and whether variants of

a gene that affects CRP are associated with cognition, which was the goal of the present study.
**Methods:** Genotype data for 41 African-American women aged 22-63 years were collected for a single nucleotide polymorphism (SNP) of the CRP gene (rs1130864). The T allele for this SNP has been previously associated with affective disorder as well as Alzheimer's disease risk (Ancelin et al., 2015; Michopoulos et al., 2015; Kok et al., 2011). A battery of neuropsychological tests (Penn Computerized Neuropsychological Battery), which included tests of attention, working memory, and executive functioning, was administered. Serum CRP data was available for 15 of these participants.
**Results:** Univariate ANOVA results revealed that those with one or two copies of the T allele had significantly fewer correct responses on the letter-n-back task on all conditions (F1, $39 = 6.89$, $p < .05$), including the 2-back condition (F1, $39 = 4.98$, $p < .05$), compared to individuals without the risk allele. A significant negative correlation was observed between CRP level and correct responses on two-back trials ($r = -.57$; $p < .05$). Carriers of the risk allele also had more false positive responses on a visual memory task (delayed recall of visual stimuli; F1, $39 = 4.31$, $p < .05$).
**Conclusions:** Certain CRP polymorphisms and resulting high levels of CRP may increase risk for memory decline, particularly following exposure to a major stressor. These data suggest that individuals who carry a particular polymorphism of this gene are likely to experience significantly higher levels of systemic inflammation and are at greater risk for developing memory problems, which are prominent features of several medical and psychiatric conditions.
**Keywords:** inflammation, Cognition, CRP, genetics
**Disclosures:** Nothing to disclose.

## M121. Contrasting Non-Linear Dynamic Analyses of EEG in Alert and Sedated States

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**Background:** Complex systems, such as the brain, involve non-linear neuronal interactions. These in turn translate into self-organization and emergence of distinct patterns of activity. And although a loss of integrated neuronal activity or increased entropy during anesthesia has been described in the literature, it has not yet been well characterized. These in turn could serve as the basis to better understand pathophysiological states like depression.
**Methods:** Task free resting 64 channel EEG recording (Neuroscan, Compumedics) was collected before and during anesthesia with Etomidate (0.2 mg/kg) or Propofol (2 mg/kg) from patients scheduled to undergo Electro-Convulsive Therapy (ECT).
An 81 second window was chosen for each of the awake and anesthesia states. The Symmetric Sensor Difference Series (SSDS) was generated by subtracting sensor pair outputs (e.g. the left frontal F3 sensor from the right frontal F4 sensor) thus resulting in a difference wave from frontal, central, parietal and temporal leads. The following parameters were extracted: power spectral scaling indices (alpha); topological, metric, and fuzzy entropies and Lyapunov exponents.
**Results:** To date, we acquired 38 recordings from 19 patients with major depressive disorder (9 females, age $= 47 \pm 9$ years). Frontal power spectral scaling index (alpha) showed significant differences awake and under anesthesia state ($1.79 \pm 0.7$, $1.97 \pm 0.9$, $p < 0.001$). Frontal Entropy measures and Lyapunov exponent were also significantly different ($0.38 \pm 0.07$, $0.34 \pm 0.04$ and $0.43 \pm 0.1$, $0.22 \pm 0.1$ respectively; $p < 0.001$). These differences were also present at the central, temporal and parietal leads.
**Conclusions:** Neuronal activities exhibit a decreased in complexity after onset of anesthesia in depressed individuals. The changes in non-linear dynamics appear spatially consistent. Ongoing studies are investigating the potential for complex emergence between depressed and healthy individuals. These could serve as future biomarkers for treatment response.
**Keywords:** EEG, non-linear, emergence, anesthesia, depression
**Disclosures:** Nothing to disclose.

## M122. Differential Effects of Oxytocin on Social and Monetary Reward Processing

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**Background:** Emerging evidence suggests that the hypothalamic neuropeptide oxytocin plays a modulatory role in the processing of reward. Significant anatomical and functional interactions between oxytocinergic and reward systems have been previously identified. Though widely considered a 'social hormone', work done by our lab and others have demonstrated that oxytocin administration can influence activity within reward-networks in anticipation of receipt of a variety of social as well as non-social incentives (e.g., social rewards such as happy faces, monetary rewards). One question that remains is whether oxytocin administration differentially affects reward-related neural activity depending on the type of incentive being offered (e.g., social versus non-social). To investigate this, we utilized fMRI to compare the effects of oxytocin on neural activity specific to the anticipation of social reward (i.e. friendly, happy faces) to those activities elicited by the anticipation of potential monetary reward.
**Methods:** We tested 16 healthy male participants in a double-blind, placebo-controlled, randomized, crossover, pharmaco-fMRI study. We explored the effects of intranasal oxytocin on brain activity elicited during the performance of two incentive delay tasks: the social incentive delay task (SID) and the monetary incentive delay task (MID). During the incentive delay tasks, subjects are given an opportunity to gain reward (SID: happy, smiling faces; MID: gain money) or to avoid loss (SID: angry faces; MID: loss of money) depending on their performance. To test our hypothesis that oxytocin may differentially influence

activity depending on the type of incentive being offered (i.e. social versus non-social) we performed two separate factorial analyses (reward and loss) with task and drug as within subject factors using SPM8 software. To assess whether there were regions where oxytocin similarly influenced on anticipatory reward related activity during both the MID and SID we performed conjunction analyses through the SPM8 interface.
**Results:** During losses, group level analyses indicated oxytocin administration influenced anticipatory reward-related activity within the ventral tegmental area (VTA) to a greater extent when the incentive at stake was social (faces) compared to non-social (monetary) ($p = 0.029$, FWE-SVC). We did not observe any differential effects of oxytocin based on task for the reward condition.
Conjunction analyses revealed that loss-related activity within the superior parietal cortex was influenced by oxytocin administration during both the MID and SID task ($p < 0.0001$ uncorrected, $Z = 3.67$, x,y,z: 50, -60,32). No effects were observed for the reward condition.
**Conclusions:** Since there are both shared and distinct neural networks which process social and non-social rewards, it is possible that oxytocin may have specific regional effects depending on the nature of the incentive at stake. We find that oxytocin impacts neural activity within the VTA to a greater degree in response to a potential social incentive than when compared to a potential monetary incentive. This finding is of interest as the VTA is known to play a significant role in the processing of reward incentives and dopaminergic activity within this area has been previously demonstrated to be influenced by oxytocin administration. We also identified one region, the superior parietal cortex, an area recruited during attention demanding tasks, that was commonly activated in response to the oxytocin challenge in both the MID and SID task. These data indicate that oxytocin's impact on the neural mechanisms associated with the processing of reward can be dependent on the type of incentive employed.
**Keywords:** oxytocin, Reward, fMRI, social neuroscience
**Disclosures:** Nothing to disclose.

### M123. A Novel Genetic Method of Measuring the Receptor-Specific Component of PET Radioligand Binding in Human Brain Without Pharmacological Blockade

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**Background:** One of the most important performance characteristics of a positron emission tomographic (PET) radioligand is its 'signal-to-noise' ratio – i.e., the amount of radioligand specifically bound to its target receptor compared to that which adsorbs nonspecifically to proteins and lipids in brain. The typical way to measure this ratio is to displace the specifically bound radioligand with pharmacological doses of similarly binding drug and then measure the residual (or nondisplaceable) uptake. Here, we describe a genomic method that can measure the specific and nondisplaceable components of radioligand uptake based on the relative regional density of the mRNA transcript of the target receptor but does not require pharmacological blockade.
**Methods:** This genetic method was tested using brain imaging results from 12 healthy volunteers injected with tracer doses of 18F-FIMX, a radioligand we recently developed to quantify metabotropic glutamate receptor 1 (mGluR1) (Xu et al., J Med Chem, 56, 2013). Regional values of total brain uptake (VT) were quantified with the 'gold standard' compartmental method that includes serial concentrations of the parent radioligand (separated from radiometabolites) in arterial plasma. The relative density of mGluR1 gene transcripts in brain regions were obtained from the Allen Brain Atlas (Hawrylycz et al., Nature, 489:391, 2012). A modification of the Lassen plot was used to correlate some measure of specific binding —in this case, the relative density of mGluR1 gene transcript—with VT and then estimate the amount of nondisplaceable uptake (VND) by extrapolating the value of VT when the specific binding equals zero (Cunningham et al., J. Cereb Blood Flow Metab, 30:46, 2010).
**Results:** Because gene transcript and expressed protein are not always linearly related, we first performed a linear regression for multiple brain regions of the mGluR1 gene transcript (y-axis) and VT (x-axis). The two variables were strongly correlated (Pearson's $r = 0.965$; $p < 0.0001$). The x-intercept of this plot equaled VND (0.5 mL • cm-3), as it was the residual value of VT when specific binding was extrapolated to be zero. Because VND is usually the same for all brain regions and even between species, we performed a standard receptor-blocking study (using pharmacological doses of a related drug) in monkey and found a similar VND value (0.6 mL • cm-3) after correcting for differences in plasma protein binding of the radioligand.
**Conclusions:** Regional PET values of mGluR1 binding were strongly correlated with mGluR1 transcript density; thus, the VND of the radioligand could be estimated without pharmacological blockade, which is often impossible or even dangerous to do in human subjects. Furthermore, because VND for this and most PET radioligands is uniform throughout the brain, the specific binding—and thus the "signal-to-noise" ratio—of this radioligand can be determined for all brain regions. In addition to introducing a novel method to determine the receptor-specific component of radioligand binding, this study also shows how a publicly available database (i.e., the Allen Brain Atlas) can be used to determine if a gene transcript is linearly related to the expressed protein across brain regions for any previously published PET radioligand. One common assumption of postmortem studies of mRNA densities is that they reflect the density of the expressed protein. This method can now test that assumption for targets that have been imaged with PET. We are now studying several other targets measured with PET and are finding, as might be expected, that gene transcript is sometimes, but not always, correlated with the density of expressed protein.
**Keywords:** PET, metabotropic glutamate receptor, Genomics
**Disclosures:** Nothing to disclose.

### M124. Becoming an Academic Researcher in Psychiatry: A View from the Trenches

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**Background:** Success in academic psychiatry requires a combination of talent, hard work, luck, and strong collaborative relationships. As competition for research funding intensifies, even these factors are not always sufficient and junior investigators face enormous challenges in academic psychiatry.
**Methods:** We draw on our own experiences of the National Institute of Mental Health (NIMH)-funded Career Development Institute for Psychiatry (CDI) and self-reports in response to a short questionnaire given to the CDI Class of 2015 participants. We qualitatively identified overarching themes related to both the problems and solutions that retain scientific researchers.
**Results:** We outline four major challenges faced by junior investigators: 1) maintaining the motivation to pursue an academic research career; 2) identifying and maintaining strong mentoring relationships; 3) coping with the lack of career security; and 4) maintaining an adequate work-life balance. We identified a number of solutions including the CDI and CDI-like programs, but also recognize a need for institutional and policy levels changes to retain junior investigators.
**Conclusions:** The CDI was established to help junior investigators to successfully confront these challenges. It is a two-year program that begins with an intensive four-day seminar series, includes mentoring from an assigned mentor, and online "webinars" on various topics of relevance to junior investigators over two years. We emphasize that while the solutions provided by the CDI are important and impactful for a small number of fortunate individuals, other critical changes must occur at institutional and policy levels to prevent the loss of junior investigators from academic psychiatry.
**Keywords:** Training, Career Development, Mentoring, Funding
**Disclosures:** Nothing to disclose.

### M125. Glutamate Neurons in the Ventral Tegmental Area Co-Release GABA and Promote Positive Reinforcement

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**Background:** In addition to the well-known dopamine cells in the ventral tegmental area (VTA), there are GABA and glutamate releasing neurons, as well as cells that release more than one small molecule transmitter. The function of the excitatory glutamate VTA neurons, defined by the expression of the vesicular glutamate transporter (VGLUT2), has been scarcely examined. Previous work has demonstrated that VGLUT2+ VTA neurons co-release glutamate and dopamine in the nucleus accumbens (NAc) and send robust non-dopamine excitatory projections from VTA to the ventral pallidum (VP) and lateral habenula (LHb). But how these excitatory VTA projections functionally integrate with other mesolimbic circuits is unknown. Here we aim to define the functional relevance of VTA VGLUT2+ neurons to motivated behaviors.
**Methods:** To selectively label VGLUT2+ neurons we stereotactically delivered Cre recombinase-dependent viral vectors in to the medial VTA of mice expressing Cre under the control of Slc17a6 (VGLUT2Cre) regulatory elements. These injections were titrated to induce selective expression of Channelrhodopsin2 fused with a red fluorescent protein (ChR2:mCherry) in VGLUT2+ medial VTA neurons. In behavioral experiments mice were coincidently implanted with a chronic indwelling optic fiber just dorsal to the medial VTA for in vivo light delivery. We used several assays to test whether stimulation of VTA VGLUT2+ neurons is reinforcing, including self-stimulation operant tasks and 2-bottle choice assays. For electrophysiological experiments: 1) acute slices were made through the NAc, VP, LHb, and VTA, 2) cell-attached or whole-cell recordings made from ChR2:mCherry+ neurons or postsynaptic neurons innervated by ChR2:mCherry+ terminals, and 3) light-evoked responses were assessed.
**Results:** Optogenetic stimulation of VGLUT2+ VTA neurons produces robust positive reinforcement. Within a single session (45-60 min) mice discriminate and acquire robust preference for a nosepoke hole coupled to optical stimulation (20-40 Hz, 10ms pulse width, 10-80 pulses). Similar results were obtained with a 2-bottle choice assay where water-deprived mice demonstrate strong preference for a water bottle coupled to optical stimulation of VGLUT+ VTA neurons, even when competed against sucrose solutions. Further, these preferences persist following systemic administration of dopamine D1 or D2 antagonists suggesting that optogenetic stimulation of VGLUT2+ VTA neurons is reinforcing independent of dopamine co-release.
Whole cell voltage-clamp recordings reveal only excitatory postsynaptic currents (EPSCs, mediated by both AMPA and NMDA glutamate receptors) following stimulation of VTA VGLUT2+ terminals in the NAc and local collaterals in VTA. Conversely, VGLUT2+ VTA terminals in the VP and LHb revealed both EPSCs and GABA-receptor mediated IPSCs. Interestingly, the GABA/AMPA ratio was much larger in the LHb when compared to the VP. Consistent with this observation, cell-attached and current-clamp recordings showed that photostimulation of VTA VGLUT2+ terminals in the NAc and VP are largely excitatory; but inhibitory in the LHb.
**Conclusions:** These data support the hypothesis that VGLUT2+ neurons can reinforce behaviors, independently of dopamine, by activating postsynaptic neurons in the NAc and VP but inhibiting neurons in the LHb. These findings are copasetic with previous observations showing that electrical or optogenetic activation of the VTA, NAc, or VP, and inhibition of the LHb, are sufficient to drive positive reinforcement; and place VGLUT2+ VTA neurons at a potential node in coordinating these limbic regions. These data also add to previous observations demonstrating

GABA and glutamate co-release in the LHb, extend this observation to the VP, and provide the first evidence for a functional role of VGLUT2+ VTA neurons in behavioral reinforcement.
**Keywords:** Ventral tegmental area (VTA), vesicular glutamate transporter (VGLUT), neurotransmitter co-release, motivation, glutamate
**Disclosures:** Nothing to disclose.

### M126. Notch Signaling in the Hypothalamic Arcuate Nucleus Controls Differentiation of NPY, Pomc and Kisspeptin Neurons

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**Background:** The hypothalamic arcuate nucleus (ARC) controls body size, energy balance and reproduction. Dysregulation of this critical homeostatic mediator underlies diseases ranging from growth failure to obesity. Despite considerable investigation regarding hypothalamic control of energy and metabolism, the mechanisms governing the development of this circuit remain unclear. The ARC contains POMC and NPY neurons, which have opposing effects on appetite. Kisspeptin ARC neurons are critical for the onset of puberty and reproductive function. Studies have shown that downstream targets of Notch signaling are important for proper specification of hypothalamic neurons. However, how Notch signaling may control progenitor populations within this region, or if Notch is necessary or sufficient to direct hypothalamic neuron specification remains unclear.
**Methods:** To elucidate the role of Notch signaling in the development of the ARC, we analyzed loss of function (LOF) mice lacking a necessary co-factor of Notch signaling, Rbpjk, in Nkx2.1-CRE expressing cells, as well as mice with constitutive expression of activated Notch1 in Nkx2.1-CRE expressing cells (GOF). Neurons and glial cells that populate the ventral hypothalamus, including the ARC, originate from the Nkx2.1 positive region of the proliferating third ventricle. Given the timing and spatial restriction of Nkx2.1 expression, these mice are a useful model for hypothalamic-specific Notch pathway loss and constitutive activation.
**Results:** We found that Notch signaling influences formation of the hypothalamic progenitor zone. In LOF mice, a reduction of the hypothalamic progenitor zone, marked by SOX-2-positive and Nestin-positive cells at embryonic day 18.5 (e18.5) is observed. In contrast, GOF mice show an expansion of the hypothalamic proliferative zone and coincident increase in SOX-2 and Nestin-positive cells at e18.5. The hypothalamic progenitors that give rise to ARC POMC neurons appear to be particularly affected by Notch. LOF mice show an increase in Pomc-positive and NPY cells and decrease in Kisspeptin neurons in the ARC, while there is no evidence of differentiation of Pomc, NPY or Kisspeptin neurons in the ARC of GOF animals.
**Conclusions:** Taken together, our results suggest that Notch signaling may regulate hypothalamic neurogenesis and differentiation of ARC neurons. Ongoing studies will elucidate the signaling mechanisms by which Notch controls neurogenesis in the hypothalamic proliferative zone, as well as ARC POMC, NPY and Kisspeptin neuron specification.
**Keywords:** POMC, Notch, Arcuate, Hypothalamic development
**Disclosures:** Nothing to disclose.

### M127. Do Religious Involvement and Religious Beliefs Protect Against Suicidality as Assessed by the Sheehan-Suicidality Tracking Scale

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**Background:** The literature suggests an inverse relationship between religious involvement and suicidality (1). Similarly, a lack of religious belief has been linked to higher rates of suicidal behavior (2). In the current study, we utilized a standardized suicide assessment to examine the relationships between religious involvement, religious behavior, suicidal ideation, and suicidal behavior.
**Methods:** Adults admitted to an urban psychiatric hospital were invited to participate in a psychometric evaluation study. 199 patients consented to participate and completed all study procedures, including the Sheehan-Suicidality Tracking Scale (S-STS) (3) and an investigator-designed Risk Assessment Measure (RAM). The S-STS is a standardized suicide assessment which produces a composite score of suicidality and produces scores on suicidal ideation and suicidal behavior subscales. The RAM included questions about patients' belief in God, attendance of religious services, and moral objections to suicide. Two-way analyses of variance were employed to compare scores on the S-STS composite scale and subscales between patients who reported religious involvement and belief and patients who did not report religious involvement and belief.
**Results:** Most patients reported that they believed in God (86.9%, $n = 172$) and believed that suicide is an immoral act (62.4%, $n = 123$). Fewer patients reported that they regularly attended religious services (38.7%, $n = 77$). Patients who believed in God did not score significantly lower on the suicidal ideation subscale ($p = 0.21$), but they did score significantly lower on the suicidal behavior subscale ($p = 0.02$) and trended to score significantly lower on the total scale ($p = 0.06$). Patients who regularly attended religious services scored significantly lower on the suicidal ideation subscale ($p = 0.04$), the suicidal behavior subscale ($p = 0.04$), and the total scale ($p = 0.03$). Patients who indicated that they believed suicide to be immoral scored significantly lower on the suicidal ideation subscale ($p = 0.02$) and the total scale ($p = 0.02$) and trended to score significantly lower on the suicidal behavior subscale ($p = 0.10$).
**Conclusions:** Our findings suggest that while both religious involvement and religious beliefs may be protective against suicidal ideation and behavior, involvement may be more protective. This study confirms findings from non-clinical

samples and adds to the more limited literature on religious involvement, religious belief, and suicidal ideation. Future studies should continue to examine these factors in clinical samples and should utilize standardized suicide assessments to capture the full spectrum of suicidality.
**Keywords:** Suicide, Religion, Sheehan Suicidality Tracking Scale
**Disclosures:** Nothing to disclose.

### M128. Pregnenolone in the Treatment of Irritability in Autism Spectrum Disorder: A Post-Hoc Metabolomic Analysis

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**Background:** One proposed model of autism suggests that the condition is a result of an altered ratio of excitation/inhibition in key neural systems. As GABAergic systems are the most important inhibitory neural pathways, modulators of GABA(A) receptors may correct the imbalance between excitation and inhibition in the brain. Pregnenolone (PREG) is the precursor of GABAergic neurosteroids such as PREG sulfate and allopregnanolone. We hypothesize that individuals with autism spectrum disorder (ASD) receiving PREG will show reduction in disruptive behaviors as measured by a standard rating scale for irritability. The objectives of this open-label study are: (1) To conduct a pilot, open-label, 12-week trial to assess the effectiveness of PREG in reducing irritability in adults with ASD. (2) To examine the safety and tolerability of PREG in adults with ASD. (3) To explore the association between response to PREG and plasma concentrations of metabolites of PREG before and after 12-week trial of oral PREG.
**Methods:** This was a 12-week, prospective open-label study of PREG in adults with ASD. PREG was initiated at a dose of 50 mg twice daily in weeks 1 and 2, then 100 mg twice daily in weeks 3 and 4, then 150 mg twice daily in weeks 5 and 6, then 200 mg twice daily in weeks 7 and 8, then 250 mg twice daily from weeks 9 to 12. If subjects could not tolerate a specific dose, s/he would be maintained at the highest tolerated dose. This initial phase was followed by a 4-week washout period to allow a better appreciation of the benefits observed while subjects were taking PREG, by monitoring the effect of tapering and discontinuation of the medication. Primary outcome measures included the irritability subscale of the Aberrant Behavior Checklist (ABC-I). Secondary measures included the other subscales of ABC, Social Responsiveness Scale, Sensory Profile Questionnaire (SPQ), and Vineland Adaptive Behavior Scale. In addition, vital signs and Dosage Record and Treatment Emergent Symptom Scale were monitored for adverse events. Paired, 2-tailed student t-tests were performed to compare outcome measures between baseline and 12 weeks.
Concentrations of PREG and its metabolites in human plasma samples were quantified by a sensitive and specific liquid chromatography-mass spectrometry (LC-MS/MS) method. Analytes [PREG, PREG sulfate, progesterone, allopregnanolone, dehydroepiandrosterone (DHEA), DHEA sulfate, testosterone, estradiol, cortisol] were extracted by methyl tert-butyl ether. Separation was achieved by using a Shimadzu Ultra-Fast Liquid Chromatography system and C18 columns and quantification was performed on a triple quadrupole MS with electron spray ionization. Analytes were run in positive mode (PREG, progesterone, allopregnanolone, DHEA, testosterone, cortisol) and negative mode (PREG sulfate, DHEA sulfate, estradiol) in two independent LC-MS/MS runs. For each analyte, two multiple reaction monitoring transitions were used with deuterated analogues as internal standard in order to confirm identity and perform quantification.
An exploratory post-hoc analysis was performed to determine the association between medication response (defined as reduction in ABC-I score of 7 or more) and plasma concentrations of PREG and its metabolites before and 12 weeks after oral administration of PREG. Two-tailed student t-tests with equal variance were performed to compare plasma concentrations of PREG and its metabolites between the responder and non-responder groups.
**Results:** Ten men and two women with ASD (mean age 22.5 $\pm$ 5.8 years; range 18.1-35.5 years; 9 Caucasians and 4 Asians) met the study criteria for inclusion in this open-label study. PREG yielded a statistically significant improvement in the primary measure, ABC-I [from 17.4 $\pm$ 7.4 at baseline to 11.2 $\pm$ 7.0 at 12 weeks ($p = 0.028$, $df = 11$, $t = 2.5$)]. Secondary measures were not statistically significant with the exception of ABC-lethargy ($p = 0.046$) and total SPQ score ($p = 0.009$). During the 12-week treatment period, two participants dropped out of the study. No significant vital sign changes occurred during this study. PREG was not associated with any severe side effects. Single episodes of tiredness ($n = 1$), diarrhea ($n = 1$) and depressive affect ($n = 1$) that could possibly be related to PREG were reported.
Six participants were found to be responders to PREG treatment, while the rest of the six participants did not reach the responder criteria. Mean baseline plasma concentration of PREG in the responder group (1.49 $\pm$ 0.41 ng/mL) was found to be lower ($p = 0.039$) than the non-responder group (2.23 $\pm$ 0.71 ng/mL), while mean baseline plasma concentrations for metabolites of PREG of the responder and non-responder groups were statistically indistinguishable. Furthermore, week 12 plasma concentration of testosterone in the responder group (1.80 $\pm$ 1.34 ng/mL) was found to be lower ($p = 0.0097$) than the non-responder group ($3.79 \pm 0.74$ ng/mL), while responder plasma concentrations of PREG and other metabolites were similar to non-responder levels.
**Conclusions:** In this pilot study, PREG was modestly effective and was overall safe and well tolerated in individuals with ASD. Post-hoc analysis found that baseline PREG and 12-week testosterone levels were associated with reduction of ABC-I. A randomized controlled trial of PREG is underway.
**Keywords:** Clinical trial, Autism spectrum disorder, Metabolomics
**Disclosures:** Nothing to disclose.

## M129. A Preclinical Evaluation of the Potential of CR845 to Induce Tolerance and a Syndrome of Dependence on Withdrawal

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**Background:** CR845 is a potent peripheral κ-opioid receptor agonist with a high degree of selectivity over other opioid receptor subtypes. CR845 exhibits potent analgesic and anti-inflammatory properties in preclinical models and is being developed by Cara Therapeutics as a candidate drug to treat acute and chronic pain, and pruritus. Unlike currently marketed opioids, CR845 does not appear to produce inhibition of intestinal transit (ileus) and does not induce respiratory depression. The aim of this study was to determine whether CR845 induces physical dependence on withdrawal by recording the behavioural and physical signs during 28 days of administration of CR845 to rats and monitoring the behavioural and physical signs that occurred after abrupt cessation of treatment. The μ opioid receptor agonist, morphine, which produces rapid tolerance and physical dependence on withdrawal, was used as the positive control in this experiment.
**Methods:** The experiment was conducted in 46 male, Sprague Dawley rats (150-175g; Charles River, UK). They were individually-housed on a 12h/12h light/dark cycle (lights on 07.00h). Food and water were available ad libitum. After acclimatising the rats to for ~1 week, they were divided into 4 groups (N values): CR845 5 mg/kg/day (15); saline 1 ml/kg/day (11); morphine 60 mg/kg/day [30 mg/kg, bid] (10); deionised water 5 ml/kg, bid (10). CR845 and its vehicle control were given intravenously (iv) and morphine and its vehicle control were given orally. After a 7 day baseline period, rats were administered drugs or vehicle for 28 days (on-dose phase), after which treatment was terminated and the rats were monitored for a further 7 days (withdrawal). Physiological measurements of body weight, food and water intake and rectal body temperature were taken once daily during the baseline, drug administration and withdrawal phases of the study. For the first 4 days of withdrawal, temperatures were measured twice daily. A battery of 40 behavioural and physical signs (e.g. locomotor changes, stereotypy, teeth chattering, aggression, vocalisation) and physical signs (e.g. loss of condition) were recorded daily during a 7 day baseline period and twice-daily in the on-dose and withdrawal phases.
**Results:** Animals were initially dosed with CR845 at 25 mg/kg iv, that resulted in a rapid loss of ~12% of their bodyweight in the first 24 hours (likely a consequence of the known aquaretic effect of κ opioid agonists). It was therefore decided to reduce the dose. Following a 7 day washout period, animals were tested with 5 mg/kg/day dose of CR845 iv. In the on-dose phase, the rats gradually regained weight back to control group levels, food and water intake was unchanged relative to controls. Body temperature was increased in Weeks 3 and 4 by a mean of 0.3°C ($p<0.05$).
Behavioural changes observed on-dose in a significant proportion of CR845-treated rats included ataxia, hunched body posture, Straub tail, increased locomotor activity and body tone, piloerection and exopthalmos. On withdrawal of CR845 treatment, there was no effect on bodyweight, food and water intake or body temperature when compared with the vehicle control group. The behavioural signs observed on-dose with CR845 gradually reduced during the 7-day withdrawal period with no signs of physical dependence.
Relative to its vehicle controls, morphine (30 mg/kg/day, bid) decreased mean bodyweight by 26g ($p<0.001$) at the end of Week 1 attenuating to 6.2g (N.S.) by the end of Week 4. Food and water intake and temperature were reduced throughout the on dose phase by an average of 15.2% ($p<0.001$), 21.8% ($p<0.001$) and 0.8°C ($p<0.001$), respectively. Morphine produced unequivocal signs of treatment, including exopthalmos, altered locomotor activity (initially decreased, then increased), subdued behaviour, increased body tone, Straub tail, increased reactivity to sound and piloerection. About 20% of rats were irritable, possibly due to dependence/withdrawal emerging between the 2 daily morphine doses due to its short half-life. Cessation of morphine treatment produced characteristic physiological withdrawal signs of weight loss, hypophagia, hypodipsia and hyperthermia. As the rats recovered, hyperphagia, hyperdipsia, and weight regain occurred and hypothermia disappeared. Behavioural signs of withdrawal included irritability, tail rattling and aggression. Other signs that emerged during this period were subdued behaviour, hunched posture, writhing, increased reactivity to sound, increased body tone and respiratory abnormalities. The most severe withdrawal effects after cessation of morphine treatment were observed in the first 3 days. Residual effects of morphine dependence gradually reduced in frequency over the following withdrawal days.
**Conclusions:** CR845 5 mg/kg iv is predicted to produce plasma exposures that are 10-fold (Cmax) and 50-fold (AUC) greater than the targeted human clinical exposures. CR845 produced clear signs of drug effect in the on-dose phase, but no behavioural, physiological or physical signs of dependence on withdrawal. In contrast, repeated morphine administration produced rapid tolerance and unequivocal physical and behavioural signs of dependence on withdrawal. If these preclinical findings translate to man, they predict that CR845 will not produce physical dependence with repeated administration.
**Keywords:** kappa agonist, CR845, Physical dependence, Preclinical, Rat
**Disclosures:** D.J. Heal is an employee and shareholder of RenaSci Ltd. S. Goddard, J. Gosden, S. Dykes, and R. Brammer are employees of RenaSci Ltd. R. Spencer and F. Menzaghi are employees and shareholders of Cara Therapeutics, Inc.

## M130. Super-Cholinergic Mice and Humans: Cholinergic-Cognitive-Affective Resiliencies

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**Background:** Cortical cholinergic inputs mediate attentional functions, including attention-dependent learning and

memory. In the brain, the choline transporter (CHT) is uniquely expressed in cholinergic neurons. The transport of choline into presynaptic terminals via the high-affinity, hemicholinium-3 (HC-3)-sensitive CHT represents the rate-limiting step for acetylcholine (ACh) synthesis and release. We have investigated the cholinergic-cognitive-affective consequences of mice and humans expressing genetically-imposed CHT low-capacity variants (Parikh et al., 2013; Paolone et al., 2013; Hahn et al. 2008; Berry et al., 2014, 2015). Humans with low-capacity CHT gene variants display increased distractability and risk for depression. Here we report findings from mice and humans with enhanced CHT function.

**Methods:** We determined the cholinergic-cognitive capacities of transgenic mice overexpressing the CHT (CHT-OXP; Holmstrand et al., 2014) and humans expressing a SNP in the 3'UTR region of the CHT gene (rs333229) that has been hypothesized to confer enhanced cholinergic function.

**Results:** 1. Optogenetic stimulation of cholinergic neurons of CHT-OXP mice expressing channelrhodopsin in cholinergic neurons (ChR2; Ai32 + /-/ChAT Cre + /-/BAC + /-) indicated a more sustained ACh release capacity. We implanted laser fibers into the basal forebrain and a dialysis probe into medial prefrontal cortex and stimulated cholinergic neurons for 3 min. Post-stimulation ACh release in wild type (WT; Ai32 + /-/ChAT Cre + /-/BAC-/-) mice decreased below baseline. In contrast, in CHT-OXP mice, ACh release during the post-stimulation period remained at the level seen during stimulation. 2. Performance in the sustained attention task did not differ between CHT-OXP and WT mice. However, following the presentation of a distractor, CHT-OXP's performance recovered faster and more completely. 3. In the cortex of CHT-OXP, exogenous choline is more rapidly cleared than in WT mice. Pharmacological blockade or inhibition of CHT function further indicated the greater cholinergic resilience of CHT-OXP, reflecting in part enhanced surface CHT function in response to CHT interference. 4. Compared with WT humans, those with a T allele at the 3'UTR site showed dramatically reduced distractibility on a laboratory attention task. They also self-reported less boredom in everyday life and better sleep, and they showed a moderate trend ($d = 0.68$) for lower scores on a self-report measure of depressive symptoms

**Conclusions:** Mice and humans expressing high-capacity CHT variants are guided by internal task representations and greater top-down control. This is in contrast with animals expressing limited-capacity CHT variants (CHT + /- mice and I89V humans) that show greater sensitivity to both relevant (target) and irrelevant (distractor) inputs and reduced top-down control. The presence of high capacity CHT variants may also protect against affective disorders. Therefore, pharmacological approaches to enhance CHT function promise to enhance the brain's resilience to a wide range of disorders.

**Keywords:** acetylcholine, Cognitive Enhancement, affective disorders, Transporters, genetics

**Disclosures:** Nothing to disclose.

## M131. Neural Activity in Basolateral Amygdala Encodes Reward Magnitude and Risk of Punishment in a Risky Decision-Making Task in Rats

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**Background:** Elevated levels of risk-taking behavior are characteristic of substance addiction, and have the potential to precipitate and exacerbate substance use. A potential treatment strategy for addiction could be to attenuate such maladaptive choice behavior, so as to mitigate drug-seeking and potential relapse. To realize this goal, however, a thorough understanding of the neurobiology underlying normal risk-taking behavior is required. Using a rodent model of risk-taking, in which rats choose between a small, "safe" reward and a large, "risky" reward accompanied by a variable probability of punishment, we showed previously that an intact basolateral amygdala (BLA) is critical for integration of risk- and reward-related information to guide adaptive risk-taking. However, it does not reveal how cells within this structure encode reward and risk-related information.

**Methods:** In the present experiment, we evaluated how neural activity in the intact BLA during performance in a risky decision-making task. Rats were trained in a modified version of the original risky decision-making task in which reward magnitude and probability of punishment were independently manipulated in separate blocks of trials. After rats were shaped and trained to stability, two drivable microwire bundles were then implanted immediately above the BLA. Upon recovery, neural activity was recorded while rats performed the risky decision-making task.

**Results:** During stable performance, rats chose the large reward significantly more than the small reward and chose the reward associated with risk of punishment significantly less than the reward associated with no punishment. Initial neural analyses indicate that during reward magnitude trials, there was an increase in BLA activity in anticipation of the large reward and a decrease in activity in anticipation of the small reward relative to baseline. A difference was also observed during risk of punishment trials, such that BLA activity increased in anticipation of the risky reward and decreased in anticipation of the safe reward relative to baseline. Importantly, the reward magnitude associated with each choice during the risk of punishment trials was held constant, indicating that differences in firing were due to encoding of risk-related information.

**Conclusions:** Together, these data suggest that BLA neurons track the most salient option during choice behavior. Future work will compare BLA neural activity in this task between rats that have undergone cocaine self-administration vs. control procedures, to determine whether dysfunctional BLA activity contributes to drug-induced maladaptive risk-taking behavior.

**Keywords:** Amygdala, decision-making, Risk, electrophysiology

**Disclosures:** Nothing to disclose.

### M132. Hormonal Contraception Diminishes Oxytocin-Induced Brain Reward Responses in Women Viewing the Faces of Their Romantic Partners

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**Background:** Converging evidence from studies in monogamous species suggests that the hypothalamic peptide oxytocin (OXT) critically modulates pair-bonding behaviors.
**Methods:** OXT may contribute to romantic bonds in men by enhancing their partner's attractiveness and reward value compared with other women. Here, we used functional MRI (fMRI) in order to examine whether a similar reinforcing mechanism also exists in pair-bonded women (N = 40).
**Results:** Intranasal OXT (24 IU) led pair-bonded women to rate their male partners as more attractive relative to unfamiliar men. On the neural level, this was paralleled by enhanced responses to pictures of the partner's face in reward-associated brain regions. Strikingly, however, the observed effects of OXT were blunted in women using hormonal contraception (HC) – a finding consistent with, and perhaps mechanistically underlying, reduced sexual satisfaction and partner attraction in women using HC.
**Conclusions:** Collectively, our findings provide evidence for a common functional role of OXT in boosting partner attractiveness representations in the reward systems of both sexes. This proximal mechanism may be disturbed in women using HC.
**Keywords:** oxytocin, fMRI, reward system
**Disclosures:** Nothing to disclose.

### M133. Regulation of Brain Susceptibility to Inflammatory Responses by Protein S-Glutathionylation in Glia

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**Background:** Accumulating evidence suggests that inflammation and oxidative stress are involved in major mental illness such as schizophrenia and depression. The underlying biological mechanisms, however, are not fully understood. Although microglia and astrocytes have been extensively studied in brain inflammatory changes, less is known about their mutual regulations. Here we have focused on astrocyte-microglia interaction and characterized the role of glutathione-S-transferase theta 2 (GSTT2), which has been implicated in major mental illness, in astrocytic control of brain immune responses.
**Methods:** Mouse models are used to address the role of GSTT2 in immune/inflammatory responses. Primary mouse glial cell culture was employed to address immune/inflammatory responses to defined sets of immune stimuli such as lipopolysaccharides (LPS). mRNA expression of inflammatory mediators was measured by quantitative RT-PCR. Systemic LPS-induced neuroinflammation was assessed in mice with astrocyte-specific modulation of GSTT2 expression levels.
**Results:** GSTT2 was shown to facilitate the production of pro-inflammatory cytokines such as TNF-$\alpha$ and IL-6 in primary mouse glial cells against various immune stimuli. Enhanced inflammatory responses were in part mediated by a chemokine CCL2. In an in vivo neuroinflammation model, the modification of astrocytic expression of GSTT2 resulted in altered inflammatory responses in the brain without dramatically changing microglial morphology. Further characterization of molecular changes in astrocytes by altered GSTT2 expression and their effects on microglia are in progress.
**Conclusions:** Our data suggest that protein S-glutathionylation in astrocytes may regulate the susceptibility to immune/inflammatory stimuli in the brain. This study provides biological insight into the mechanisms underlying immune/inflammatory changes in major mental illness.
**Keywords:** inflammation, astrocytes, S-glutathionylation
**Disclosures:** Nothing to disclose.

### M134. Evaluation of 5-HT2C Receptor in the Human Brain in Vivo: A [$^{11}$C]Cimbi-36 PET Study

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**Background:** The 5-$HT_{2C}$ receptor has considerable interest for CNS drug development, however evaluation of potential candidates has been hampered by the lack of suitable tools to evaluate this target in the living human brain. [$^{11}$C]Cimbi-36 is a 5-$HT_2$ agonist PET radioligand with similar affinity *in vitro* for the 5-$HT_{2A}$ and 5-$HT_{2C}$ sub-types [1]. The 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors are generally co-distributed in the human brain, with 5-$HT_{2A}$ $>>$ 5-$HT_{2C}$ in most areas. The areas of highest 5-$HT_{2C}$ mRNA expression in the post-mortem human brain are the choroid plexus, hypothalamus, hippocampus and the striatum [2]. Consistent with this, non-human primate PET studies estimated that almost 100% of [$^{11}$C]Cimbi-36 specific binding in the choroid plexus, and between 30% and 70% in the hippocampus may be attributable to the 5-$HT_{2C}$ component [3]. Due to the lack of selective 5-$HT_{2C}$ blockers that can be administered to humans, we evaluated the 5-$HT_{2A}$ and 5-$HT_{2C}$ components of the [$^{11}$C]Cimbi-36 PET signal in the human brain, before and after the administration of the antipsychotic risperidone. Risperidone and its major metabolite 9-OH-risperidone, have a 50-100 fold higher affinity for the 5-$HT_{2A}$ than for the 5-$HT_{2C}$ receptors *in vitro*, and are not expected to bind to the 5-$HT_{2C}$ at doses producing moderate 5-$HT_{2A}$ occupancy.
**Methods:** Four healthy male volunteers underwent 2 x 90 minute [$^{11}$C]Cimbi-36 PET scans each (at baseline and 2 hours following a single oral dose of risperidone (1 or 2 mg). Arterial blood was collected from a radial artery cannula during the PET scan, and a metabolite corrected arterial plasma input function was generated. Regional volume of distribution ($V_T$) was calculated via a 2 tissue

compartment model (2TCM), and regional binding potential ($^{2TCM}BP_{ND}$) calculated using the cerebellum as a reference region. In addition, the PET data was also quantified using a simplified reference tissue model (SRTM) with the cerebellar time-activity curve as an input function, to generate regional $^{SRTM}BP_{ND}$ values. Regional occupancy (Occ) was estimated as
$Occ_{ROI} = 100 \times (1 - {}^{risperidone}BP_{ND} / {}^{baseline}BP_{ND})$
Occupancy values from a large cortical region (defined as frontal, parietal and occipital cortices containing minimal 5-$HT_{2C}$ expression) and the hippocampus, for all subjects, were fitted to the equations below to estimate $^{risperidone}K_i^{5\text{-}HT2A}$ (affinity constant for risperidone at the 5-$HT_{2A}$) and the $^{hipp}f_{2C}$ (the fraction of [$^{11}$C]Cimbi-36 PET signal in the hippocampus attributable to the 5-$HT_{2C}$)
$Occ_{Hipp} = (1 - {}^{hipp}f_{2C})Dose/(Dose + {}^{risperidone}K_i^{5\text{-}HT2A})$
$Occ_{Cx} = Dose/(Dose + {}^{risperidone}K_i^{5\text{-}HT2A})$
**Results:** 1. The cerebellar $V_T$ was not changed by the administration of risperidone ($\Delta^{cerebellum}V_T = -3.6 \pm 9.2\%$), and $^{SRTM}BP_{ND}$ correlated well with $^{2TCM}BP_{ND}$ ($^{SRTM}BP_{ND} = 0.87\ {}^{2TCM}BP_{ND} + 0.03$, $r^2 = 0.91$). 2. $Occ_{Cx}$ (assumed to represent the 5-$HT_{2A}$ occupancy by risperidone) was significantly higher than $Occ_{Hipp}$, $p = 0.01$, paired Student's t-test. 3. The estimated $^{hipp}f_{2C}$ was 0.37 (95% CI, 0.25-0.49), while the estimated $^{risperidone}K_i^{5\text{-}HT2A}$ was 8.1 ug/kg (95% CI, 7.1-9.7).
**Conclusions:** 1. The 5-$HT_{2C}$ receptor represents between 1/3 and 1/2 of the total [$^{11}$C]Cimbi-36 $BP_{ND}$ in the hippocampus. 2. The hippocampus can be used as an index region to estimate the occupancy of compounds binding to the 5-$HT_{2C}$ receptor in [$^{11}$C]Cimbi-36 PET studies.
**Keywords:** Positron emission tomography, Serotonin 5-HT2C Receptor, Human Neuroimaging
**Disclosures:** Nothing to disclose.
**References:**
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## M135. Functional Independence of Brain Regions at Rest Broadly Predicts Individual Differences in Higher-Order Cognition

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**Background:** Growing evidence suggests that intrinsic functional connectivity (i.e. highly structured patterns of communication between brain regions during wakeful rest) may encode cognitive ability. However, the generalizability of these findings is limited by between-study differences in statistical methodology and cognitive domains evaluated. To address this barrier, we evaluated resting-state neural representations of multiple cognitive domains within a relatively large normative adult sample.
**Methods:** Forty-five participants (mean (sd) age = 31(9.6) years; 18 male and 27 female) were enrolled in the Cognitive Connectome project, an initiative to bridge functional neuroimaging and clinical neuropsychology to translate functional MRI (fMRI) into clinical decision-making. Participants completed resting-state fMRI scanning and neuropsychological assessments spanning motor, visuospatial, language, learning, memory, attention, working memory, and executive function performance. Robust linear regression related cognitive performance to resting-state connectivity among 200 a priori determined functional regions of interest (ROIs). Multiple comparison correction was conducted using false-discovery rate ($q < 0.05$) to identify significant regressions of cognitive performance to pairwise functional connectivity.
**Results:** All regressions of performance to resting-state connectivity which survived FDR correction for multiple comparisons were negative; furthermore, 95% of these negative regressions were for correlations between regions located in different hemispheres or lobes. Only higher order cognitions (working memory, learning, and executive function) showed significant brain-behavior regressions after FDR correction. Scatterplot analyses revealed moderate connectivity (mean = 0.29) among low performers and negligible anti-correlation (mean = -0.20) among high performers, suggesting that functional independence among regions at rest facilitates higher-order cognitive performance.
**Conclusions:** Our findings are consistent with graph theory analyses, which suggest the brain consists of functionally independent nodes that undergo dynamic reorganization with task demand. Our use of a well-characterized sample allowed us to circumvent many of the methodological barriers in functional neuroimaging research, such as between-study differences in sample recruitment or cognitive assessments administered. Our extensive cognitive assessment also allowed direct comparison these cognitive domains, and we report significant brain-behavior relationships only higher-order cognitions (working memory, learning, and executive function). We thus conclude that complex cognitions require a greater degree of inter-regional communication than simpler cognitions, and regions which are tightly integrated ("yoked") at rest are less able to adaptively reorganize with cognitive demand, resulting in poorer performance. Future work will extend these findings to clinical populations, such as predicting post-surgical cognitive impairments in patients receiving surgical resections for treatment of temporal lobe epilepsy.
**Keywords:** individual differences, Resting State Functional Connectivity, executive function, working memory, Translational Neuroscience
**Disclosures:** CDK served as a member of a scientific advisory meeting for Allergan Pharmaceuticals, served as a member of the national advisory board for Skyland Trail, and is also a co-holder of U.S. Patent No. 6,373,990 (Method and device for the transdermal delivery of lithium). The other authors have no financial interests to disclose.

## M136. Strain Dependency of the Effects of Nicotine and Mecamylamine in a Rat Model of Attention

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**Background:** Several disease states marked by cognitive deficits have been suggested to benefit from nicotinic acetylcholine receptor (nAChR) agonist treatment. Cognitive benefits of the prototypical agonist nicotine are well established and are reported most consistently in tests of attention. To facilitate the successful development of compounds that share these therapeutic but not unwanted side-effects of nicotine, the mechanisms mediating the effects of nicotine on attention need to be clarified. This endeavor depends on the availability of a preclinical model of attention in which beneficial effects of nicotine are seen reliably. Previously, nicotine has been shown to improve performance of rats in the 5-Choice Serial Reaction Time Task (5-CSRTT), a rodent paradigm in which a brief light stimulus appears randomly in one of five apertures, and the animal obtains a food pellet for a nose-poke response into the correct hole. Across a range of studies employing this paradigm, nicotine increased the % of responses into the correct whole (accuracy), reduced omission errors and the latency to correct responses, and increased anticipatory responses in the intertrial interval. The increase in response accuracy was the strongest indicator of an improvement in attention because this measure is not affected by non-specific changes in the rate or speed of responding. The majority of studies reporting this effect utilized Hooded Lister rats, a strain not available in the United States. The purpose of the present study was to identify a rat strain sensitive to the attention-enhancing effects of nicotine as reflected by an improvement in response accuracy. Additional experiments with the nAChR antagonist mecamylamine were aimed at aiding the interpretation of results obtained with nicotine.
**Methods:** 12 Long Evans, 12 Sprague Dawley, and 12 Wistar rats were trained on the 5-CSRTT for 4.5 months. Once performance was stable at a stimulus duration of 1 s, three experiments were performed in the following sequence. Experiment 1 tested the effects of nicotine (0, 0.05, 0.1, 0.2 mg/kg s.c.), Experiment 2 tested the effects of mecamylamine (0, 0.75, 1.5, 3.0 mg/kg s.c.), and Experiment 3 tested the effects of low-dose mecamylamine (0, 0.05, 0.1 mg/kg s.c.). Each subject was tested with each dose in a randomized sequence. Each experiment was analyzed by two-factor ANOVA with group (Long Evans, Sprague Dawley, Wistar) as a between-subjects factor and dose as a within-subject factor.
**Results:** Experiment 1: Nicotine significantly reduced omission errors and response latency and increased anticipatory responding in all strains, with no group x dose interaction. In contrast, nicotine produced a dose-dependent increase in response accuracy in Wistar rats ($P = 0.001$), but not in Long Evans ($P > 0.8$) or Sprague Dawley rats ($P > 0.8$), resulting in a trend-level group x dose interaction ($P < 0.1$). Experiment 2: The largest dose of mecamylamine robustly increased omission errors, slowed response latency, and reduced anticipatory responding. More subtle effects were also seen at the middle dose. This was supported by a main effect of dose on each of these measures. The effects of mecamylamine interacted with group on omission errors ($P = 0.004$) and anticipatory responding ($P < 0.05$): the rate-suppressant effects of the largest dose of mecamylamine, although significant in all groups, were reduced in Long Evans rats as compared with the other two strains. There were no effects on response accuracy. Experiment 3: Low-dose mecamylamine produced a trend level main effect on response accuracy in Wistar rats ($P < 0.06$), reflecting an increase in accuracy that reached significance at the 0.05 mg/kg dose. There was no effect of low-dose mecamylamine on accuracy in Long Evans ($P > 0.9$) or Sprague Dawley rats ($P > 0.9$). A main effect of dose on omission errors was due to the larger dose of mecamylamine producing a subtle increase in omissions across groups.
**Conclusions:** Nicotine-induced improvements in rate- and speed-dependent performance indices were identical between strains. Only Wistar rats displayed an increase in response accuracy, the measure most closely related to attention. This result points to Wistar rats as the strain of choice when studying the mechanisms underlying the attention-enhancing effects of nAChR agonists. Surprisingly, mecamylamine had no effect on accuracy in any strain at a dose range bordering rate-suppressant effects. A possible explanation is that the increase in response accuracy seen with nicotine in Wistar rats was mediated by receptor desensitization. Consistent with this possibility, previous preclinical studies of cognition have suggested paradoxical performance benefits with ultra-low doses of nAChR antagonists. We observed an increase in response accuracy by low-dose mecamylamine in Wistar rats only. This suggests that the attention-enhancing effects of nicotine seen in the Wistar strain may, at least in part, be the result of nAChR desensitization, although other neuropharmacological mechanisms are conceivable. Future research on the attention-enhancing effects of nAChR agonists would benefit from a characterization of the genetic differences between the rat strains tested here that may explain their differential sensitivity to the attention-enhancing effects of nAChR ligands.
**Keywords:** nicotinic acetylcholine receptors, attention, rat strains, nicotine, mecamylamine
**Disclosures:** Nothing to disclose.

## M137. Input and Output-Specific Regulation of a Learned Action Sequence by Corticostriat al Circuits

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**Background:** Corticostriatal circuits play a central role in choreographing movement, including both individual actions and more complex behavioral routines involving multiple distinct actions performed in sequence. While synaptic plasticity in the striatum is important for learning to perform many types of actions, the striatum contains several different cell types that serve as postsynaptic targets for presynaptic

input arising from many sources. The specific source and target of synapses that undergo plasticity while learning an action sequence have yet to be identified.
**Methods:** We used a combination of retrograde and anterograde viral and optogenetic manipulations in mice to study synapses connecting motor cortex and dorsolateral striatum. Striatal medium spiny neurons that form the direct and indirect pathways were targeted using transgenic mice expressing Cre recombinase driven by Drd1a and Adora2a regulatory sequences. Monosynaptic projections to the striatum were targeted using retrogradely transported deletion-mutant rabies virus. Cells were inhibited by expression of a Kir2.1 potassium channel, and activated through optogenetic stimulation.
**Results:** The completion of a simple action sequence was impaired by inhibiting striatal medium spiny neurons that form the direct pathway. This phenotype was not due to impairments in movement, motivation, or perseveration. Action sequence initiation was mediated by cells in the secondary motor cortex that send monosynaptic projections to the striatum. Slice physiology experiments revealed that synapses connecting these corticostriatal circuit elements became stronger after mice learned a sequence of actions. This led to a disparity in striatal output that favored the direct pathway and was necessary for completion of an action sequence.
**Conclusions:** While striatal outputs through the direct and indirect pathway have opposite behavioral functions, both pathways are active during action initiation. Our data suggest the completion of an action sequence requires dynamic modulation of striatal output, leading to a disparity that favors the direct pathway. This disparity may be driven by the strengthening of excitatory synaptic inputs from secondary motor cortex onto direct pathway medium spiny neurons - a form of synaptic plasticity that may encode the chunking or concatenation of sequential actions.
**Keywords:** Synaptic Plasticity, Dorsal striatum, cortex, optogenetics, Direct pathway
**Disclosures:** Nothing to disclose.

### M138. Erasure of Recent and Remote Fear Memory by Enhancing Forgetting Through Increase in Adult Hippocampal Neurogenesis

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**Background:** Erasure of fear memory is thought to be a therapeutic target for emotional disorders such as post-traumatic stress disorders (PTSD). Memantine (MEM) is a noncompetitive N-methyl-D-aspartate (NMDA) glutamate receptor antagonist and has known to enable to increase in adult hippocampal neurogenesis. Previous study has shown that increasing adult hippocampal neurogenesis enhanced forgetting of hippocampus-dependent memory. In this study, we tried to examine effects of MEM treatment on forgetting of hippocampus-dependent fear memory.
**Methods:** Mice were contextual fear-conditioned with an electrical footshock and then received systemic injections of MEM once a week for 4 weeks.
**Results:** We found that MEM-treated group displayed disruption of contextual fear memory following the MEM treatment, while control group treated with saline displayed normal contextual fear memory. Importantly, MEM-treated group did not show spontaneous recovery of fear memory even a month after the MEM treatment. Moreover, we observed similar effects of MEM treatment on inhibitory avoidance memory, another type of hippocampus-dependent fear memory. In contrast, MEM-treated group showed normal amygdala-dependent cued fear memory. We next examined the relationship between erasure of hippocampus-dependent memory and enhancement of adult hippocampal neurogenesis by MEM. Mice received an injection of MEM followed by injections of 5-bromo-2-deoxyuridine 2 days later, and then were contextual fear-conditioned. Interestingly, there was a significant negative correlation between the number of BrdU-positive cells increased by MEM and differences of freezing scores before and after the MEM treatment.
**Conclusions:** These observations suggest that MEM treatment enables to erase hippocampus-dependent fear memory by enhancing memory forgetting through the increase in adult hippocampal neurogenesis. Thus our findings suggest a possible therapeutic treatment to weaken traumatic memory. We are examining effects of the MEM treatment on remote fear memory.
**Keywords:** PTSD, Fear conditioning, Fear extinction, mice, Neurogenesis
**Disclosures:** Nothing to disclose.

### M139. Aberrant Nocturnal Cortisol as a Vulnerability Trait for More Rapid Progression of Advanced Breast Cancer

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**Background:** There is wide variability in the progression of advanced breast cancer (ABC) in humans. One of the factors that has been posited as a moderator of cancer progression is an abnormality in the pattern of cortisol. Specifically, previous research has found that a flattened diurnal slope of salivary cortisol is associated with more rapid progression of ABC and shorter survival. Whether this corresponds to abnormalities in plasma cortisol is currently unknown.
**Methods:** We examined the patterns of 24-hour and overnight plasma cortisol in a cohort of 97 women with advanced breast cancer and 24 age-matched controls. Plasma samples were obtained every 15-60 minutes via an indwelling, intravenous catheter from outside the room so as to not disturb sleep. Diurnal patterns in cortisol were characterized with a cosinor analysis. Overnight patterns were characterized with 9-base Fourier functions analyzed with functional principal component analysis (fPCA). Concomitant data on polysomnographically-defined sleep were also collected.
**Results:** We could detect no differences between women with ABC and controls in the circadian pattern of cortisol, as described by its absolute timing (phase) or timing relative to sleep (phase angle), amplitude (half fitted peak-to-trough concentration), or mesor (average fitted concentration)

(p's > 0.11, t-tests). However, a prominent and unusual aberration in the overnight cortisol pattern, an additional early night spike in plasma cortisol, was evident in a subset of participants (approximately 20% of both controls and those with ABC). During this aberrant cortisol spike, there was an 8-fold increase in the amount of objectively-measured wake time ($p < 0.004$, Wilcoxon Signed Rank). The amplitude of this aberration was quantified by fPCA. In doing so, we found that the greater the amplitude of this cortisol aberration, the shorter was the disease-free interval (DFI; $r = -0.30$, $p = 0.004$, $n = 69$ of 97 with sufficient overnight cortisol data; linear regression) - time from initial diagnosis to recurrence, a marker of poorer prognosis for disease progression.
**Conclusions:** The occurrence of an atypical, early night spike in cortisol was associated with shorter DFI and acute disruption of sleep among women with ABC. We could find no overall difference in circadian timing, as captured by the absolute and relative timing of the diurnal cortisol rhythm, between women with ABC and controls. The aberrant early night cortisol spike appears to be a vulnerability trait that is associated with faster progression of ABC.
**Keywords:** Cortisol, circadian rhythm, sleep, breast cancer, disease progression
**Disclosures:** Nothing to disclose.

### M140. Distinct Roles of Melanin Concentrating Hormone in Maternal Behavior and Postpartum Depression

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**Background:** In order to prepare mothers for the demands of pregnancy and lactation, a number of brain adaptations take place. The expression of maternal behavior, such as nursing, pup retrieval, nest building and maternal aggression, is regulated neuronal circuits some of which express high levels of melanin concentrating hormone receptor (MCHR1). Furthermore, ablation of MCH has been shown to increase mortality and cannibalism rates. Moreover, while the lateral hypothalamus (LH) is the main location of MCH neurons, MCH expression is induced in the medial preoptic area (mPOA) only in the late postpartum period and has been associated with waning maternal behavior. Finally, MCH injection into mPOA in the early lactation inhibits maternal behavior. These data point at the MCH system as having an important role in maternal behaviors.
**Methods:** We used genetic and pharmacological approaches to investigate the role of MCH system in regulating maternal behavior. Female wild type (WT) and MCHR1 knockout (KO) mice were allowed to mate with genotype matching male mice for a period of 3 days. Following this mating period male mice were removed from the cage and the female mice were subsequently monitored for signs of pregnancy daily by visual examination and weight measurements. The date of birth of pups was considered postpartum day 0 (P0). Pup mortality was measured as percentage of initial litter which died between days 0 and 2 post-partum. Both wild type and knockout mothers were observed for instances of cannibalism. The following maternal behaviors were assessed in WT and KO by observers who are blinded to the condition of the experimental subjects: nest building, maternal aggression, pup Retrieval, milk Production, and forced swim test. The effects of MCHR1 selective antagonist GW803430 on pups retrieval, milk production, and forced swimming were assessed. c-Fos immunostaining was performed to localize the active neurons in the maternal neural circuit following pups retrieval test on P2.
**Results:** We first found that MCHR1KO female mice display a distinct behavioral phenotype characterized by disruption of maternal behavior. Early postpartum MCHR1KO females failed to display several aspects of maternal behaviors such as nesting, warming and protecting pups (causing decreased survival), retrieving pups, and maternal aggression. Milk production was also reduced in the MCHR1KO postpartum female mice. When injected with a MCH antagonist mice show a decrease in pups retrieval and milk production which are in line with the results of MCHR1 KO mice. However, beyond the second day after parturition, the MCHR1KO mice showed improvement in maternal care towards their pups. This was reflected by an increase in the survival rate and decreased pups retrieval time. Of particular interest is that genetic ablation of MCHR1 caused reduction in postpartum depression-like behavior in the MCHR1KO mice in the late lactation period but not in early lactation period or in virgin female mice. Accompanied with the maternal behavior deficits, we found decrease in the c-Fos-reactive neurons in MCHR1KO in regions involved in maternal behavior including: ventral tegmental area (VTA), nucleus accumbens shell (NAcsh), lateral septum (LS), ventral pallidum (VP), and amygdala (Am), but not in the mPOA. The expression of c-Fos suggests that MCH regulates more than one neural circuit, which is in line with the distinct anatomical localization of MCHR1 in the brain, and the impairment of specific maternal behaviors that are regulated by different neural circuits.
**Conclusions:** According to our data, MCH system might play a dual role in regulating maternal behaviors. We propose a model for this role. MCH inputs into the maternal circuits originate from two different sources: the LH and the mPOA. LH-MCH neurons in the early postpartum period mediates the maternal behavior facilitating action of mPOA neurons through activating the dopamine reward circuit. In the late postpartum period, mPOA MCH neurons which are GABAergic in nature act to wane the maternal behavior through suppressing the dopamine reward circuit.
**Keywords:** MCH, Maternal, Behaviors, postpartum, depression
**Disclosures:** Nothing to disclose.

### M141. Impact of Metabolic Aberrations on Biophysical Integrity of the Default Mode Network

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**Background:** The brain's default mode network (DMN), most active at rest with high levels of basal metabolism, is

particularly vulnerable to the insult of type 2 diabetes mellitus (T2DM) due to altered glucose metabolism associated with insulin resistance and chronic hyperglycemia. Recent neuroimaging studies demonstrate that functional connectivity within the DMN is disrupted in T2DM patients compared with healthy control subjects (HCs). Impaired white matter fibers that connect DMN nodal regions, including the cingulum bundle, are implicated. However, it is unknown whether the DMN nodal regions themselves are also compromised, thereby contributing to the disrupted functional connectivity. Using magnetization transfer (MT) imaging, this study examined the impact of metabolic aberrations on biophysical integrity of the DMN in patients with T2DM and HCs.

**Methods:** Subject: 21 patients with T2DM and 27 non-diabetic HCs. Diagnosis of T2DM was confirmed using the American Diabetes Association guidelines. All participants had no history of depression, a score of 8 or lower on the 17-item Hamilton Depression Rating Scale (HAM-D), and were free of unstable medical conditions. For all participants, vascular comorbidities were assessed using the Framingham Stroke Risk Profile (FSRP) score and glycated hemoglobin (HbA1c) levels were evaluated as an indicator of the glycemic control status.

Magnetization Transfer Imaging: MRI scans were performed on a Philips Achieva 3T scanner with an 8-element phased-array head coil. MT images were acquired using a 3D spoiled gradient-echo sequence with multi-shot EPI readout: TR/TE = 64/15ms, flip angle = 9°, FOV = 24 cm, 67 axial slices, slice thickness/gap = 2.2 mm/no gap, EPI factor = 7, reconstructed voxel size = $0.83 \times 0.83 \times 2.2$ mm3, with a nonselective five-lobed Sinc-Gauss off-resonance MT prepulse (B1/Δf/dur = 10.5μT/1.5kHz/24.5ms). Parallel imaging was utilized with a reduction factor of 2.

Image Processing: The magnetization transfer ratio (MTR) values were calculated on the voxel-by-voxel basis. The ROIs encompassed nodal regions of the DMN, including posterior cingulate cortex (PCC), precuneus (PCu), medial prefrontal cortex (mPFC), lateral inferior parietal cortex, and medial and lateral temporal lobes in both hemispheres (major nodal regions of DMN obtained using independent component analysis (ICA) on 36 healthy controls from a third party). The generation of the ROIs in the images and the calculation of MTR in each ROI were performed using in-house developed programs.

**Results:** The macromolecular protein pools in a key DMN hub region, PCC, is impaired in patients with T2DM, represented by significantly lower MTR compared with HCs. In contrast, there were no significant group differences of MTR in the other core DMN regions: medial prefrontal cortex, lateral inferior parietal cortex, and precuneus and in non-core DMN regions: medial and lateral temporal cortices. The reduced MTR in PCC correlated inversely with T2DM-related clinical measures, including hemoglobin A1c level and increased cerebrovascular risk factors.

**Conclusions:** Our results demonstrate that the biophysical integrity of macromolecular protein pools in PCC is uniquely compromised in T2DM, thus independently or synegistically contributing to the disrupted functional connectivity observed in the DMN of affected individuals. We anticipate that examining the biophysical integrity of macromolecular protein pools in the DMN in T2DM patients may shed light on the molecular neurobiology of diabetes and help clarify the pathophysiology of diabetes-related cognitive comorbidities and risk for dementia.

**Keywords:** type 2 diabetes mellitus, default mode network, magnetization transfer

**Disclosures:** Nothing to disclose.

### M142. Selective Modulation of Forebrain Function

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**Background:** Pharmacological manipulation of specific neural circuits to optimize therapeutic/side-effect profile is an unrealized goal in neurology and psychiatry.

**Methods:** Here we take advantage of an AMPA receptor auxiliary protein, TARP γ-8, to selectively reduce excitatory neurotransmission in forebrain, an action with rich potential therapeutic prospects.

**Results:** The benzothiazole, LY3130481 (6-((S)-1-{-(1-[-(5-(2-hydroxy-ethoxy)-pyridin-2-yl)]-1H-pyrazol-3-yl)}-ethyl)-3- H-1, 3-benzothiazol-2-one), selectively antagonized recombinant $\gamma - 8$-containing AMPA receptors but not other TARP members and was predicted to have anticonvulsant properties without the motoric side-effects inherent in non-gamma-8-selective AMPA receptor antagonists. LY3130481 attenuated synaptic transmission, glutamate-evoked currents of native rodent and human AMPA receptors in hippocampus and cerebral cortex, but not those in $\gamma - 8$ lacking Purkinje neurons of the cerebellum. As hypothesized, LY3130481 prevented convulsions in rats without motoric side-effects. The negation of anticonvulsant efficacy in gamma-8-null mice demonstrates that LY3130481 transduces its selective anticonvulsant activity via AMPA receptors in forebrain while sparing AMPA receptor function in motor-coordinating cerebellar regions (gamma-2 enriched). In cortical tissue slices from two epileptic patients, LY3130481 reduced seizure frequency and size and abolished epileptiform-like oscillatory network activation.

**Conclusions:** The present findings provide proof of principle for the selective targeting of specific neural circuitry via specific protein-receptor associations for therapeutic advantage.

**Keywords:** epilepsy, AMPA antagonist, Pain

**Disclosures:** All employees of Eli Lilly and Company are employees of the pharmaceutical company of discovery.

### M143. Patients Receiving Regular Oral Lithium Therapy have a Reduced Incidence of Severe Neurological Disease and Myocardial Infarction

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**Background:** Lithium has been a standard treatment for Bipolar Disorder for more than 50 years. A variety of studies have examined the medical consequences of long-term

lithium treatment. There have been some in-vitro and animal studies demonstrating that lithium has cytoprotective actions in nerve cells and other tissue. Long-term lithium treatment has been reported to reduce the occurrence of Alzheimer's Disease and some other neurological disorders in humans as well. It is possible that a disease-sparing effect of lithium may be observable in patients taking regular oral lithium therapy. This study compares the prevalence of cardiovascular and neurological disease in psychiatric outpatients receiving and not receiving lithium.
**Methods:** This study consists of a retrospective chart review of adult psychiatric outpatients treated at the New York State Psychiatric Institute Lithium Clinic and two affiliate lithium clinics of the Columbia University Medical Center and the Foundation for Mood Disorders. These clinics specialize in the treatment of mood disorders and the majority of patients were diagnosed with either Major Depression or Bipolar Disorders. All patient diagnoses were made by a board-certified psychiatrist, and patients were assigned to lithium treatment as appropriate for their individual diagnoses. All patients in the practice underwent yearly physical exams and blood chemistries performed by a separate medical practice. The chart review included patient demographic information, diagnosis, treatment information, and any reported medical disorders. Odds ratios were calculated to assess the risk of having a disorder for patients receiving lithium compared to patients not receiving lithium.
**Results:** To date, 1021 patients have been entered in the database (54.2% female, and 45.8% male), ranging in age from 18 to 88 years old (mean = 42.1 yrs.; sd = 14.8 yrs.). Of these, 570 patients (55.8%) received lithium treatment, with the average duration of lithium therapy being 3.2 years (range 0.1 – 30.0 yrs.; sd = 5.43 yrs.). The frequency of any neurological disease in this group was low: there were 182 unique disorders occurring in 107 patients (10.4% of all patients). The frequency of specific conditions ranged from 37 patients with a history of Migraine Headache, to zero patients with Huntington's Chorea, Down's Syndrome, Lewy Body Disease, and Multi-Infarct Dementia. For seizures, the OR was 0.097 (95% CI: 0.022 – 0.426). For dementia – not otherwise specified (D-NOS), the OR was 0.113 (95% CI: 0.014 – 0.924). For amyotrophic lateral sclerosis (ALS), the OR was 0.113 (95% CI: 0.014 – 0.922). For myocardial infarction (MI), the OR was 0.293 (95% CI: 0.114 – 0.754). Odds ratios for all other disorders (AD, Cerebrovascular Disease, CNS Neoplasm, Down's Syndrome, Huntington's Disease, Lewy-Body Disease, Migraine Headache, Multi-Infarct Dementia, Multiple Sclerosis, Optic Atrophy/Neuritis, Parkinson's Disease, Stroke, Syncope) were found to be equivalent to unity.
**Conclusions:** Patients receiving long-term lithium treatment for psychiatric illness have a significantly lower likelihood of seizure, ALS, Dementia (NOS), and myocardial infarction, compared to psychiatric outpatients not receiving lithium therapy. These results suggest that long-term lithium treatment may protect against some neurological disorders and myocardial infarction.
**Keywords:** Lithium, Neurological disorder, Disease prevalence, Cytoprotection
**Disclosures:** Nothing to disclose.

### M144. Comparing Dynamic SUV and Cortical Thickness Between Healthy Controls and Epilepsy Patients Using Simultaneous PET/MR

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**Background:** A combined PET/MR scanner with simultaneous acquisition allows direct correlations of PET data with MR-detected parameters on the same subject at the same time. This multi-modal analysis will facilitate the identification of an optimal biomarker. Here we report our study to compare dynamic SUV and cortical thickness between controls (HC) and epilepsy patients (Ep) using simultaneous PET/MR.
**Methods:** Subjects (11 HC and 27 Ep) were imaged on a combined PET/MR scanner (Biograph mMR, Siemens). After FDG injection, dynamic PET scans and simultaneous MR imaging (including T1, T2 and other sequences) were acquired for ~90 minutes. Dixon sequence was acquired for attenuation correction. PET data were reconstructed using the e7tools provided by Siemens. Images were processed using Freesurfer, a fully automated image analysis tool. Over 100 masks (ROIs), including left and right, for cortical and subcortical regions were generated. Statistical analyses on mean SUV for entire study (SUVmean_all), meanSUV derived from the last three frames (SUVmean_late), and mean cortical thickness were compared between groups.
**Results:** Based on Mann-Whitney U tests, SUVmean_late values showed significant differences between groups for most ROIs, while no difference was seen with SUVmean_all. Temporal_Mid_tempocci consistently showed significant difference when normalized SUV values were compared ($p < 0.01$, by individual subject's mean cortical, white matter or global brain). Significant cortical thinning (Epi vs. HC) was detected bilaterally (left, right) within localized regions, such as precentral ($p = 0.017$, 0.012) and superiorfrontal ($p = 0.016$, 0.001). Binary logistic regression indicated that both SUVmean_late and cortical thickness were independent predictors for epilepsy.
**Conclusions:** Our results suggest that simultaneous PET/MR imaging provides a useful imaging tool to identify regional abnormality, and that SUVmean_late and cortical thickness are independent biomarkers for epilepsy.
**Keywords:** PET, MRI, epilepsy
**Disclosures:** Nothing to disclose.

### M145. NPI Agitation/Aggression Domain Scores Demonstrate Clinically Meaningful Changes in a Phase 2 Study of Dextromethorphan/Quinidine (DM/Q) in Patients with Agitation in Alzheimer's Disease

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**Background:** Neuropsychiatric symptoms in dementia, such as agitation and aggression, adversely impact patient and caregiver quality of life, are associated with increased disability,

cost of care and institutionalization risk. Testing of pharmacologic treatments for agitation in Alzheimer disease (AD) has employed various efficacy assessments including the Neuropsychiatric Inventory (NPI), Cohen Mansfield Agitation Inventory and the Neurobehavioral Rating Scale. Although drug-placebo treatment effects have been demonstrated using the NPI and other scales, clinically meaningful improvement is not well defined. The NPI website suggests a 30% reduction or a 4 point change from baseline constitutes a relevant improvement (http://npitest.net/about-npi.html), but the threshold may be lower for more disruptive symptoms (e.g. agitation). Recently, a combination of dextromethorphan hydrobromide and quinidine sulfate (DM/Q) was associated with a significant improvement in AD-related agitation vs placebo, as assessed by NPI Agitation/Aggression (NPI A/A) score reduction (primary efficacy). This analysis explores the relationship between NPI A/A score improvements and global measures of clinical change.

**Methods:** Multicenter, randomized, double-blind, placebo-controlled, Sequential Parallel Comparison Design (SPCD) Phase 2 study of DM/Q vs placebo comprised of two 5-week stages. Eligible patients had probable AD, Mini-Mental State Examination (MMSE) scores of 8–28 and clinically meaningful agitation for which pharmacologic intervention was clinically indicated. Primary endpoint: change from baseline on the NPI A/A score (range: 0-12 points). Numerical and percent changes in the NPI A/A score were examined via a receiver operating characteristic (ROC) curve analysis, assuming that Alzheimer's disease Cooperative Study-Clinical Global Impression of Change (ADCS-CGIC Agitation; 7-point scale ranging from "marked worsening" to "marked improvement") - agitation scores of "moderate" or "marked" improvement from baseline were clinically meaningful. Box plots were created to show a relationship between NPI A/A change and ADCS-CGIC Agitation. Additional analyses included comparisons of change in the NPI A/A to the caregiver-rated Patient Global Impression of Change (PGI-C), and a cumulative frequency distribution of percentage of patients showing any given level of change in NPI A/A score at week 10.

**Results:** 220 patients (DM/Q N = 93; Placebo N = 127) enrolled; 194 (88%) completed the study. Mean (standard deviation) baseline NPI A/A scores were 7.1 (2.6) for DM/Q vs 7.0 (2.4) for placebo. NPI A/A scores significantly improved for DM/Q vs placebo-treated patients at week 5 ($P \leq 0.001$, standardized effect size = 0.51), week 10 ($P = 0.02$, standardized effect size of the change in NPI A/A score = 0.48), and overall (primary outcome: SPCD, ordinary least squares $P < 0.001$). In a 10-week analysis of patients remaining on their original randomized treatment throughout, DM/Q was associated with a 51% reduction in NPI A/A score (baseline 7.1; n = 93) compared with 26% for placebo (baseline 7.2; n = 66). In the 10-week analysis of cumulative frequency distribution, treatment difference (DM/Q minus placebo) in the proportion of patients achieving an improvement of $\geq 1$ to 4 points on the NPI A/A domain was approximately 15%-30%, respectively. In the ROC analysis, a 4 point improvement (50% to 60% reduction) in NPI A/A score was associated with an approximate 71% to 76% sensitivity and specificity for a "marked" or "moderate" improvement on the ADCS-CGIC Agitation scale. Changes from baseline to week 5 and to week 10 produced similar results. For the box plot analysis, most patients rated as having any improvement from baseline on ADCS-CGIC Agitation (minimal to marked improvement) had $\geq 2$ point improvement in NPI A/A score; almost all patients with moderate to marked improvement on ADCS-CGIC Agitation had NPI A/A improvement, with most demonstrating $\geq 3$ point reductions. For the ROC analysis for PGI-C, a 4 point improvement (or 50% to 60% reduction) in NPI A/A score was associated with a 64% to 76% sensitivity and specificity for a rating of "very much" or "much" improved on the PGI-C. For the box plot analysis for PGI-C, most patients showed a 3 point NPI A/A score improvement for the "much" and "very much" improved categories. For the 10-week analysis of NPI A/A response ($\geq 50\%$ decrease in NPI A/A from baseline), 56% of patients randomized to DM/Q were responders vs 38% in the placebo group.

**Conclusions:** DM/Q significantly reduced NPI A/A score vs placebo (primary outcome) with clinically important and meaningful changes in the NPI A/A based on several analytical methods. Almost all patients assessed as having "moderate" or "marked" improvement from baseline on the ADCS-CGIC Agitation score had some decrease in NPI A/A score; most had a decrease of $\geq 3$ points from a baseline score of 7.1. In the ROC analysis, the NPI A/A domain also demonstrated good sensitivity and specificity that was associated with clinically meaningful change in global improvement measures. Similar results were seen for the PGI-C. Significantly more patients on DM/Q had a $> 50\%$ improvement in the NPI A/A (responders) compared with placebo. These results lend evidence for construct validity to the NPI A/A domain as a sensitive measure for clinically important and meaningful symptom improvement in AD-associated agitation.

Study supported by: Avanir Pharmaceuticals, Inc.
**Keywords:** Alzheimer's disease, neuropsychiatric inventory, ROC analysis, Dextromethorphan/Quinidine

**Disclosures:** Sanjay Dubé, Harry Cui, and Joao Siffert are employees of Avanir Pharmaceuticals, Inc. Jeffrey Cummings has served as a consultant and/or received consulting fees or honoraria for/from Abbott Laboratories, Acadia, Adamas, Anavex, Astellas Pharma US, Inc., Avanir Pharmaceuticals, Inc., Avid, Baxter Healthcare, Bristol-Myers Squibb, Eisai, Elan Pharmaceuticals, EnVivo, Forest Pharmaceuticals, Inc., GE Healthcare, Genentech, GlaxoSmithKline, Lilly, Lundbeck, MedAvante, Medtronic, Merck & Co., Inc., Neurokos, Neuronix, Neurotrax, Novartis, Ortho McNeil Janssen, Otsuka Pharmaceuticals, Pain Therapeutics, Inc., Pfizer, Inc., Plexxicon, Prana, QR, Sanofi Aventis, Signum Bioscience, Sonexa, Takeda, Toyama, and UBC, holds a copyright for The Neuropsychiatric Inventory, and owns stock or stock options in Adamas, MedAvante, Neurokos, Neurotrax, Prana, QR, and Sonexa.

## M146. [11C]Neuroflux: In Vivo Measurement of Neuron Population Flux

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**Background:** Neuron population flux is the longitudinal alteration in a neuron population as a result of net neuron

influx or efflux. Neurodevelopmental and neurodegenerative diseases often result in aberrant neuron flux that, if measured, could be a biomarker of disease progression or disease treatment efficacy. Regions with the highest neuron turnover are ideally targeted for measurements of neuron population flux due to their dynamic state, as changes in neurogenic or neuron death rates will be apparent more quickly. The tissue with the highest rate of adult neuron turnover is the olfactory epithelium (OE), which contains the olfactory sensory neurons (OSNs) that provide the sensory input for our sense of smell. Across lifespan, the OSN proliferation rate is several hundred to several thousand fold higher than that found in the subgranular zone of the dentate gyrus, making it the ideal candidate for monitoring neuronal population flux. To measure flux, longitudinal measurements of the neuron population are required; thus, we focused on non-invasive imaging techniques that offer the possibility for repeat measurements within individual subjects. We selected positron emission tomography (PET) due to its biological target selectivity, human translational potential, and capacity for quantitative comparisons. We developed a novel neuron-population-monitoring PET radiotracer, [11C]neuroflux, and applied it to biological models to demonstrate its facility for neuron flux monitoring and highlight its diagnostic potential.

**Methods:** After we synthesized and validated [11C]neuroflux, it was utilized in several rodent models through intravenous administration, 60 minute dynamic PET scans, and Logan image analysis. Ex vivo evaluation of the OSN population in all animal models was achieved with Western Blot (WB) of the olfactory marker protein (OMP), which is selectively expressed in the OSNs. The animal models studied include: 1) olfactory bulbectomy 2) stimulus-induced neurogenesis, 3) normative post-natal development, 4) normative aging, and 5) a neurodegenerative tauopathy model. The olfactory bulbectomy model was chosen for its well-documented ability to selectively remove mature OSNs from the OE, resulting in net neuron efflux. In this study, we completed unilateral and bilateral olfactory bulbectomies on groups of WT mice with [11C]neuroflux imaging after 2-3 days. To model the reciprocal process of neurogenesis, we utilized intranasal zinc sulfate treatment, which strips the OSNs from the OE, resulting in a neurogenic-predominant state for OSN replenishment. This model is differentiated from the bulbectomy model because newly born neurons are able to mature by forming axonal connections with the intact olfactory bulb. Subsequently, normative alterations in neuron population flux were monitored, beginning with post-natal neuron population development. This was achieved through [11C]neuroflux imaging of 1.3, 2, 3, 5.5, 9 and 12 month old rats, including tracking individual rats between 5.5 and 12 months of age. Turning to aging-induced neuron death, we imaged cohorts of 7, 12, and 23 month old mice with [11C]neuroflux. To highlight the disease diagnosis potential for [11C]neuroflux imaging, we moved to a murine tauopathy neurodegeneration model, rTg4510, which reproduces many of the features of Alzheimer's disease and frontotemporal dementia. [11C]Neuroflux imaging was completed in 3.7 and 7 month old rTg4510 animals and age-matched controls.

**Results:** Exploiting olfactory bulbectomy and zinc sulfate models, which respectively produce OSN death and OSN neurogenesis, we validated [11C]neuroflux imaging for sensitive monitoring of the complete neuron efflux-influx cycle. A switch to the assessment of [11C]neuroflux imaging in animal models of normative development and aging reproduced the anticipated neuron growth curve that predominates into early adulthood; thereafter, [11C]neuroflux imaging monitored the neuron efflux resulting from aging-induced neuron death. This study displayed the sensitivity of [11C]neuroflux to normative OSN population changes, supporting the capacity for [11C]neuroflux imaging to measure neuron flux during disease. This hypothesis was validated through successful stratification of control and tauopathy (rTg4510) mice by [11C]neuroflux at early stages of the disease, prior to neuronal loss in the cortex. Additional validation of all animal models through ex vivo WB analysis of OMP levels recapitulated the [11C]neuroflux imaging results for each model.

**Conclusions:** Our novel [11C]neuroflux radiotracer monitors changes in neuron flux resulting from altered neurogenic and neuron death rates. Based on initial studies, there are expected applications for early neurodegenerative disease diagnosis as well as monitoring the in vivo, longitudinal efficacy of novel neuroprotecive therapeutics. Of note, a recent study on human olfactory dysfunction indicates that loss of smell predicts 5-year mortality in older adults, a result that may be correlated with neuron efflux from the olfactory tissue. This highlights the potential contribution of OSN flux imaging to the understanding of human mortality.

**Keywords:** PET Imaging, Neurodevelopment, Aging, Neurodegenerative Disease, Radiotracer

**Disclosures:** Nothing to disclose.

## M147. The Effects of Aging and Psychiatric Disease on Circadian Patterns of Gene Expression in the Human Prefrontal Cortex

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**Background:** With aging, significant changes in circadian rhythms occur, including a shift in phase toward a "morning" chronotype and a loss of rhythmicity in circulating hormones. There are also well documented disruptions to circadian rhythms that are associated with several psychiatric disorders. However, the effects of aging and psychiatric conditions on molecular rhythms in the human brain have remained elusive.

**Methods:** Here we employed a previously described time-of-death analyses to identify transcripts throughout the genome that have a significant circadian rhythm in expression in the human prefrontal cortex (Brodmann's areas (BA) 11 and 47). Expression levels were determined by microarray analysis in 146 individuals.

**Results:** Rhythmicity in expression was found in $\sim$10% of detected transcripts ($p < 0.05$). Using a meta-analysis across the two brain areas, we identified a core set of 235 genes

($q < 0.05$) with significant circadian rhythms of expression. These 235 genes showed 92% concordance in the phase of expression between the two areas. In addition to the canonical core circadian genes, a number of other genes were found to exhibit rhythmic expression in the brain. Notably, we identified more than one thousand genes (1186 in BA11; 1591 in BA47) that exhibited age-dependent rhythmicity or alterations in rhythmicity patterns with aging. Interestingly, a set of transcripts gained rhythmicity in older individuals, which may represent a compensatory mechanism due to a loss of canonical clock function. We are currently analyzing samples from subjects with either bipolar disorder or schizophrenia and these data will also be presented.
**Conclusions:** We confirm that rhythmic gene expression can be reliably measured in human brain and identified for the first time significant changes in molecular rhythms with aging that may contribute to altered cognition, sleep and mood in later life.
**Keywords:** circadian rhythm, aging, Postmortem Brain Tissue, Bipolar Disorder, schizophrenia
**Disclosures:** Nothing to disclose.

### M148. Gender Differences in the Clinical Features and Substance Use Disorder Comorbidities in Patients with Antisocial Personality Disorder

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**Background:** Gender is an important variable in the study of mental health because of the actual and perceived differences between men and women. Relatively little is known how males and females differ in their manifestations of antisocial personality disorder (ASPD). We have conducted a study to compare demographic and clinical characteristics of men and women with ASPD.
**Methods:** Our sample consisted of 323 participants with ASPD, 253 men and 70 women. The study was performed in an urban academic medical center. Demographic and clinical features of participants with ASPD were assessed and recorded.
**Results:** Women had fewer episodes of antisocial behavior involving ($p = 0.01$) or not involving ($p = 0.003$) police, higher scores on the Childhood Trauma Questionnaire (CTQ) ($p = 0.009$) and on Emotional Abuse ($p = 0.007$) and Sexual Abuse ($p = 0.003$) subscales of the CTQ compared to men. CTQ scores positively correlated with the number of episodes of antisocial behavior involving police in men ($p = 0.015$) but not in women. The percentage of patients with comorbid borderline ($p = 0.004$) and histrionic ($p < 0.001$) personality disorders was higher and the percentage of participants with cocaine use disorder ($p = 0.01$) was lower among women compared to men. Comorbid alcohol use disorder was frequent in both groups (50.5% in men and 43.6% in women), while a higher percentage of women had comorbid mood disorders compared to men ($p = 0.04$). Logistic regression analysis demonstrates that CTQ scores, histrionic personality disorder, and antisocial behavior involving the police drive the difference between the groups.
**Conclusions:** Our findings indicate that treatment of individuals with ASPD should focus on the following: a) management of comorbid substance use disorders which is important both for men and for women; b) prevention of antisocial acts which is more important for men; and c) management of comorbid mood and personality disorders which is more important for women.
**Keywords:** antisocial personality disorder, gender, Psychiatric Comorbidity
**Disclosures:** Nothing to disclose.

### M149. Cognitive Reappraisal Training Enhances Emotion Regulation in Borderline Personality Disorder

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**Background:** Borderline personality disorder (BPD) is the prototypical disorder of emotion dysregulation. We have shown previously that BPD patients do not activate brain regions known to participate in cognitive reappraisal as healthy subjects (HCs) do. This may contribute to their impaired emotion regulation. This study tests the hypothesis that BPD patients can be trained to enhance cognitive reappraisal, thereby normalizing neural activity during reappraisal and improving reappraisal success.
**Methods:** On each of 5 study days, BPD ($n = 14$) and HC ($n = 16$) subjects were shown 60 negative social emotional pictures and instructed to employ reappraisal-by-distancing to half and to look, without reappraising, at the other half. Day 1 was baseline and days 2 through 5 (spaced 2 days apart) afforded training through practice on novel pictures. Behavioral ratings were obtained each day. 3T fMRI images were obtained on days 1 and 5. Functional connectivity to an amygdala seed was assessed with PPI analysis.
**Results:** BPD patients showed significantly decreased negative ratings of aversive images ($p < 0.008$) on day five compared to day one. Neurally, when reappraising they showed greater increases in DLPFC and decreases in amygdala activity ($p < 0.05$) after training, demonstrating a pattern similar to HCs at baseline. With training, BPD patients increased negative connectivity between amygdala and ventrolateral prefrontal cortex and an extensive medial and lateral prefrontal region, showing a pattern of connectivity similar to that seen in HC's at baseline.
**Conclusions:** This is the first study to demonstrate that brief longitudinal training can increase reappraisal success and normalize reappraisal neural activity in BPD. It suggests that focused reappraisal training may have a role in the treatment of BPD.
**Keywords:** cognitive reappraisal, Borderline Personality Disorder, Reapprasial Training
**Disclosures:** Nothing to disclose.

### M150. Low Activity Monoamine Oxidase-A Allelic Variants Relate to Abnormal Amygdala Morphology in Antisocial Personality Disorder with High Psychopathic Traits

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**Background:** Antisocial personality disorder (ASPD), especially when high psychopathic traits are present, is linked to violent offending. Morphological abnormalities of the amygdala, a key emotion processing region, are seen in individuals with high psychopathic traits, and low levels of monoamine oxidase A (MAOA), a brain protein involved in metabolism of amine neurotransmitters, are also observed in limbic structures of ASPD males with high psychopathy. Among healthy males, MAO-A genetic variants associated with low transcription in vitro (MAOA-L) are related to structural abnormalities of the amygdala. However, it is currently unknown whether amygdala morphology in ASPD relates to specific MAOA genetic polymorphisms. We hypothesized that amygdala surface area abnormalities would be associated with MAOA-L genotype in ASPD males with high psychopathic traits.
**Methods:** We studied 18 males with ASPD and 20 healthy male controls. Groups did not differ on age. All subjects were clinically assessed by a forensic psychiatrist (NJK) using the SCID I and II. All subjects were medication-free, non-smoking, and were free of illicit substance use. ASPD subjects additionally had no history of mood or psychotic illness. Genomic DNA was extracted from peripheral leukocytes with MAOA genetic polymorphisms determined using standard PCR procedures. Each subject underwent a T1-weighted anatomical brain scan on a 3.0-T GE Discovery MR750 scanner (TE = 3.0 ms, TR = 6.7 ms, flip angle = 8°, slice thickness = 0.9 mm, 200 slices, FOV = 240 mm, matrix = 256 x 256, voxel size = 0.9 mm x 0.9 mm x 0.9 mm). Amygdala morphology was estimated using MAGeT Brain (Pipitone et al., 2014), a novel multi-atlas technique that bootstraps segmentation using Multiple Automatically Generated Templates. Outcome measures were vertex-wise measures of amygdala shape and surface areas, where shape was measured as a series of surface displacement metrics that describe the inward and outward displacement along a surface normal required for the atlas to match each subject and any corresponding local surface area differences. Surface area at each vertex was divided by the total surface area of each structure for an individual to account for global effects of volume on local vertex-wise measures. Analyses were run within a vertex-wise linear model with IQ as a covariate. Results were corrected for multiple comparisons using the false discovery rate (FDR) correction.
**Results:** Results revealed a group by genotype interaction for the left amygdala ($t = 4.14$, $p = 0.0002$, FDR 5%, $df = 32$), such that ASPD subjects with MAOA-L had regions of decreased surface area on the anterolateral aspect of the left amygdala and increased surface area on the posteromedial aspect of the left amygdala ($t = 2.89$, $p = 0.0069$, FDR 10%). Additionally, there was a cluster of decreased surface area on the superior aspect of the right amygdala and clusters of increased surface area on bilateral posterior regions of the right amygdala ($t = 2.67$, $p = 0.0118$, FDR 10%) in ASPD with MAOA-L. No group differences were observed among carriers of the high MAOA allele.
**Conclusions:** This is the first study to describe genotype-related morphological differences of the amygdala in a clinical population marked by high aggression and violence. Deficits in emotional regulation that contribute to the violence of ASPD may relate to morphological abnormalities of emotion processing regions under genetic control.
**Keywords:** antisocial personality disorder, monoamine oxidase A, neuroimaging
**Disclosures:** Nothing to disclose.

### M151. Maintenance Antipsychotic Dose can be Decreased in Late-life Schizophrenia: A Prospective DopamineD2/3 Receptor Occupancy Study with [11C]-raclopride

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**Background:** Schizophrenia is a life-long illness that typically requires maintenance antipsychotic treatment over the life span. Positron Emission Tomography (PET) studies have established that striatal dopamine D2/3 receptors (D2/3R) occupancy by antipsychotics for a safe therapeutic window is between 65% and 80% in younger patients with schizophrenia, which has been successfully employed in predicting the clinically effective doses for antipsychotics. On the other hand, patients with late-life schizophrenia (LLS) are highly susceptible to antipsychotic adverse effects. Treatment guidelines endorse lower antipsychotics dosages. However, optimal dose of antipsychotics and associated D2/3R occupancy remain largely unexplored in patients with LLS. Therefore, the aim of the current longitudinal study was to evaluate the effects of antipsychotic dose reduction on striatal dopamine D2/3R occupancy, clinical variables and blood pharmacokinetic measures in patients with LLS.
**Methods:** The study was approved by the local institutional ethics board and authorized by Health Canada. All participants were recruited and provided a written informed consent at the Centre for Addiction and Mental Health (CAMH), Toronto, between August 2007 and July 2013. The current open-label prospective PET study included stable outpatients with schizophrenia (the Positive and Negative Syndrome Scale (PANSS) scores $\leq 3$ for positive symptoms), aged 50 years or older, treated on the same dose of oral olanzapine or risperidone for at least 6-12 months. Each patient was scanned with [11C]-raclopride PET before and after antipsychotic dose reduction. The patients had a gradual dose reduction of up to 40% of the baseline dose to a target dose not lower than the recommended minimal maintenance dose for olanzapine (7.5 mg/day) or risperidone (1.5 mg/day). Patients were clinically followed up for 3-6 months after the dose reduction was

completed. Outcome measures included antipsychotic D2/3R occupancy, PANSS, Brief Psychiatric Rating Scale (BPRS), Targeted Inventory on Problems in Schizophrenia (TIP-Sz), Simpson Angus Scale (SAS), Barnes Rating Scale for Drug-Induced Akathisia (BAS), and Udvalg for Kliniske Undersøgelser Side Effect Rating Scale (UKU). Prolactin and antipsychotics blood levels were also measured. D2/3R occupancy was estimated using age and gender corrected measures of nondisplaceable binding potential derived from 10 antipsychotic-free patients with schizophrenia.

Clinical data was analyzed using Generalized Estimation Equation for repeated measures. Linear or Poisson regression was used for continuous or discrete variables, respectively. Multiple comparisons were conducted with Bonferroni correction. Baseline and follow-up D2/3R occupancy were compared by paired t-tests. Baseline and follow-up D2/3R occupancies were compared between participants with and without clinical deterioration during the follow-up phase and between participants with and without extrapyramidal symptoms by Mann-Whitney tests or t-tests based on their distribution.

**Results:** 35 patients were included (age = 60 ± 7 years, age of onset = 26.0 ± 9.0 years, duration of illness = 33.1 ± 9.3 years, PANSS = 61.3 ± 14.4). D2/3R occupancy of the entire sample decreased by 6.2 ± 8.2% following dose reduction (70 ± 12% to 64 ± 12%, $p < .001$). The lowest D2/3R occupancy associated with clinical stability was 50%. Extrapyramidal symptoms were more likely with D2/3R occupancies higher than 60%. The baseline D2/3R occupancies were lower in patients with clinical deterioration (N = 5) than in those who remained stable (N = 29) (58 ± 15% vs. 72 ± 10%, $p = .02$). Following dose reduction, TIP-Sz increased ($p = .04$) and PANSS ($p = .02$), BPRS ($p = .03$), SAS ($p < .001$), BAS ($p = .03$), UKU ($p < .001$), and prolactin ($p < .001$) and blood antipsychotic levels ($p < .001$) all decreased.

**Conclusions:** Antipsychotic dose reduction is feasible in most stable patients with LLS. Antipsychotic dose reduction can improve extrapyramidal symptoms, hyperprolactinemia, and some symptoms through decreases in D2/3R occupancy. The striatal D2/3R occupancy threshold for antipsychotic therapeutic effects is lower (i.e. 50%) in patients with LLS than in younger patients (65%), which has a significant implication on the management of this specific and ever growing population.

**Keywords:** Schizophrenia, Dopamine, Antipsychotics, PET, aging

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## M152. Selective Estrogen Modulation Increases Dorsolateral Prefrontal Cortex Activity During Emotional Inhibition in Schizophrenia

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**Background:** People with schizophrenia show impaired response inhibition in conjunction with decreased neural activity in the dorsolateral prefrontal cortex (DLPFC). DLPFC activity during emotional response inhibition correlates positively with circulating estrogen levels in healthy females and with circulating testosterone in men with schizophrenia. Here, we tested the extent to which the selective estrogen receptor modulator (SERM) raloxifene could modify neural activity during a language-based emotional go/no-go task in men and women with schizophrenia. We also predicted that the neural response to raloxifene will vary depending on estrogen receptor alpha (ESR1) genotype in people with schizophrenia.

**Methods:** Twenty-one men and women with schizophrenia participated in a 13-week, randomized, double-blind, placebo-controlled, crossover adjunctive treatment trial of the SERM raloxifene administered orally at 120mg daily. Effects of raloxifene versus placebo on brain activity were assessed using functional magnetic resonance imaging (fMRI) during an emotional inhibition test. Functional ESR1 genotype changes within intron 1 were determined by TaqMan allelic discrimination assay.

**Results:** Relative to placebo, treatment with raloxifene increased neuronal activity in the DPLFC during inhibition of negative words in men and women with schizophrenia. The increased BOLD signal in the DLPFC was more pronounced in ESR1 genotype that predicted higher ESR1 levels in the DLPFC. A separate confirmatory Region of Interest analysis comparing 21 people with schizophrenia to 23 healthy controls demonstrated that raloxifene restores DLPFC activity to normal levels in people with schizophrenia.

**Conclusions:** Selective estrogen receptor modulation by raloxifene facilitates activation of the DLPFC during inhibition of negative emotions in men and women with schizophrenia. People with schizophrenia having a specific ESR1 genotype displayed increased DLPFC activity during inhibition of emotional words with raloxifene administration relative to those carrying the ESR1 risk genotype. These results support a role for estrogen receptor modulation of prefrontal neural activity in both men and women with schizophrenia and suggest that ESR1 genotype may be informative of treatment response to raloxifene.
**Keywords:** schizophrenia, emotional response inhibition, dorsolateral prefrontal cortex, raloxifene, selective estrogen receptor modulator
**Disclosures:** CSW has received consultation fees from Lundbeck and Roche, CSW and TWW have received support from Roche. PRS has received speaking fees from Janssen Pharmaceuticals. None of the above relationships are related to work described in the present project.

## M153. Comparison of Subjective Experiences between Patients with Schizophrenia and Bipolar Disorder Receiving Long-Acting Injectable Antipsychotics

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**Background:** Long-acting injectable antipsychotic (LAI) provides a potential solution to overcome poor adherence to medication in patients with schizophrenia and bipolar disorder. In this study, we compare the subjective experiences and clinical feature in patients receiving LAI treatment between schizophrenia and bipolar disorder.
**Methods:** 434 patients with schizophrenia and 33 patients with bipolar disorder received LAI treatment for at least 6 months regularly at Taipei City Psychiatric Center were administered with Chinese Health Questionnaire (CHQ), World Health Organization's Quality of Life (WHOQOL), Visual Analogue Scale (VAS) from 0 (no pain ever) to 100 (worst pain) of injection pain, Personal and Social Performance scale (PSP), Clinical Global Impression of Severity (CGI-S) and Drug-Induced EPS Scale (DIEPSS).
**Results:** The Frequency of hospitalization (times/year) were significantly decreased after LAI treatment in schizophrenia ($0.26 \pm 0.42$ to $0.09 \pm 0.24$) and bipolar disorder ($0.56 \pm 0.67$ to $0.15 \pm 0.39$). There was no difference in CHQ, WHOQOL, CGI-S and DIEPSS between the two groups, while the bipolar patients had slightly higher PSP scores ($74.6 \pm 5.8$ vs $71.0 \pm 5.9$).
**Conclusions:** LAI treatment can efficaciously prevent the relapse of psychotic or mood episodes in patients with poor adherence to medications. The subjective experience and quality of life were almost the same in patients with schizophrenia and bipolar disorder receiving LAI treatment.
**Keywords:** adherence, depot, schizophrenia, bipolar disorder
**Disclosures:** Nothing to disclose.

## M154. Effects of Extended Cannabis Abstinence on Clinical and Cognitive Symptoms in Cannabis Dependent Patients with Schizophrenia and Non-Psychiatric Controls

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**Background:** Chronic cannabis use occurs at high rates in patients with schizophrenia, especially among males. The principal psychoactive ingredient of cannabis—Δ9-tetrahydrocannabinol (THC)— acts on CB1 receptors. These receptors are differentially distributed throughout the brain with the highest receptor densities found in the basal ganglia, hippocampus, cingulate cortex and the cerebellum. While cannabis has been demonstrated to have a negative effect on the clinical presentation of schizophrenia, evidence for associations between cannabis and cognition is mixed. Given that most studies have employed cross-sectional designs to examine these relationships, we sought to investigate the effects of cannabis using a prospective longitudinal laboratory model. Therefore we examined the effects of a 28-day period of cannabis on clinical and cognitive outcomes in cannabis dependent male outpatients with schizophrenia versus cannabis dependent non-psychiatric controls.
**Methods:** Seventeen cannabis dependent schizophrenia patients and 19 cannabis dependent controls underwent 28-days of cannabis abstinence supported by contingency management and weekly cognitive behavioral therapy sessions. Clinical symptoms were assessed weekly using the PANSS and depression scales (CDSS, HAM-D), while a comprehensive cognitive battery was administered biweekly. Twice weekly urine assays were used to confirm abstinence, which later was tested by GC/MS to obtain quantitative cannabis metabolite level (THC-COOH).
**Results:** To date, 10/17 schizophrenia patients and 11/19 controls achieved end-point cannabis abstinence and complete elimination of cannabis by day 28 (normalized THC-COOH $< 50$ng/mg). Among patients, PANSS sub- and total scores [$F(4,60) = 1.57$, $p = 0.19$)] remained stable over the course of the 28-day abstinence period. Cognitive performance assessed by the HVLT percent retention [$F(2,18) = 6.92$, $p < 0.01$] and Grooved Pegboard Task [$F(2, 18) = 4.51$, $p = 0.03$] demonstrated improvements over time in patients; significant improvements were not observed in control subjects.
**Conclusions:** Twenty-eight days of cannabis abstinence had no effect on clinical symptomatology in cannabis dependent schizophrenia patients. In contrast, verbal memory and motor performance improved over time. Improved cognitive functioning occurred in tasks facilitated by areas that correlated with high concentrations of CB1 receptors such as the hippocampus and cerebellum, while those tasks mediated by areas of moderate concentration such as the prefrontal cortex remained unchanged. Given that no change in performance was observed in controls on any cognitive tests suggests that cannabis-dependent schizophrenia patients may have heightened sensitivity to the cognitive-impairing effects of cannabis, which supports

previous studies. Future research should investigate whether longer abstinence periods may lead to further remediation of cognitive performance as well as clinical symptom improvement in patients. Similarly, longer abstinence periods may be warranted to initiate cognitive change among cannabis dependent controls.

**Keywords:** Cannabis Dependence, Cognition, Schizophrenia

**Disclosures:** T. George reports that in the past 12 months he has been a consultant to Pfizer on smoking cessation medications, and recipient of grant support for multi-center and investigator-initiated studies from Pfizer, as well as a member of a Data Monitoring Committee (DMC) for Novartis. The authors alone are responsible for the content and writing of this paper. The other authors have no disclosures to report. Supported by a CIHR Doctoral Fellowship to Ms. Rabin, a Brain and Behavior Research/ NARSAD Young Investigator Award to Dr. Barr and CIHR grant MOP#1151145 to Dr. George.

### M155. Reduced Rostrolateral Prefrontal Cortex Activity Associated with Exploration in Schizophrenia

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**Background:** Motivational deficits are a key component of the negative symptoms of schizophrenia and are viewed as a reduced tendency to engage in goal-directed behavior. In laboratory studies of motivation in schizophrenia (SZ), goal-directed behavior is usually assessed using tasks requiring participants to learn about and exploit favorable reward contingencies. A second type of goal-directed behavior is the tendency to explore the environment when reward contingencies are uncertain. Basic neuroscience research has linked uncertainty-driven exploration to the function of rostrolateral prefrontal cortex (RLPFC; 1). Behavioral results from our group (2) indicate that, in people with SZ, exploration is less tied to uncertainty about reward contingencies than it is in healthy volunteers. Furthermore, the degree to which exploration is driven by uncertainty in people with SZ has been shown to correlate with the severity of motivational deficits. Using functional MRI, our goal was to investigate whether uncertainty-related RLPFC activity is attenuated in schizophrenia.

**Methods:** We acquired event-related MRI data (81 2-mm axial slices; 128 x 128 matrix; FOV = 22 x 22 cm; TR = 2 s; TE = 30 ms; FA = 90°) from 27 participants with SZ and 30 controls performing a decision-making task (the Temporal Utility Integration Task, or TUIT). The TUIT required participants to decide WHEN to respond, in order to maximize reward receipt, as a hand took 5 seconds to revolve around a clock face. Task conditions were designed such that expected value (probability × magnitude) increased, decreased, or remained constant with increasing response times. Computational analyses were applied to generate trial-by-trial estimates of expected value, certainty about value, and the degree to which each choice was exploratory. These analyses were also used to estimate parameters for each individual corresponding to learning rates associated with both positive and negative prediction errors (alphaG and alphaN), and the degree to which decisions to explore or exploit tracked relative uncertainty (epsilon). Participants were classified as "uncertainty-driven explorers" or not, depending on whether their epsilon parameter estimate was positive or not. Following standard preprocessing of data, functional datasets for individual subjects were submitted to general linear models using AFNI (Cox, 1996). Trial-by-trial parameter estimates from computational modeling were used to construct amplitude-modulated regressor functions for modeling fMRI data. For group analyses, we performed whole-brain analyses using t-tests and multivariate models (the AFNI 3dMVM function; Chen et al., 2014). Whole-brain analyses were corrected for multiple comparisons with cluster size thresholds, determined with Monte Carlo simulations to provide an overall false-positive rate of 0.05.

**Results:** Analyses of fMRI data revealed that participants with SZ classified as uncertainty-driven explorers showed significantly reduced RLPFC activity associated with uncertainty processing, relative to the group of healthy volunteers classified as uncertainty-driven explorers. The entire sample of participants with SZ showed reduced uncertainty-related activity in superior parietal lobule – another brain region associated with exploratory decision making – relative to the entire sample of healthy volunteers. Consistent with our previous results, we found that the 14 participants with SZ classified as uncertainty-driven explorers showed a strong trend toward less severe motivational deficits than the 13 participants with SZ not classified as uncertainty-driven explorers ($p = 0.061$).

**Conclusions:** These results suggest that motivational deficits in schizophrenia are associated with a specific type of goal-directed behavior: the tendency to explore reward contingencies based on uncertainty. Furthermore, people with schizophrenia show aberrant activity in the neural circuitry associated with the performance of uncertainty-driven exploration. These findings suggest an alternate source of motivation deficits in schizophrenia, aside from a reduced tendency to exploit known reward contingency. In addition they link a specific deficit to abnormal activity in a specific circuit, which might serve as a biomarker for future studies of candidate risk genes or potential interventions.

**Keywords:** Reinforcement learning, motivation, Negative Symptoms

**Disclosures:** Nothing to disclose.

**References:** 1. Badre, D., Doll, B.B., Long, N.M., Frank, M.J. (2012). Rostrolateral prefrontal cortex and individual differences in uncertainty-driven exploration. Neuron, 73, 595-607.

2. Strauss, G.P.*, Frank, M.F.*, Waltz, J.A., Kasanova, Z., Herbener, E.S., Gold, J.M. (2011). Deficits in Positive Reinforcement Learning and Uncertainty-Driven Exploration are Associated with Distinct Aspects of Negative Symptoms in Schizophrenia. Biological Psychiatry, 69, 424-431 (*authors contributed equally to this work).

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### M156. Metabolic Safety of Cariprazine in Patients with Schizophrenia

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**Background:** The metabolic safety of antipsychotics is an important concern since weight gain, glucose dysregulation, and lipid abnormalities have been associated with some of these medications. Cariprazine is a potent dopamine D3 and D2 receptor partial agonist with preferential binding to D3 receptors. Cariprazine has been evaluated in short- and long-term schizophrenia trials. A pooled analysis of safety data from these studies was conducted to evaluate the effects of cariprazine on metabolic parameters in patients with schizophrenia.
**Methods:** Data were pooled from four 6-week Phase II/III studies and two 48-week open-label studies. Patients in these short- and long-term studies received cariprazine 1.5 to 12 mg/d (fixed or flexible doses) or cariprazine 1.5 to 9 mg/d (flexible), respectively; cariprazine dosages were pooled in this analysis. Assessments included mean change from baseline in metabolic parameters and the percentage of patients with potentially clinically significant (PCS) postbaseline values for weight (PCS, $\geq$7% from baseline), total cholesterol (1.3 x upper limit of normal [ULN]), triglycerides (1.2 x ULN), and glucose (1.2 x ULN). Incidence of metabolic syndrome before and after long-term cariprazine was determined using National Cholesterol Education Program criteria as a guideline.
**Results:** In the short-term studies, mean change from baseline in weight was greater for cariprazine vs placebo (1.1 vs 0.3 kg); changes in other metabolic parameters were generally small and similar for cariprazine and placebo: total cholesterol (-2.5 vs 2.0 mg/dL), triglycerides (-1.1 vs 2.6 mg/dL), and glucose (4.7 vs 4.9 mg/dL). The percentage of patients with PCS weight increase was higher with cariprazine than placebo (9.2% vs 4.7%); shifts to PCS values for other metabolic parameters were similar between groups (total cholesterol, 4.0% vs 5.1%; triglycerides, 5.2% vs 5.1%; glucose, 4.6% vs 5.9%).
Mean change in weight and metabolic parameters following long-term cariprazine treatment was comparable to the acute studies. The percentage of patients meeting PCS postbaseline criteria for metabolic parameters (weight, 27.2%; total cholesterol, 6.7%; triglycerides, 12.3%; and glucose, 12.7%) was higher in the 48-week studies than in the 6-week studies; however, incidence of metabolic syndrome after treatment (12.8%) was similar to baseline (14.4%).
**Conclusions:** In patients with schizophrenia who received 6 weeks of cariprazine, mean changes in cholesterol, triglycerides, and glucose were generally small and similar to placebo; mean change in weight was slightly higher with cariprazine relative to placebo. Comparable mean changes were found in patients after 48 weeks of treatment though incidence of PCS postbaseline values were higher relative to the short-term studies. Cariprazine was not associated with increased incidence of metabolic syndrome.
**Keywords:** cariprazine, schizophrenia, metabolic safety

**Disclosures:** Supported by funding from Forest Laboratories, LLC, an affiliate of Actavis, Inc., and Gedeon Richter Plc. I, Suresh K. Durgam, am an employee and shock shareholder of Forest Research Institute.

### M157. Development of AUT00206, a Novel and Selective Kv3 Channel Modulator for the Treatment of Schizophrenia

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**Background:** Antipsychotic drugs are the mainstay of treatment for patients diagnosed with schizophrenia. However, while psychotic symptoms are reasonably well controlled by antipsychotic medication, negative symptoms and cognitive deficits remain an unmet clinical need. Accumulating evidence supports glutamatergic dysfunction in the pathophysiology of schizophrenia, leading to disinhibition of cortical circuitry, dysregulation of gamma oscillations and reductions in the calcium binding protein parvalbumin (PV), located on fast spiking GABAergic interneurons. The voltage gated potassium channel Kv3.1 is co-localised on PV interneurons, and is closely involved in this brain circuitry and found to be reduced in unmedicated patients... Modulation of the Kv3.1 channel may therefore provide a novel target for restoration of function in schizophrenia patients. The aim of this project was to identify selective modulators of the Kv3.1 channel, assess their efficacy for negative symptoms and cognitive deficits in animal models and validate their mechanism of action.
**Methods:** Cohorts of adult female Lister-Hooded (LH) rats received PCP (2 mg/kg, sub-chronic-scPCP) or saline i.p. for 7 days, followed by 7 days washout. Rats were then tested for cognitive and social behaviour deficits following the lead Kv3.1 channel modulator, AUT00206, at 10-60 mg/kg ip or po or vehicle. AUT00206 was given acutely 30-60 min prior to testing or once daily for 21 days. Effects of AUT00206 on PV interneurons and Kv3.1 channel expression were examined using immunohistochemistry on brain free-floating sections. Prefrontal cortical slices of the prelimbic and infralimbic regions were prepared from one cohort of these rats and effects of AUT00206 at 10 and 20uM to modulate kainate-induced fast (20-80 Hz) network oscillations in vitro were examined. Effects of AUT00206 at 10uM on gamma oscillations in human temporal neocortex slices were also investigated. Male Sprague-Dawley rats were pre-treated with AUT00206 at 10 or 60 mg/kg or vehicle (ip) and imaged in a 7T magnet with pharmacological challenge fMRI (phMRI) before and after in-magnet administration of 30 mg/kg ketamine sc.
**Results:** In acute studies, AUT00206 (10-60 mg/kg) restored cognitive and social behaviour deficits induced by scPCP in female LH rats. Specifically AUT00206 significantly reversed the scPCP-induced deficit in recognition memory and reversal learning at all doses ($P<0.05$-$P<0.001$). In a chronic study, AUT00206 treatment for 21 consecutive days consistently reversed the scPCP-induced recognition memory deficit when

tested on days 1, 7, 14 and 21 ($P<0.01$); this effect was not sustained following drug washout. 21 days treatment with AUT00206 was also accompanied by a reversal of the scPCP-induced reduction in PV interneuron density in hippocampus and infralimbic cortex ($P<0.05$-$P<0.01$). Kv3.1 channel-positive cell density was significantly reduced in the prefrontal cortex ($P<0.05$) in the AUT00206 group only. Using immunofluorescence analysis, we verified that Kv3.1b channels were co-localised on PV interneurons in rat brain control tissues. Eighty to 90% co-localisation was measured in the hippocampus and prefrontal cortex, respectively. In vitro, AUT00206 at 10 and 20 uM significantly enhanced the power of fast network oscillations in both prelimbic and infralimbic cortex slices from scPCP treated rats ($P<0.05$), but caused a small, but significant ($P<0.05$) decrease in oscillations in slices taken from vehicle treated animals. At 10uM AUT00206 enhanced gamma oscillations in human temporal neocortical slices exposed acutely to PCP. Finally, in rats, in vivo phMRI showed that AUT00206 significantly reduced ketamine-induced BOLD signal changes in cortical and sub-cortical regions of the brain. Blood pressure, blood gases and other plasma related biological markers and electrolytes were unaffected by the drug.

**Conclusions:** AUT00206 improved cognitive and social behaviour deficits in an animal model of schizophrenia symptomatology. Cognitive enhancement was sustained over 21 days' treatment and accompanied by a reversal of the PV interneuron deficit. AUT00206 reversed BOLD activation induced by ketamine and enhanced gamma oscillations in cortical slices from scPCP treated rats and from humans. The modulation of Kv3 channels on PV neurons by AUT00206 could thus be an important novel approach for improving symptoms and function in schizophrenia patients and is an exciting new development in the treatment of this currently poorly managed illness.

**Keywords:** schizophrenia, Animal Models, parvalbumin interneurons, Antipsychotic, ion channel

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Declaration of interests: Steve Williams has received research grants from pharmaceutical companies researching antipsychotic drugs. Jo Neill and Bill Deakin have received expenses to attend conferences and honoraria for lecturing, consulting and attending advisory boards from the manufacturers of antipsychotic drugs; Frank Tarazi has received research grants from Lundbeck and Shire. Charles Large and Giuseppe Alvaro are full time employees and share-holders in Autifony.

## M158. Efficacy and Safety of the Glycine Transporter Type-1 Inhibitor AMG 747 for the Treatment of Negative Symptoms Associated with Schizophrenia

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**Background:** AMG 747 is a nanomolar potent, orally bioavailable inhibitor of the glycine transporter type-1 (GlyT1), with pharmacokinetic properties that enable once daily dosing. AMG 747 was evaluated in 2 phase 2 studies of near-identical design ($N = 270$ each) as an add-on to antipsychotic therapy in clinically stable participants with schizophrenia with enduring negative symptoms.

**Methods:** These international, randomized, double-blind studies (NCT01568216 and NCT01568229) enrolled participants with a Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision diagnosis of schizophrenia who were stabilized on antipsychotic medication, with a PANSS negative symptom factor score (PANSS NSFS) $\geq$ 20 and a PANSS positive symptom factor score (PANSS PSFS) $\leq$ 30. After screening and a 2-week placebo lead-in, participants were stratified by sex and randomized 3:2:2:2 to placebo or AMG 747 5 mg, 15 mg, or 40 mg daily for up to 12 weeks. AMG 747 doses were selected based on phase 1 safety and tolerability data and predicted increases in cerebrospinal fluid (CSF) glycine levels of approximately 12%, 31%, and 76%, respectively. The primary endpoint was the change from baseline to week 12 in negative symptoms as measured by the Negative Symptom Assessment Total Score (NSA-16t). Selected secondary and exploratory endpoints included the effect of AMG 747 treatment on the PANSS NSFS, PANSS total score (PANSSt), NSA Global Score (NSA-16g), the Sheehan Disability Scale (SDS), the Quality of Life Enjoyment and Satisfaction Questionnaire-18 item version (Q-LES-Q-18), and cognition measures (Measurement and Treatment Research to Improve Cognition in Schizophrenia Consensus Cognitive Battery or CogState batteries). Selected rating scale interviews were videotaped and reviewed by a blinded third party as a continuous quality control measure; feedback from the third party reviewers was provided to the raters. The studies were pooled for efficacy and safety analyses following a prespecified analysis plan. The change from baseline at continuous endpoints was analyzed using mixed effect repeated measures including baseline values as covariates and assuming an unstructured variance-covariance matrix structure; multinomial logistic regression utilizing generalized estimating equations was used for categorical endpoints. Hypothesis testing was 2-sided; nominal p values are reported.

**Results:** When 232 (43%) subjects enrolled, enrollment was stopped for both studies after a case of Stevens-Johnson Syndrome was reported in 1 participant receiving AMG 747 40 mg. At the time enrollment was halted, 76 participants were randomized to placebo, 54 to AMG 747 5 mg, 51 to AMG 747 15 mg, and 51 to AMG 747 40 mg. Overall, 153 (66%) enrolled participants completed 12 weeks of treatment. The study populations were predominantly male (67%) and Caucasian (53%), with a mean age at baseline of $43.9 \pm 10.5$ years and mean age at first diagnosis of $27.5 \pm 9.3$ years. The disease characteristics at baseline were generally comparable among the treatment groups. The mean (standard deviation [SD]) NSA-16t score at baseline was 57.2 (9.8). The baseline mean (SD) NSFS score was 25.1 $\pm$ 3.8, and PSFS score was 19.5 $\pm$ 4.4. At week 12, the NSA-16t change from baseline showed no significant differences between groups. The PANSS NSFS showed greater change from baseline to week 12 for subjects who received AMG 747 15 mg compared with those who received placebo (mean [standard error] change $-1.8$ [0.9]; $p < .05$;

Cohen's d = 0.36). The changes in the PANSS NSFS from baseline for the 5 mg and 40 mg doses were −1.0 [0.9] and 0.7 [0.9], respectively ($p > .05$ for both). Greater improvement with the AMG 747 15 mg dose compared to placebo was also observed in the NSA-16g (−0.3; $p < .02$). Changes in the PANSSt and patient-reported outcome measures (SDS and Q-LES-Q-18) showed trends consistent with greater efficacy of AMG 747 15 mg compared with placebo ($p \leq .1$). There was no evidence of treatment effects on measures of cognition. The rate of reported adverse events was similar across all placebo and treatment groups, with no clear differences observed. There was no evidence of meaningful treatment effects on laboratory values or electrocardiograms.

**Conclusions:** Pooled results from the 2 phase 2 AMG 747 studies replicate previously reported findings of an inverted-U dose response; negative symptom efficacy was observed with doses of AMG 747 yielding approximately a 30% increase in CSF glycine levels, but not at a higher dose of investigational product. The variability in efficacy measures and the inverted-U dose response may partly account for inconsistent findings in previous studies of GlyT1 agents in people with schizophrenia. The use of GlyT1 inhibitors for the treatment of negative symptoms associated with schizophrenia should be further evaluated.

**Keywords:** Negative Symptoms, schizophrenia, NMDA glycine-site receptor

**Disclosures:** Eduardo Dunayevich: Employee and shareholder of Amgen; Robert W Buchanan: Advisory Boards: AbbVie; Amgen; EnVivo(now Forum); Janssen Pharmaceutical, Inc.; Pfizer; Roche; Takeda; Consultant: AbbVie; Amgen; Bristol-Myers Squibb; EnVivo (now Forum); Omeros; Pfizer; DSMB: Pfizer; Chao-Yin Chen: Former employee and shareholder of Amgen; Julie Dietrich:Employee and shareholder of Amgen; Hong Sun: Employee and shareholder of Amgen; Stephen Marder: Advisory Boards: Forum, Targacept, Otsuka, Roche, Takeda, Lundbeck
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## M159. Central Dopamine D2/3 Receptor Occupancy Following Dose Reduction is Predictable with Minimal Plasma Antipsychotic Concentrations: An Open-Label Clinical Trial

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**Background:** Population pharmacokinetics can predict antipsychotic blood concentrations at a given time point prior to a dosage change. Those predicted blood concentrations could be used to estimate the corresponding dopamine D2/3 receptors (D2/3R) occupancy by antipsychotics based on the tight relationship between blood and brain pharmacokinetic parameters. However, this two-step prediction has never been tested.

**Methods:** Two blood samples were collected at separate time points from 32 clinically stable outpatients with schizophrenia (DSM-IV) (mean +/-SD age: 60.1 +/-7.3 years; 9 women; 4 Africans, 2 Asians, and 26 Caucasians; 20 receiving olanzapine and 12 receiving risperidone) to measure plasma concentrations of olanzapine or risperidone at baseline. Then, subjects underwent a dose reduction of olanzapine or risperidone and completed a [11C]-raclopride positron emission tomography scan to measure D2/3R occupancy in the putamen. The plasma concentration at the time of the scan was predicted with the two samples based on population pharmacokinetic model, using NONMEM. D2/3R occupancy was then estimated by incorporating the predicted plasma concentration in a hyperbole saturation model. Mean prediction error and mean root squared prediction error were used to assess the predictive performance of our two-step procedure. The prediction error refers to the difference between the true and predicted values, so that the mean prediction error is considered a measure of "bias". The root mean squared prediction error refers to the root of the mean squared prediction error, which is a measure of "precision". Pearson's correlation analysis was also used to examine the relationship between the observed and predicted values. A two-tailed p-value of $<0.05$ was considered statistically significant.

**Results:** Using the predicted plasma concentration of olanzapine, the mean (95% CI) prediction error and root squared prediction error (%) for the prediction of D2/3R occupancy were -1.76 (-5.11 - 1.58) and 7.21 (5.05 - 9.36), respectively. The observed and predicted D2/3R occupancy levels with olanzapine were highly correlated ($r = 0.67$, $p = 0.001$). The mean (95% CI) prediction error and root squared prediction error (%) for the prediction of D2/3R occupancy with the predicted plasma concentration of risperidone plus 9-hydroxyrisperidone, were 0.64 (-6.18 - 7.46) and 10.40 (5.95 - 14.85), respectively. There was also a significant correlation between the observed and predicted D2/3R occupancy with risperidone ($r = 0.67$, $p = 0.02$). When results from these two antipsychotic drugs were combined, the mean (95% CI) prediction error and root squared prediction error (%) for the prediction of D2/3R occupancy were -0.86 (-5.98 - 4.26) and 8.55 (5.05 - 12.04), respectively. Again, the correlation was significant between the observed and predicted D2/3R occupancy levels ($r = 0.67$, $p = 0.02$).

**Conclusions:** Central D2/3R occupancy levels can be predicted from blood drug concentrations collected prior to dosage change. Although this two-step model is subject to a small degree of error, it could be used to select oral doses aimed at achieving optimal D2/3R occupancy on an individual basis and obviate trial-and-error dose titration of antipsychotic drugs. Although these findings require replication and extension to a more diverse patient population and antipsychotics other than olanzapine and risperidone, they have important clinical implications for individualized treatment strategies in schizophrenia.

**Keywords:** schizophrenia, Antipsychotic, Dopamine (D2, D3) receptors, PET

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### M160. Bi-Phasic Effect of Ketamine on Auditory Steady-State Response in Awaking Rats

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**Background:** Electroencephalogram (EEG)-detectable event-related potentials have proven useful in detecting sensory processing deficits in patients with schizophrenia. In particular, a reduction in generation and synchronization of 40 Hz gamma oscillation in auditory steady-state response (ASSR) is observed in a wide range of disease states. This perturbation is presumably linked to NMDA receptor hypofunction, a widely-accepted marker of schizophrenia pathogenesis. To this end, ASSR dysfunction has been proposed as a potential biomarker for schizophrenic pathophysiology. However, several studies have reported controversial effects of NMDA antagonists on ASSR. In this investigation, we assessed event-related spectral perturbations (ERSP) and inter-trial coherence (ITC) in ASSR under a wide dose range of the NMDA antagonist ketamine.
**Methods:** Recording electrodes were placed on the surface of temporal auditory cortex (recording), frontal sinus (reference) and cerebellum (ground) of each rat for electrocorticographic ASSR. EEG recording and entrained click sounds stimuli (40 Hz, 80dB, 20clicks/500msec, ITI = 600msec, repeated 200times/trial) were performed by programing script on Spike2 (CED). Rats were exposed to sound stimuli 10-, 30-, 70- and 110 min after drug administration to evaluate the PK/PD relationship. Ketamine dosage was divided into two categories: low dose (0.3–3 mg/kg, sc) and high dose (10–100 mg/kg, sc including anesthesia dose). ERSP and ITC analyses were performed using EEGLAB, a MATLAB (MathWorks) toolbox.
**Results:** The recordings of vehicle-treated animals showed stable auditory steady-state response for both ERSP and ITC during 40 Hz stimuli. These stable responses persisted without marked alteration through all recording points. Low-dose ketamine showed a trend toward increased power of ERSP and ITC in a manner both dose- and time-dependent. 30 min after administration, 3 mg/kg augmented the power of ERSP significantly, while the dose only induced significant increase in ITC at 10 min. These augmented effects were attenuated with time. In high dose range of ketamine, 10 mg/kg still significantly augmented power of ERSP 30 min after injection. On the other hand, 30- and 100 mg/kg doses, which reportedly achieve (sub) anesthetic effects, showed rapid, significant reduction of ERSP power 10- and 30 min after administration. 100 mg/kg ketamine also significantly decreased ITC at the same time points. Curiously, animals treated with 30 mg/kg ketamine showed significant rebound effects on both ERSP and ITC when evaluated 70 and 110 min after administration.
**Conclusions:** Our results suggest that NMDA antagonists have bi-phasic effects on gamma-synchronous activity in ASSR. This may be attributed to regulation of E/I balance between glutamatergic and GABAergic neurons and/or reflect differential effects induced by NMDA antagonists in various pharmacological, behavioral, and neurophysiological studies.
**Keywords:** ketamine, ASSR, ERPs, electroencephalography, NMDA Antagonists
**Disclosures:** Astellas Pharma Inc., Astellas Research Technologies Co., Ltd.

### M161. Overexpression of a Schizophrenia-Associated Missense Mutation in Kalirin-9 in Primary Neuronal Culture

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**Background:** Kalirin (Kal) is a Rho GEF that is highly involved in regulation of the actin cytoskeleton within dendritic spines. There are several isoforms of the protein that arise from differential splicing of kalirin's 66 exons. A missense mutation (P2255T, PTKAL9) located within a region shared by two of the longer Kal isoforms has been shown to be associated with schizophrenia. We sought to determine the biological effects of this mutation on dendritic spine morphology and synaptic structure in mature neurons.
**Methods:** We transfected rat embryonic hippocampal neurons to test the effects of wildtype and PTKAL9 overexpression on excitatory synapse formation and dendritic spine morphology. Cells were transfected with either GFP-only vector, GFP/wildtype myc-KAL9, or GFP/myc-PTKAL9 at DIV 8 and fixed at DIV14. They were triple labeled for myc, PSD95, and synapsin1. Overexpression of KAL was quantified based on myc label intensity. Spines were characterized into various subtypes based on morphological features. Mature synapses were defined as areas of overlap between GFP/PSD95/synapsin1. A total of 2 coverslips with 3 neurons per coverslip and 2 dendritic segments per neuron were evaluated for each condition.
**Results:** There was no significant difference in relative overexpression of wildtype KAL9 and PTKAL9 ($p = .423$). The density of mature synapses in PTKAL9 overexpressing hippocampal neurons was significantly decreased compared to that of KAL9 wildtype overexpressing neurons ($p = 0.004$). There was a trend reduction in density of PSD95 positive puncta in PTKAL9 vs wildtype KAL9 ($p < 0.1$). There was no significant change in the size of PSD95 positive puncta between groups. Analysis of spine morphology and extension of these findings to cortical neurons is ongoing.

**Conclusions:** Overexpression of the PTKAL9 mutation appears to decrease the number of established excitatory synapses in mature hippocampal neurons compared to wildtype KAL9 overexpression without impacting PSD size, though the mechanism through which this occurs remains unknown. Further studies are required to determine which aspects of spine dynamics and which signaling pathways may be altered by this mutation, thus providing potential sites for therapeutic intervention to alleviate disease burden of schizophrenia.
**Keywords:** Kalirin, Dendritic Spine, schizophrenia
**Disclosures:** Nothing to disclose.

### M162. Adolescent Suppression of Prefrontal Nicotinic Signaling Shapes Attentional Function

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**Background:** Attention is a major cognitive function impaired in various neurodevelopmental disorders and psychiatric illnesses, but the developmental mechanism essential to shape attentional function is poorly understood. In visual cortex, a well characterized model of developmental critical period, adolescent suppression of nicotinic acetylcholine receptors (nAChRs) by an increased expression of an endogenous nAChR inhibitor Lynx1 is essential to complete cortical maturation (Morishita et al Science 2010). Given that the nAChR system is also implicated in attention and neurodevelopmental disorders, we tested a hypothesis that Lynx1 plays a key role in establishing frontal cortex-dependent attentional function.
**Methods:** By employing a 5-choice serial reaction time task on an automated touchscreen system, we combined genetic, viral, pharmacological, and histological approaches to investigate when and where in the brain Lynx1 exerts its effects to establish attention in mice.
**Results:** We found that Lynx1 knock-out mice displayed attention deficits in adulthood. This functional deficit was associated with reduced task-dependent-activation of anterior cingulate cortex (ACC) neurons that increasingly express Lynx1 during the peri-adolescence period and throughout adulthood. Viral knockdown of Lynx1 in the ACC from peri-adolescence into adulthood phenocopied the impairment. Strikingly, this attention deficit was rescued by a chronic 10 day pharmacologic nAChR blockade both during peri-adolescent and adult periods –but not by acute blockades during attention testing.
**Conclusions:** These data suggest that, in the absence of Lynx1, excess nAChR signaling across adolescent development may "freeze" attentional circuit maturation, rendering immature cortical circuits that underlie aberrant frontal cortex activity and long-lasting impairment in attention. Developmental regulation of attentional function by Lynx1 may prove a novel mechanism and therapeutic target for a major cognitive function disturbed in psychiatric disorders characterized by disrupted nAChR signaling including autism, ADHD, and schizophrenia.
**Keywords:** Attention, Adolescence, nicotinic acetylcholine receptors, prefrontal cortex, mouse behavior
**Disclosures:** Hiroyuki Koike is an employee of Taisho Pharmaceutical Co., Ltd.

### M163. Reduced Amplitude Low-Frequency BOLD Signal Oscillations in Early Illness Schizophrenia Patients and Individuals at Clinical High Risk for Psychosis

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**Background:** Low-frequency oscillations (LFO) of the blood oxygen level-dependent (BOLD) signal assessed with resting state functional magnetic resonance imaging (FMRI) are gaining interest as potential biomarkers sensitive to neuropsychiatric pathology. Schizophrenia has previously been associated with intrinsic LFO activity alterations that covary with cognitive deficits and symptoms. However, the extent to which LFO activity dysfunction is present prior to schizophrenia illness onset remains unknown. Accordingly, this study examined the amplitude of resting LFO activity in youth at clinical high-risk (CHR) for psychosis, relative to healthy controls (HC) and early illness schizophrenia patients (ESZ).
**Methods:** Resting-state FMRI data were collected from CHR ($n = 59$), ESZ ($n = 74$), and HC ($n = 85$) adolescents and young adults, ages 12-35. Age-adjusted voxelwise fractional amplitude of low frequency fluctuations (fALFF) within the .01 to .08 Hz frequency band of the BOLD signal was compared between the three groups. Main effects of group ($p < .005$ height threshold, family-wise error cluster-level corrected $p < .05$) were followed up via Tukey-corrected pairwise comparisons.
**Results:** Significant main effects of group (height threshold, $p < .005$; cluster $p \leq .05$) revealed decreased fALFF in ESZ and CHR groups relative to HC, with values in the CHR group falling between those of ESZ and HC groups. These differences were identified primarily in posterior cortex, including temporoparietal regions, extending into occipital and cerebellar lobes. Furthermore, lower LFO activity was related to higher symptom severity in CHR and ESZ groups (height threshold, $p < .005$; cluster $p \leq .05$).
**Conclusions:** These data support an intermediate phenotype of reduced posterior cortical LFO amplitude in CHR individuals, with resting fALFF values smaller than in HC but higher than in ESZ patients. Findings indicate that LFO activity alterations, measured by fALFF, predate psychosis onset but are more pronounced in the early stages of schizophrenia. Furthermore, these LFO abnormalities in both CHR and ESZ groups are related to clinical symptoms.
**Keywords:** schizophrenia prodrome, ultra-high-risk youth, amplitude of low frequency fluctuation, first episode schizophrenia, fMRI resting state
**Disclosures:** Dr. Mathalon has received compensation as a consultant from Bristol-Myers Squibb, Amgen, and Hoffmann-La Roche.

### M164. Altered Intrinsic Prefrontal Activity and Connectivity is Associated with Impaired Cognitive Abilities in Patients with Schizophrenia

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**Background:** Cognitive dysfunction is considered a core feature of schizophrenia, which often precedes the onset of psychosis, is stable over the course of the illness, and is a key determinant of the long-term morbidity and mortality of the patients affected by this disorder. Among cognitive abnormalities, impaired performances in episodic memory (EM) and executive function (EF) tasks are consistently reported in schizophrenia patients. Traditional functional Magnetic Resonance Imaging (fMRI) and electroencephalogram (EEG) studies have helped identifying the brain areas involved in such tasks, such as the prefrontal cortex (PFC). However, it is difficult to establish whether intrinsic defects in the neuronal activity and connectivity of those areas contribute to reduced cognitive performance in schizophrenia patients, given the presence of confounds like reduced motivation, fluctuation in the level of attention, and occurrence of psychotic symptoms. TMS with simultaneous high density (hd)-EEG allows directly and non-invasively perturbing a cortical region while recording TMS-evoked local and long-range brain responses. Furthermore, TMS/hd-EEG measurements can be obtained without any cognitive effort from the patients, and TMS-evoked neuronal activity and connectivity can be assessed and measured in virtually any cortical area.
**Methods:** In this study we performed TMS/hd-EEG recordings in four cortical areas, parietal, motor, premotor, and prefrontal cortex in healthy individuals (N = 20) and patients with schizophrenia (N = 20). Source modeling analysis of TMS-evoked brain responses was also calculated, and two synthetic indices of cortical activity (significant current density, SCD) and connectivity (significant current scattering, SCS) were computed for both groups. Schizophrenics also performed two episodic memory/executive function (EM/EF) tasks, the Computerized Penn Word memory test delayed (CPWd) and the Penn Conditional Exclusion Test (PCET). The CPWd evaluates WM and involves primarily PFC, whereas the PCET provides a measure of EF and implicates PFC as well as other cortical and subcortical regions, including the thalami, which are interconnected with PFC.
**Results:** We found no difference in SCD and SCS values after TMS of parietal and motor cortex between the two groups, whereas those parameters were significantly reduced in both premotor and prefrontal areas of schizophrenia patients compared to healthy controls. Furthermore, in the prefrontal cortex, where those indices were most defective, SCD predicted performance in the CPWd task whereas SCS was associated with shorter reaction time and more errors in the PCET.
**Conclusions:** Altogether, those findings indicate that schizophrenia patients have intrinsic defects in both the activity and connectivity of anterior frontal areas, and especially in PFC. Furthermore, these intrinsic prefrontal defects are associated with impairments in cognitive abilities known to implicate PFC and commonly found to be reduced in patients with schizophrenia.
**Keywords:** schizophrenia, prefrontal cortex, TMS, EEG, Source Localization
**Disclosures:** Nothing to disclose.

### M165. A Unique Dual Cortico-Striatal Action of a Beta-Arrestin Biased Dopamine D2 Receptor Ligand

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**Background:** β-arrestin2 (βarr2) signaling at D2 receptors (D2Rs) plays an important role in antipsychotic responses, allowing development of signaling biased therapies. In preclinical studies βarr2 biased D2R ligands behave as efficacious antipsychotic compounds. The dopamine (DA) hypothesis of schizophrenia postulates hypodopaminergia in the prefrontal cortex (PFC) and hyperdopaminergia in the striatum. Current antipsychotics effectively reverse excess striatal activity, but do not fully reverse cortical deficits. Using cell-specific βarr2KO mice and β-arrestin biased ligands we address this problem here.
**Methods:** To achieve cell type-specific deletion of βarr2 we crossed βarr2 floxed mice to D1R, D2R or A2aR CRE mice. We then tested the ability of clinically effective antipsychotics haloperidol (HAL), clozapine (CLOZ), aripiprazole (ARI) and the β-arrestin-biased D2R ligands UNC9994A (94A) and UNC9975A (75A) to inhibit psychostimulant-induced hyperlocomotion in these neuron-specific βarr2KO mice. We employed in vitro GPCR signaling assays to test ARI, 94A and 75A for their antagonist/partial agonist activity at D2Rs.
**Results:** Deletion of βarr2 in striatal D2R + (A2aCRE) or all D2R + (D2CRE) but not D1R + (D1CRE) neurons causes β-arrestin-biased D2R ligand 94A but not 75A to lose its antipsychotic activity against amphetamine. However other antipsychotics tested (HAL, CLOZ and ARI) were still effective in all βarr2KO mouse lines. Interestingly, unlike AMPH, when tested against phencyclidine (PCP), 94A lost its antipsychotic activity only in D2R + (D2CRE) but not striatal D2R + (A2aCRE) or D1R + (D1CRE) βarr2KO mice suggesting a role for cortical βarr2 in this effect. Upon western blot analyses we observed higher expression of βarr2 and GRK2 in the PFC compared to the striatum. In vitro signaling assays revealed that upon over-expression of GPCR Kinase2 (GRK2) - ARI and 94A but not 75A have partial agonist activity at βarr2 recruitment at the D2R. However, with endogenous expression levels of GRK2 - ARI, 75A and 94A antagonize βarr2 recruitment to the D2R but that only ARI and 75A antagonize Gi mediated D2R signaling.
**Conclusions:** Using neuron-specific βarr2KO mice and the β-arrestin-biased D2R ligand 94A, we show that βarr2 antagonism in striatal D2R + neurons is sufficient for antipsychotic activity against amphetamine. However, for antipsychotic activity against phencyclidine, 94A displayed a unique regional selectivity, suggesting a role for PFC D2R/

βarr2 agonism. The switch of 94A from antagonism to agonism is due to higher PFC expression of βarr2 and GRK2 compared to striatum. Therefore, unlike current antipsychotics, β-arrestin-biased D2R ligands that behave as agonists in the cortex but antagonists in the striatum may be sufficient for clinical antipsychotic efficacy, with a superior ability to correct cortical hypodopaminergia. Such a mechanism would allow for the amelioration of not only psychosis but also cognitive and negative symptoms observed in schizophrenia.
**Keywords:** Dopamine, beta arrestin, Schizophrenia, Dopamine, Antipsychotics, Functional Selectivity, prefrontal cortex
**Disclosures:** Nothing to disclose.

### M166. Effects of Endurance Training in Combination with Cognitive Remediation in Multi-Episode Schizophrenia and Healthy Controls

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**Background:** The objective of this longitudinal magnetic resonance (MR) imaging study was to examine the effects of endurance training in combination with cognitive remediation on the symptomatology, function and neurobiological variables in schizophrenia patients and healthy controls. 20 multi-episode patients with schizophrenia and 21 age- and gender-matched healthy controls underwent 3 months of endurance training (30 min, 3 times per week). 19 additionally recruited schizophrenia patients played table soccer (''football'' in the USA) over the same period.
**Methods:** MR imaging with 3D-volumetric T1-weighted sequences was performed on a 3T MR scanner at baseline, after 6 weeks and after the 3-month intervention and 3 additional training-free months. In addition to voxel-based morphometry (VBM), we performed manual and automatic delineation of the hippocampus and its substructures. Endurance capacity, psychopathological symptoms and neuropsychological as well as functional variables were measured as secondary endpoints.
**Results:** No significant increases in the volumes of the hippocampus or hippocampal substructures were observed in schizophrenia patients or healthy controls. However, VBM analyses displayed an increased volume of the left superior, middle and inferior anterior temporal gyri compared to baseline in schizophrenia patients after the endurance training, whereas patients playing table soccer showed increased volumes in the motor and anterior cingulate cortices. In addition, patients forming the endurance training combined with cognitive remediation showed a highly significant improvement of their functions over a period of 3 months, measured by the GAF and SAS.
**Conclusions:** In summary, this second study of our group showed that endurance training, possibly boosted by cognitive remediation, demonstrates a plasticity effect on the brain in multi-episode schizophrenia and furthermore, helps to improve the functional outcome in this severe mental disorder.
**Keywords:** endurance training, cognitive remediation, multi-episode schizophrenia
**Disclosures:** Nothing to disclose.

### M167. Mortality and Cumulative Exposure to Antipsychotics, Antidepressants and Benzodiazepines: An Observational Follow-Up Study

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**Background:** Mortality related to psychotropic medications has gained much attention. However, there are very little data on the risk of death and cumulative antipsychotic load, and nothing is known about mortality versus cumulative exposure to antidepressants or benzodiazepines.
**Methods:** We identified all individuals with schizophrenia diagnosis (N = 21,492) aged 16 to 65 years, in Sweden by using prospectively collected nation-wide databases, and calculated all-cause and cause-specific mortality as function of cumulative low (< 0.5 DDD/day), moderate (0.5–1.5 DDD/day), and high (> 1.5 DDD/day) antipsychotic, antidepressant and benzodiazepine exposures from January 2006 to December 2010.
**Results:** When compared with no exposure, both moderate (adjusted HR 0.59, 95%CI 0.49–0.70) and high (0.75; 0.63–0.89) antipsychotic doses were associated with substantially lower overall mortality. Moderate antidepressant use was associated with a lower mortality (0.85, 0.73-0.98), and the risk of death was even lower for high dose (0.71, 0.59–0.86). Exposure to benzodiazepines showed a dose response for increased mortality (HR up to 1.74, 1.50–2.03 for high exposure). In a sensitivity analysis among first episode patients, the highest risk was observed for high-dose benzodiazepine use, with almost 4-fold mortality compared to the majority of patients with no benzodiazepine use.
**Conclusions:** Moderate and high dose antipsychotic and antidepressant use were associated with about 15–40% lower overall mortality, whereas high-dose, chronic use of benzodiazepines was associated with up to a 70% higher risk of death when compared to no exposure. Since patients with anxiety and depressive symptoms may have a higher intrinsic risk of death, the finding for benzodiazepines may be attributable in some extent to residual confounding. On the contrary, that implies a robust causal relationship between antidepressant use and decreased mortality. Presently, and with scant scientific data, adverse events of antipsychotics are considered the main issue in excess mortality, but it appears that the lack of antipsychotic usage is associated with the highest overall and cardiovascular mortalities. It is important to realize that although monitoring of patients with moderate or high dose antipsychotic treatment is relevant, it is essential to focus the preventive interventions on those patients who have even higher risk of death, i.e. patients not using antipsychotics and patients using high doses of benzodiazepines. The results of the present study suggest that these characteristics of patients are useful markers for high risk of death. Irrespective of the causal mechanisms, these patient groups should receive close monitoring and active treatment of their physical and mental health, suggesting a radical paradigm shift in the treatment of schizophrenia.
**Keywords:** schizophrenia, Antipsychotic, Antidepressant, benzodiazepine

**Disclosures:** Dr. Tiihonen has served as a consultant to Lundbeck, Organon, Janssen-Cilag, Eli Lilly, AstraZeneca, F. Hoffman-La Roche, and Bristol-Myers Squibb, he has received fees for giving expert opinions to Bristol-Myers Squibb and GlaxoSmithKline and lecture fees from Janssen-Cilag, Bristol-Myers Squibb, Eli Lilly, Pfizer, Lundbeck, GlaxoSmithKline, AstraZeneca and Novartis, and he is a member on the advisory board of AstraZeneca, Janssen-Cilag, and Otsuka, and he has received a grant from the Stanley Foundation. Other authors report no financial relationships with commercial interest.

### M168. Impact of Withdrawal from Haloperidol, Clozapine, or Aripiprazole Treatment on Dopamine System Activity in MAM Rodent Model of Schizophrenia

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**Background:** Novel compounds for the treatment of schizophrenia that appear efficacious based on promising preclinical studies often fail during the clinical phase of testing. Consequently, optimizing preclinical models for the testing of pharmacotherapies is a continuing challenge. Typically, preclinical trials are performed on either naïve rats or in rodent models of schizophrenia that have never been exposed to antipsychotic drugs. The methylazoxymethanol acetate (MAM) neurodevelopmental model of schizophrenia in the rodent recapitulates many of the neurochemical, anatomical, and behavioral hallmarks of schizophrenia. Our lab has previously demonstrated with the MAM model that withdrawal from repeated haloperidol (HAL) treatment can persistently alter the state of the dopamine system to the degree that previously effective drugs are rendered ineffective. Importantly, the impact of withdrawal from repeated HAL on the dopamine system was not the same in normal and MAM rats. The present study sought to determine whether these findings could be extended to other antipsychotic drugs. The effects of withdrawal from repeated HAL were compared to similar treatment with clozapine (CLO) and aripiprazole (ARI). CLO, like HAL, is frequently prescribed in the clinical setting but is classified as atypical in its binding to both dopamine and serotonin receptors. Aripirazole (ARI) is a more recent addition to the antipsychotic arsenal and has a unique mechanism as a partial D2 receptor agonist. It was anticipated that the mechanistic differences would distinguish ARI from CLO and HAL in terms of impact on the dopamine system following a brief withdrawal from repeated treatment.
**Methods:** Saline (SAL) and MAM-treated offspring received 21 days of HAL (0.6 mg/kg, p.o), CLO (10 mg/kg, p.o.), ARI (10 mg/kg, p.o.), or vehicle (2% glacial acetic acid, p.o.) followed by 7 days of withdrawal. The number of spontaneously active DA neurons in the VTA was measured using in vivo extracellular recordings from anesthetized animals. Additional electrophysiological recordings were conducted in a separate group of SAL and MAM rats following acute treatment with ARI (10 mg.kg, p.o).
**Results:** When given acutely, ARI was effective in reducing dopamine system hyperactivity in MAM animals while having no effect in SAL animals, consistent with earlier studies utilizing HAL. After repeated treatment, compared to drug-naïve animals, withdrawn HAL- and CLO-treated SAL rats demonstrated reductions in the number of spontaneously active dopamine neurons, likely due to depolarization block. This effect was not observed following withdrawal from repeated ARI treatment. In contrast, in MAM animals, withdrawal from all three compounds caused a reduction in the number of spontaneously active dopamine neurons compared to vehicle-treated animals. Preliminary data show that the reduction in spontaneously active dopamine neurons observed following acute and repeated ARI in MAM rats was unlikely the result of depolarization block since low doses of apomorphine (40 μg/kg) failed to change the number of spontaneously active neurons after treatment.
**Conclusions:** In normal rats, these data suggest that HAL and CLO produce a similar reduction in the dopamine system activity following a brief withdrawal period after repeated treatment. Consistent with the mechanistic difference, withdrawal from ARI did not cause a reduction in dopamine activity in SAL rats. More promising was the observation of ARI-induced down-regulation of dopamine neuron firing in MAM rats without inducing depolarization block. These initial results suggest that ARI treatment may circumvent antipsychotic drug-induced supersensitivity that is proposed to interfere with novel drug action.
**Keywords:** Dopamine, Antipsychotic Treatment, Schizophrenia, Dopamine, Antipsychotics
**Disclosures:** For AAG only: Johnson & Johnson, Lundbeck, Pfizer, GSK, Merck, Takeda, Dainippon Sumitomo, Otsuka, Lilly, Roche, Asubio, Abbott.

### M169. β-Arrestin Signaling Increases Excitability of Fast-Spiking Interneurons in the Prefrontal Cortex

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**Background:** Activation of dopamine 2 receptors (D2Rs) not only leads to downstream effects through canonical, G-protein mediated signaling, but also through non-canonical β-arrestin-dependent signaling. Indeed, all clinically effective antipsychotics interact with D2Rs and signal through both canonical and non-canonical pathways. Parsing apart the effects of these signaling pathways in prefrontal and striatal circuits may shine light on the mechanisms of current antipsychotics and lead to the development of more effective ones.
**Methods:** Here, we examined the effects of second-generation antipsychotic, aripiprazole, and β-arrestin biased D2R ligand UNC9994, on fast spiking interneurons (FSIs) in the prefrontal cortex. We performed whole-cell recordings from GFP-labeled FSIs in acute slices from GAD1-eGFP mice. We injected depolarizing current steps in current-clamp mode and recorded the number of action potentials generated.
**Results:** Aripiprazole elicited an increase in excitability in prefrontal FSIs, consistent with agonist-like activity previously reported with the D2R agonist, quinpirole. Inter-

estingly, we found that UNC994 elicited a more robust increase in FSI excitability than the increase observed with aripiprazole. This effect was absent in FSIs recorded from β-arrestin2 KO mice, suggesting signaling through β-arrestin.
**Conclusions:** Parvalbumin-positive FSIs in the prefrontal cortex are thought to be dysfunctional in schizophrenia and enhancing their activity may reverse the cognitive deficits observed in this disorder. Thus, enhancing the activity of prefrontal FSIs by β-arrestin signaling offers an opportunity for novel antipsychotic drugs that also improve negative symptoms and cognitive deficits associated with schizophrenia.
**Keywords:** D2 receptor, beta arrestin, parvalbumin interneurons, electrophysiology, Schizophrenia, Dopamine, Antipsychotics
**Disclosures:** Employed by Pfizer Inc.

## M170. Improvement in Depressive Symptoms Mediates Changes in Functional Capacity in Schizophrenia: A Treatment Study

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**Background:** Depressive symptoms are common in schizophrenia, and have been linked to impairment in functioning. However, some recent studies found smaller direct relationships with everyday functioning for depression compared to other symptoms. It is possible that the impact of depression is that of a mediator of other influences and that improvement in depression could still have a beneficial impact on everyday functioning. The objective of this post-hoc analysis was to investigate the effects of improvement in depressive symptoms on performance-based measures of functional capacity in patients followed for up to 6 months after acute phase treatment.
**Methods:** Data was derived from a 6-week randomized, placebo- and active-controlled trial of lurasidone in patients with an acute exacerbation of schizophrenia, followed by a 6-month, double-blind, flexible-dose, continuation study was conducted. Depressive symptoms and functional capacity were assessed with the Montgomery-Asberg Depression Rating Scale (MADRS) and the UPSA-B, respectively. Patients in the extension phase were treated with either lurasidone or Quetiapine XR. Patients treated with placebo in double-blind study were all treated with lurasidone in the extension. Assessments were performed at baseline, 6 weeks, 12 weeks and 6 months or endpoint.
**Results:** Depressive symptoms, assessed using the MADRS, improved in the lurasidone 80 mg/d and 160 mg/d groups compared to the placebo group in the acute study at week6 (LOCF) (both $p<0.001$). Similar results were observed in the quetiapine XR 600 mg/d group ($p<0.001$). Reduction in depressive symptoms mediated an improvement in functional capacity during the initial, 6-week, randomized, trial for both lurasidone and quetiapine XR-treated patients ($p<0.001$). At month 6 of the double-blind, continuation study, the association between improved depressive symptoms and increased functional capacity was significant ($p<0.05$), for both the lurasidone (40-160 mg/d) and quetiapine XR (200-600 mg/d) groups. This relationship was not found for cognitive functioning, which was not improved by quetiapine XR at any of the assessment time points.
**Conclusions:** These findings suggest that there is a significant mediating relationship between reduction in depressive symptoms and improvement in functional capacity in patients with schizophrenia in this treatment study. These relationships were found across both acute and continuation study phases. These results are different from cross-sectional studies and suggest that longitudinal effects of treatment may be critical to consider when understanding the global picture of the determinants of everyday disability in schizophrenia. Treatment of depression in schizophrenia may have wide ranging clinical and functional benefits.
**Keywords:** Schizophrenia, Functional Capacity, Antipsychotic Treatment
**Disclosures:** Philip D. Harvey has served as a consultant to: Abbvie; Boeheringer Ingelheim, Forum Pharma; Genentech; Lundbeck Pharma; Otsuka America, Roche Pharma, Sanofi, Sunovion, and Takeda Pharma in the past 3 years. Dr. Ogasa is a full time employee of Sumitomo Dainippon Pharma. Cynthia Siu has served as a consultant to Pfizer and Sunovion Pharma. Antony Loebel is a full time employee of Sunovion Pharma.

## M171. A Candidate Gene Analysis of Startle Latency in Schizophrenia and Control Subjects: A Replication Study

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**Background:** The acoustic startle response and its modulations have been very well studied in schizophrenia. In humans the eyeblink component of the startle response is easily measured by electromyographic recording of the right orbicularis oculi muscle. Latency of the acoustic startle response is the time interval from the presentation of the startling auditory stimulus until the startle response is elicited. Latency is determined by the time required for the auditory signal to travel through the 3-synapse subcortical circuit that mediates the startle response and thereby provides an index of neural processing speed. Latency is prolonged in subjects with schizophrenia (SCZ) compared to healthy controls (CON), and is highly heritable (90%) in a sample of SCZ and CON subjects and their first-degree relatives (1). We previously reported on associations of latency with selected candidate single nucleotide polymorphisms (SNPs) (2). This study was conducted as a replication of these findings in a separate population to determine potential genetic associations with latency as a contributor to schizophrenia susceptibility and severity.
**Methods:** The subjects were 185 SCZ and 139 CON individuals. Startle testing was conducted using a computerized EMG startle response monitoring system (SR-LAB, San Diego Instruments) according to published methods

from our group (2). All subjects completed a startle paradigm designed to assess magnitude, latency, and prepulse inhibition of startle (Hasenkamp et al 2010). DNA was obtained from venous blood samples. Genotyping was performed in a 384-well format using iPlex Gold kits and the Sequenom MassARRAY system. Amplification and extension primers were designed by SpectroDESIGNER software. The MassARRAY™ typer software was used to assign the genotype calls. Candidate SNPs were selected from 12 genes associated with startle, startle latency, the kynurenine pathway, and/or SCZ in the animal or human literature. Because we had no assumptions about the mechanism of latency heritability, we examined the association between latency and each SNP under two inheritance models: additive (e.g. homozygote 1 vs. heterozygote vs. homozygote 2), and dominant (combining the minor homozygote with the heterozygote, assuming a single copy of an allele is sufficient to influence the trait). For each of these potential models, we examined association using linear regressions with covariates of age, race, and sex. Of 77 SNPs analyzed, 23 had less than 10 subjects in the minor allele group. For these SNPs we considered the additive models unreliable and therefore we proceeded with the dominant model regressions. For the other 54 SNPs we performed both additive and dominant model regressions.
**Results:** Of 77 SNPs analyzed, ten SNPs in eight genes predicted slowing of latency ($p<.05$). We found a significant association of peak latency with rs963468 (Beta = 0.18, p = 0.006), replicating the association that we had reported in a completely separate population (1). This SNP is in the DRD3 gene that codes for the dopamine D3 receptor. Evaluation of the association between rs963468 genotypes and DRD3 expression in the brain (Gene Expression Omnibus Accession Number GSE15745) revealed significant genotype-dependent differences ($p = 1.47x10\text{-}4$) in the pons. Subjects with the GG genotype had faster peak latency and lower DRD3 expression in the pons compared to those with the GA or AA genotypes.
There was a strong association of onset latency with the SNP rs901561 on the neuregulin gene (NRG1; Beta = 0.18, p = 0.003), indicating that subjects with the AA or GA genotype had slower mean latency than subjects with a GG genotype. This gene has been reported as being associated with SCZ in large GWAS studies.
Of particular interest is the association of peak latency with rs3025962 in the RELN gene (Beta = 0.14, p = 0.024), indicating that subjects with the TC genotype had slower mean latency (than subjects with a TT genotype. Mice who are deficient in this gene have decreased startle latency, and this gene has been found to associate with SCZ in human studies. Furthermore, rs3025962 is a coding SNP that changes an amino acid from threonine to alanine in the protein sequence, making this a particularly important finding deserving of further study.
**Conclusions:** Latency of the acoustic startle response, an index of neural processing speed, is a potential endophenotype for SCZ: latency is prolonged (i.e. slower) in SCZ and is highly heritable. A candidate gene approach yielded potentially promising SNPs that predicted slowing of latency, particularly rs963468 (DRD3), rs901561 (NRG1), rs3025962 (RELN) and several others. The rs963468 finding is a replication in an independent sample and is concordant with gene expression data. rs3025962 is a coding SNP and mice deficient in this gene have altered latency. Although these results obviously need replication in larger datasets, these data suggest that startle latency may be a useful biological probe for genetic contributions to the risk of schizophrenia.
(1) Hasenkamp W et al. (2010) Psychiatry Res 178(2):236-43
(2) Gensler L et al. (2013) Annual Meeting, ACNP, Hollywood, FL
Supported by the VA Merit Review Program (E.D.), NIMH (MH085806 to A.K.S) and the National Center for Advancing Translational Sciences (NIMH UL1TR000454).
**Keywords:** schizophrenia, endophenotype, acoustic startle, startle latency, genetics
**Disclosures:** Nothing to disclose.

## M172. Clozapine Treatment in Patients with Benign Neutropenia

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**Background:** It has been estimated that 35% to 40% of all patients with schizophrenia should be considered for a trial of clozapine. However, in the U.S. only four to five percent of patients with schizophrenia ever receive clozapine. While the superior and unique efficacy of clozapine has been repeatedly supported by the literature, the frequency of clozapine use remains low. There are numerous reasons why clozapine is not more widely used including limitations in some patients due to White Blood Cell Count (WBC) and Absolute Neutrophil Count (ANC) monitoring. Patients with benign neutropenia (including benign ethinic neutropenia (BEN)) are often under prescribed clozapine due to their lower ANCs that preclude use or lead to early discontinuation. The purpose of this study was to determine if clozapine can be safely utilized in a population of psychiatric patients with benign neutropenia prior to treatment with clozapine. To our knowledge, this will be the first study to evaluate the safety of clozapine in a larger group of patients with benign neutropenia and evaluate ANC before and after treatment in this population.
**Methods:** A single-center evaluation of patients with benign neutropenia and treated with clozapine using modified monitoring guidelines was conducted. All available laboratory values for ANC and clinical data before clozapine initiation and during treatment were examined. The primary endpoint was the difference in ANC after initiation of clozapine compared to before clozapine treatment.
**Results:** A total of 26 patients were reviewed. Mean age was 34 years at clozapine initiation. The majority were African-American (73%), with more males than females (73% vs. 27%). The mean lowest ANC value was not significantly different after clozapine initiation compared to before (1.5 and 1.4x103 cells/mm3, respectively; $p = 0.22$). There were no cases of agranulocytosis (ANC $<0.5$ x103 cells/mm3) and no patients were discontinued for falling below limits set by modified guidelines. There were fewer occurrences of

mild neutropenia (ANC <2.0x103 cells/mm3) after clozapine initiation than before (16% and 31.4%, respectively; $p<0.001$). There were also fewer occurrences of moderate neutropenia (ANC <1.5x103 cells/mm3) with 2.1% after clozapine and 13.3% before ($p<0.001$). Occurrence of ANC <1.0x103 cells/mm3 did not differ (0.4% before, 0.3% after, $p=0.79$) before and after treatment with clozapine.
**Conclusions:** Twenty-six patients with benign neutropenia were safely treated with clozapine. Their pre-clozapine neutropenia did not predict increased risk for agranulocytosis with clozapine. Patients had significantly fewer episodes of mild and moderate neutropenia after receiving clozapine compared to before. A large prospective clinical trial is underway to test the safety and genetic biomarkers associated with BEN in a multisite study.
**Keywords:** clozapine, benign neutropenia, schizophrenia
**Disclosures:** Nothing to disclose.

### M173. Lack of Face Selectivity for Putative Neural Marker of Face Processing in Schizophrenia: Evidence from ERP and fMRI during Face Detection

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**Background:** Face detection, an ability to identify a visual stimulus as a face, is impaired in patients with schizophrenia. It is unclear whether impaired face processing in this psychiatric disorder results from face-specific processing domains or stems from more basic visual processing domains. In this study, we examined cortical face-sensitive evoked response potential (ERP) N170 in schizophrenia, taking into account deficient basic visual processing of individual patients that may impact face processing.
**Methods:** We equalized visual signals among patients ($n=20$) and controls ($n=20$) and between face and tree images, based on their individual perceptual capacities (determined using psychophysical methods). We measured N170, a putative temporal marker of face processing, during face detection and tree detection. We compared the N170 responses with fMRI responses from Fusiform Face Area (FFA), a putative spatial neural marker, that were acquired separately but using identical task paradigms.
**Results:** In controls, N170 amplitudes were significantly greater for faces than trees across all three visual salience levels tested (perceptual threshold, two times perceptual threshold and 100%). In patients, however, N170 amplitudes did not differ between faces and trees, indicating diminished face selectivity (indexed by the differential responses to face vs. tree). Significant correlations between the face selectivity indices of N170 and fMRI responses in FFA were found in controls, but not patients.
**Conclusions:** These results provide converging evidence for a lack of face-selectivity in spatial and temporal responses of the putative brain machinery responsible for face processing in schizophrenia. This neuroimaging finding suggests that face-specific processing should be targeted during the remediation of social functioning in schizophrenia.
**Keywords:** schizophrenia, evoked response potential, fMRI, face perception, face specific processing
**Disclosures:** Nothing to disclose.

### M174. Rare Variants in the Neurotrophin Signaling Pathway Implicated in Schizophrenia Risk

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**Background:** Multiple lines of evidence corroborate impaired signaling pathways as relevant to the underpinnings of schizophrenia. There has been an interest in neurotrophins, since they are crucial mediators of neurodevelopment and in synaptic connectivity in the adult brain. Neurotrophins and their receptors demonstrate aberrant expression patterns in cortical areas for schizophrenia cases in comparison to control subjects. There is little known about the contribution of neurotrophin genes in psychiatric disorders.
**Methods:** To begin to address this issue, we conducted high-coverage targeted exome capture in a subset of neurotrophin genes in 48 comprehensively characterized cases with schizophrenia-related psychosis.
**Results:** We herein report rare missense polymorphisms and novel missense mutations in neurotrophin receptor signaling pathway genes. Furthermore, we observed that several genes have a higher propensity to harbor missense coding variants than others.
**Conclusions:** Based on this initial analysis we suggest that rare variants and missense mutations in neurotrophin genes might contribute to the etiology across psychiatric disorders.
**Keywords:** Brain-Derived Neurotrophic Factor, schizophrenia, genetics, psychosis, de novo mutation
**Disclosures:** Nothing to disclose.

### M175. Glutamate, Calcium-Channel and Dopamine Genes and Brain Glutamate in Schizophrenia: A Proton Spectroscopic Imaging and Genetics Study

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**Background:** Neuroimaging and genetic studies have identified dopamine and glutamate as key neurotransmitter systems involved in schizophrenia. PET radioligand studies have found increased striatal DA release and H-MRS investigations report elevated glutamate in striatum and medial frontal cortex. The large PGS consortium reported associations with schizophrenia in 108 loci several involving brain function (specifically DrD2, glutamate and $Ca2+$ signaling). We examined associations between specific loci identified in the PGS study and glutamate brain levels in schizophrenia and healthy controls.
**Methods:** Subjects with schizophrenia ($n=63$; outpatients treated with antipsychotics) and healthy controls ($n=68$) were studied. Spectroscopic imaging (1H-MRSI) was performed

with a phase-encoded version of a point-resolved spectroscopy sequence (PRESS) both with and without water pre-saturation with: TE = 40ms, TR = 1500ms, slice thickness = 15mm, FOV = 220 × 220mm, circular k-space sampling (radius = 24), effective voxel volume of 2.4cm3. The 1H-MRSI was from immediately above the lateral ventricles and parallel to the anterior-posterior commissure axis, and included portions of the cingulate gyrus and the medial frontal and parietal lobes. Spectra were fitted with LCModel with SD ≤20% for all metabolites. Metabolites were referenced to water and partial volume corrected for voxel tissue composition. Glx was measured from voxels in medial frontal and medial parietal gray matter. The genetic data comprised 9 SNPs genotyped across either an Illumina 1M or 5M platform. These included 4 glutamate-related SNPs (rs10520163 CLCN3, rs12704290 GRM3, rs9922678 GRIN2A, rs12325245 SLC38A7), one dopamine-related SNP rs2514218 DRD2) and 4 calcium channel SNPs (rs1339227 RIMS1, rs7893279 CACNB2, rs2007044 CACNA1C, rs12691307 DOC2A). Missing genotypes at these loci were selectively imputed via IMPUTE2 software and 1000Genomes reference dataset.

**Results:** For rs2514218 DRD2 there was a group by allele interaction (age-adjusted, $F1,130 = 7.8$, $p = 0.006$; Bonferroni correction n÷9): in the SZ group there was a dose effect with subjects homozygous for the minor allele (CC) having higher Glx, the heterozygous (TC) intermediate levels, and the homozygous for the major allele (TT) having lowest Glx levels. None of the glutamate or calcium-related SNPs had any effects on Glx. No such effect was seen in the controls. The SZ group had greater increases of gray matter Glx with age than the controls ($F2,130 = 19.1$, $p < 0.001$).

**Conclusions:** Increased brain glutamate in schizophrenia has been previously described and is consistent with the NMDA hypofunction model of psychosis. NMDA blockade in animals leads to increase in prefrontal glutamate and dopamine. Here we failed to document relationships between brain Glx with glutamate or calcium-signaling genes previously found to be associated with the illness by the PGS. However, the minor allele for the dopamine D2 receptor gene, which is associated with SZ, predicted higher Glx levels only in the illness. This suggest that a complex relationship between dopamine and glutamate is present in schizophrenia.

**Keywords:** glutamate, Dopamine (D2, D3) receptors, MR spectroscopy

**Disclosures:** Nothing to disclose.

### M176. Characterization of a Novel Dopaminergic Agonist that Displays Spatial Bias and Functional Selectively at the D2 Dopamine Receptor

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**Background:** Functional selectivity, also termed biased agonism, protean agonism, agonist-directed trafficking, or collateral efficacy, can occur when a receptor is able to transduce signals through more than one intracellular pathway. In this case, most agonists, in particular the endogenous transmitter, will typically activate all signaling pathways in parallel with equal or similar efficacy. However, it is now recognized that synthetic agonists may preferentially activate one signaling pathway over another or even activate one while inhibiting another. The potential impact of functional selectivity on drug discovery is rapidly beginning to be realized. For instance, this mechanism may allow for the development of novel ligands that differentially activate (or block) a subset of signaling pathways for a single receptor, thus, optimizing therapeutic actions. In scenarios where a receptor activates multiple signaling pathways, it is possible that modulation of only one pathway is relevant to the therapeutic response. Moreover, parallel activation of other pathways may not be desirable as these activities could lead to target-related side effects. The D2 receptor (D2R) is one of the most validated drug targets in neuropsychiatry and mediates the effects of numerous therapeutics, including those for the treatment of Parkinson's disease and schizophrenia. Many of these therapeutic agents are not without adverse side effects, however, such that improved drug treatments would be highly desirable. As the D2R is known to signal through multiple pathways, our hypothesis is that functionally selective drugs that are limited in their signaling action may be just as effective as existing therapeutics, but exhibit fewer side effects. Since the rational design of such agents is not currently possible, the identification of novel chemical scaffolds for the D2R that exhibit biased signaling activities is required.

**Methods:** Our general approach was to use high throughput screening (HTS) to interrogate a small molecule library containing ~400,000 unique compounds. The HTS assay measured the ability of ligands to activate, block, or potentiate D2R stimulation of a Ca2+ response, which is G protein-mediated. Compounds that were active in this primary screen were re-screened in secondary assays to confirm their activity and to assess their receptor selectivity. As described below, a wide variety of signaling pathways associated with the D2R were assessed. The lead compound was also evaluated using ex vivo experiments that measured its ability to modulate dopaminergic neuronal activity in brain slices as well as in vivo experiments to assess behaviors in rodents that are associated with D2R and D3R stimulation.

**Results:** To identify novel ligands for the D2R, we screened small molecule chemical libraries using high throughput screening. These screens identified a hit compound that selectively activates the D2R in a functionally biased fashion. Chemical optimization resulted in a lead compound (VU207) that exhibits full agonist activity in three different D2R signaling assays: Ca2+ mobilization, inhibition of forskolin-stimulated cAMP accumulation (Gi/o), and β-arrestin recruitment. However, VU207 fails to activate D2R-Gβγ-mediated responses including GIRK potassium channel activation and adenylyl cyclase potentiation. Using the β-arrestin recruitment assay, VU207 was also found to exhibit potent D3R antagonism with no functional activity at other dopamine receptors. Further, behavioral paradigms (hypothermia and yawning) indicates that VU207 penetrates the brain and acts as a D2R agonist and a D3R antagonist in vivo. Using ex vivo brain slices, we investigated the functional activity of VU207 at D2Rs located on dopaminergic neurons. These cells express D2Rs on the cell bodies and dendrites (somatodendritic D2Rs),

which activate GIRK channels whereas the D2Rs located on the nerve terminals (presynaptic D2Rs) inhibit dopamine release through activation of Kv1 potassium channels. We found that VU207 failed to activate GIRK currents through somatodendritic D2Rs and, in fact, acted as an antagonist of this D2R response. Notably, in contrast, VU207 acted as an agonist at inhibiting dopamine release via the D2Rs at the nerve terminals. This latter response was absent in tissue from "autoD2R" knockout mice that lack D2R expression in dopaminergic neurons, thus confirming its specificity for D2Rs.
**Conclusions:** These findings suggest that VU207 is a functionally selective agonist that exhibits a unique form of spatial or location bias acting as either an agonist or an antagonist depending on where in the neuron (cell bodies vs. terminals) the D2R is located. It is expected that more cases of location/spatial bias will be observed upon further analyses of receptor-mediated signaling in cells with complex morphologies, especially neurons. Further evaluation of VU207 in behavioral assays that are predictive of various therapeutic modalities are currently in progress.
**Keywords:** Dopamine, Dopamine (D2, D3) receptors, Functional Selectivity
**Disclosures:** Nothing to disclose.

### M177. New Roles for Dopamine and Dopamine D2-Like Receptors in Pancreatic Insulin Release: Implications for Antipsychotic Drug Action Outside the Brain

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**Background:** Antipsychotic drugs (APDs) are some of the most widely used medications today. However, APDs also have prominent metabolic side effects, including substantial weight gain, glucose intolerance, and increase risks for type II diabetes and cardiovascular disease (Pramyothin and Khaodhiar, 2010). Significantly, recent studies have demonstrated that all APDs cause metabolic disturbances (Fleischhacker et al., 2012). The single unifying property of all APDs is their blockade of dopamine D2-like receptors including D2 (D2R) and D3 receptors (D3R), suggesting their potential role in mediating these metabolic disturbances. Besides expression in brain regions implicated in metabolism, D2R and D3R are also expressed peripherally in insulin-secreting pancreatic beta cells (Rubi et al., 2005). Nevertheless, the precise roles of pancreatic DA and D2R/D3R remain poorly characterized. Thus, we have begun to investigate D2R/D3R-mediated effects on glucose-stimulated insulin secretion (GSIS) in mouse islets and INS-1E cells, a well-established rodent beta cell-derived cell line.
**Methods:** INS-1E cells and wildtype C57Bl6/J mouse-derived pancreatic islets were cultured in RPMI 1640 media (37°C, 5% CO2) prior to experimental use. GSIS was measured via a homogenous time-resolved fluorescence insulin assay we developed (Farino et al., 2015). Cells or islets were first glucose-starved in Krebs-Ringer bicarbonate buffer (KRB; 1 h, 37°C) and then stimulated with 20 mM glucose in the presence or absence of additional drugs. Secreted insulin was measured from sample supernatant following drug incubation (30-90 min). To measure glucose-stimulated DA secretion, cells were pre-incubated with 30 micromolar L-DOPA (30 min, 37°C) followed by addition of additional drugs. Secreted DA in cell supernatant was quantified by high performance liquid chromatography (HPLC).
**Results:** In characterizing the DA biosynthetic machinery in beta cells, we found that human and rodent beta cells express the neuronal isoform of the vesicular monoamine transporter, VMAT2, which packages cytoplasmic DA into secretory vesicles. Consistent with this, we localized DA to insulin-containing dense core secretory vesicles. Moreover, INS-1E cells took up L-DOPA and converted it to DA, leading to a reduction in insulin secretion. Additionally, islet L-DOPA uptake and DA secretion was significantly enhanced during stimulation, suggesting metabolic coupling of GSIS with DA production and secretion. D2R/D3R agonism by quinpirole further enhanced glucose-stimulated DA release, suggesting a feed-forward mechanism to potentiate DA's inhibitory effects on GSIS and this effect was attenuated in either D2R or D3R knockout (KO) islets. Treatment with exogenous DA also dose-dependently inhibited GSIS ($IC50 = 1.28$ uM) where 10 uM DA completely blocked GSIS. D2R/D3R played a key role in DA-mediated GSIS inhibition since D2R/D3R agonism by quinpirole mimicked DA's effects. Similarly, D2R/D3R blockade by raclopride or sulpiride attenuated L-DOPA's inhibitory effects on GSIS. We also examined D3R's individual contributions to these effects using D3R-specific blocker, R22, or via genetic D3R knockout. We found that inhibition of D3R function alone significantly attenuated effects of L-DOPA and DA on GSIS inhibition. Lastly, we developed a novel beta cell-selective D2R KO mouse to study pancreatic D2R's in vivo roles in mediating insulin secretion and metabolism. Compared to wildtype littermates, we found that D2R KO mice were more resistant to high fat diet and did not exhibit the dwarfism showing in global D2R KO mice.
**Conclusions:** These results reveal that peripheral pancreatic DA and D2R/D3R receptors play important roles in metabolism through their inhibitory effects on GSIS. Consequently, by blocking peripheral pancreatic D2R/D3R, APDs override the DA-dependent negative feedback mechanism that mediates GSIS. This opens the possibility that, besides their action in the central nervous system, APDs' actions on peripheral D2R/D3R may play a key role in causing the metabolic disturbances observed clinically.
**Keywords:** Dopamine (D2, D3) receptors, Antipsychotic induced weight gain, Insulin, Dopamine
**Disclosures:** Nothing to disclose.

### M178. Tyrosine Hydroxylase, GAD67, vGLUT1, and vGLUT2 Proteins in the Substantia Nigra in Schizophrenia

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**Background:** Schizophrenia (SZ) is a serious mental illness with positive, negative, and cognitive symptoms. The

substantia nigra/ventral tegmental area (SN/VTA) provides the largest dopaminergic (DA) input to the brain, and projects to the striatum that is the primary locus of action for antipsychotic drugs (APDs). The SN also receives both GABAergic and glutamatergic inputs that regulate neuronal activity. In spite of this, there are relatively few studies of the substantia nigra in schizophrenia.
**Methods:** This study compared tyrosine hydroxylase (TH), glutamate decarboxylase (GAD67), and vesicular glutamate transporters (vGLUT1 and vGLUT2) in control and SZ postmortem human SN. The SZ cohort is tested as a whole and then divided by treatment status or treatment response. Postmortem caudal SN was obtained from the Maryland Brain collection. SZ (n = 13) cases were compared to matched controls (NC, n = 12). The SZ group was then subdivided by treatment status: no APD (SZ-Off, n = 4 or on APD (SZ-On, n = 9); or treatment response: treatment resistant (TR, n = 5) and treatment responsive (RESP, n = 4). Western blot analysis was used to measure protein levels of vGLUT1, vGLUT2, TH, GAD67, and actin. Optical density values were normalized to actin and the average NC value.
**Results:** A significant difference in protein levels of both TH and GAD67 was observed between NC and SZ groups. SZ (1.70 ± 0.72) had higher levels of TH compared to NC (1.00 ± 0.30, p = 0.01), with a similar result for GAD67 (SZ: 1.25 ± 0.25; NC: 1.00 ± 0.15, p = 0.004). vGLUT1 and vGLUT2 protein levels did not significantly differ between the NC and SZ groups.
Protein levels significantly differed among NC and SZ divided by treatment status (OFF and ON) for TH, GAD67, and vGLUT2. TH protein levels were higher in SZ on medication (ON: 1.88 ± 0.71) compared to NC (1.00 ± 0.30, p = 0.008). GAD67 protein levels were also higher in ON (1.41 ± 0.38) compared to NC (1.00 ± 0.15, p = 0.003). In contrast, a post-hoc test of vGLUT2 illuminated a difference between SZ off medication (OFF: 1.29 ± 0.10) and NC (1.00 ± 0.22, p = 0.041), but no difference between ON and NC. Protein levels of vGLUT1 did not significantly differ.
Significant differences were observed among NC and SZ divided by treatment response (OFF, RESP, TR) for both TH and GAD67 protein levels. TR (2.21 ± 0.61) had significantly higher TH levels than NC (1.00 ± 0.30, p = 0.001) and OFF (1.28 ± 0.61, p = 0.045). Similarly, GAD67 levels in TR were significantly higher than NC (p = 0.004). No significant differences among groups were observed for vGLUT1 or vGLUT2.
**Conclusions:** In summary, TH and GAD67 protein levels were increased in the caudal SN in SZ compared to controls, which could possibly be an APD effect; TR had higher levels of TH and GAD67 than NCs. vGLUT1 tended to be higher in SZ, especially in SZ-off, with no relationship to treatment response. vGLUT2 was increased in SZ-off, and had no relationship to treatment response. These data suggest abnormalities in DA and GABA transmission in SZ with a possible relation to treatment response. Glutamatergic inputs to the SN in SZ from subcortical regions (as marked by vGLUT2) may be elevated in SZ-off and normalized by APDs.
**Keywords:** glutamate, GABA, Dopamine, pathophysiology
**Disclosures:** Nothing to disclose.

## M179. Cortical Dopaminergic Deficiency in Schizophrenia while Performing a Cognitive Task: A PET Study

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**Background:** Several lines of evidence point towards deficient cortical function in schizophrenia (SCZ). However, no study has directly tested dopamine (DA) deficiency in SCZ patients while performing a cognitive task. In this work we directly examined cortical DA deficiency using [11C]FLB-457 PET, a high affinity DAD2 radioligand in antipsychotic-free patients with SCZ compared to healthy volunteers (HV).
**Methods:** Antipsychotic-free SCZ (n = 8;6 males) and age, sex/age matched HV (n = 8;5 males) underwent 2 PET scans using [11C]FLB-457, a D2/3 receptor ligand while doing the Wisconsin Card Sorting Test (WCST) and a sensorimotor control task (SMCT). Individual MRIs were obtained for image co-registration and Region of Interest delineation. Time activity curves were obtained to estimate binding potential non-displaceable (BPND) using the simplified reference tissue model. Percentage change in FLB-457 BPND between conditions (WCST/SMCT), an index of cortical dopaminergic release, was calculated using the formula [(BPND SMCT-BPND WCST)/BPND SMCT X 100].
**Results:** All patients were antipsychotic free for at least 3 months and did not have comorbid psychiatric or substance use disorders at the time of scans. All participants had negative urine drug screens. We found a significant difference between SCZ and HV in [11C]FLB-457 percent change in the Anterior Cingulate Cortex (ACC) (t = 2.45; p = 0.02), with a trend in dorsolateral prefrontal cortex (DLPFC, (t = 1.89; p = 0.07). While performing a cognitive task SCZ patients demonstrated a smaller [11C]FLB-457 percent change (-9.07 ± 14.84) than HV (6.77 ± 7.89) in ACC, suggesting reduced DA release supporting DA deficiency.
**Conclusions:** These pilot data, to be confirmed in a larger study, represent the first in-vivo PET imaging demonstration of cortical dopaminergic deficiency in SCZ while performing a cognitive task (WCST).
**Keywords:** Dopamine, Cognition, schizophrenia
**Disclosures:** Nothing to disclose.

## M180. Testing the "PACT" Strategy: Amphetamine (AMPH) Enhances Gains in Auditory Discrimination Training in Adult Schizophrenia (SZ) Patients

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**Background:** An intensive program of computerized targeted cognitive training of auditory processing and auditory working memory appears to drive improvements

in higher-order cognition in SZ patients. Potentially, such therapeutic effects might be facilitated pharmacologically, via drugs that enhance attention, sensory processing and/or working memory processes that are taxed by the training. If this "pharmacologically augmented cognitive therapy" ("PACT") strategy is successful, it might be possible to augment and accelerate the clinical benefits of cognitive training in SZ patients. Because gains in auditory psychophysical efficiency can be detected after a single one-hour session of training, it is possible to use a placebo (PBO)-controlled single dose cross-over design to assess drug effects on training response. We assessed the effects of the pro-attentional drug, d-amphetamine (AMPH; 10 mg po), on one hour of auditory cognitive training in SZ patients and healthy subjects (HS).

**Methods:** Carefully screened and characterized HS (n = 17; M:F = 12:5; age mean, range = 29.8, 20-46) and patients with a diagnosis of SZ (n = 9; M:F = 6:3; age mean, range = 37.3, 23-48) participated in this study; this sample included only individuals homozygous (AA or GG) at the rs4680 single nucleotide polymorphism (SNP) for catechol O-methyl-transferase. Subjects were tested three times with 1 week between tests: first in a screening session (no pill administered), and next in a double-blind order-balanced crossover design (test days 2 and 3), comparing PBO vs. 10 mg of AMPH. On each test day, 1 hour of Sound Sweeps training was bracketed by brief (2-4 min) pre- and post-training assessments of auditory processing speed (APS). Training consisted of a speeded auditory time-order judgment task of two successive frequency modulation (FM) sweeps (Posit Science "Sound Sweeps" exercise). On test days 2 and 3, pre-training assessments began 210 min post-pill administration, when 10 mg AMPH is known to be bioactive. Autonomic and subjective measures were collected throughout test days 2 and 3. All test days also included measures of neurocognition and sensorimotor gating, reported separately.

**Results:** AMPH was bioactive, enhancing autonomic function as well as subjective measures of alertness in HS. Baseline (screening and pre-training) performance (APS, trials completed) was impaired in SZ patients vs. HS (all p's < 0.02–0.0001). Auditory system "learning" (APS post- vs. pre-training) was enhanced by AMPH, and this effect was more pronounced in patients (main effect of diagnosis: ns; main effect of AMPH: $p < 0.0001$; diagnosis x drug interaction: $p < 0.0001$; main effect of AMPH in patients: $p < 0.006$; main effect of AMPH in HS: ns ($d = 0.2$)). Independent of diagnosis, AMPH effects on auditory system "learning" tended to be greater among rs4680 AA vs. GG subjects ($p < 0.10$); learning achieved statistical significance only among AA ($p < 0.03$) but not GG subjects (ns).

**Conclusions:** Cognitive training and other cognitive therapies are gaining empirical support for use in the treatment of SZ. While auditory system training is efficacious for SZ at a group level when given in sufficiently intensive dosing (e.g., 50 hours), there is wide variability in individual responses, and thus interventions that augment and accelerate training-induced benefits will be of great value. Here, we demonstrate that a low dose of the pro-attentional drug, AMPH, enhances auditory discrimination learning in SZ patients. This effect may not be unique to SZ: a weaker but similar effect in HS supports the notion that AMPH's benefits in SZ patients reflect an enhancement of intact brain mechanisms, in the service of the attentional demands of training. Identifying patients most sensitive to such drug effects via biomarkers is an important part of the "PACT" strategy, and the present findings provide tentative evidence that AMPH effects on auditory discrimination learning may be most pronounced among individuals homozygous for methionine at the rs4680 SNP. It is important to note that we do not yet know whether the behavioral improvement (i.e. gains in APS) observed in SZ patients after a single session of auditory discrimination training predicts clinical benefits after an entire course of 30-50 hours of auditory system exercises. However, if such benefits are demonstrated, and AMPH can enhance the therapeutic effects of training, then this "PACT" approach could represent a transformative treatment paradigm for SZ.

**Keywords:** amphetamine, targeted cognitive training, schizophrenia

**Disclosures:** Dr. Light has served as a consultant for Astellas, Forum Pharmaceuticals, Boehringer Ingelheim and Neuroverse. Dr. Vinogradov serves as a site PI on an SBIR grant to Positscience.

## M181. Investigating Schizophrenia GWAS Risk Variants in Cognitive and Brain Structural Defects in the GENUS Consortium Sample Collection

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**Background:** Recent genome-wide association studies (GWAS) have identified many genetic variants with significant evidence for association with schizophrenia (SCZ) risk. However, the case-control samples used in these analyses have limited phenotypic data to elucidate the role of these genetic variants in brain dysfunction that characterizes the disorder. The Genetics of Endophenotypes of Neurofunction to Understand Schizophrenia (GENUS) Consortium aims to clarify the neurobiological role of SCZ genetic risk variants by testing their association with cognitive and neuroanatomical intermediate phenotypes in a large patient collection.

**Methods:** Fifteen research groups have contributed a total of 4896 SCZ cases, 804 genetic high-risk (GHR) subjects, and 3331 healthy controls with clinical, genotype, and cognitive data, as well as structural MRI data in ~30% of subjects. To select robust intermediate phenotypes for genetic analyses, literature review and meta-analysis were performed to identify cognitive and brain structural traits with high heritability, reliability, and case-control differences.

Genome-wide SNP data from each site were subjected to quality control procedures in Plink and imputed to the 1000 Genomes Phase III reference panel using a standardized pipeline. Phenotype processing and quality assurance protocols were developed and validated to maximize comparability of phenotype data across sites. Cognitive data were harmonized across samples by pooling controls for each test version and fitting a linear regression model

(correcting for age, age^2, sex, and interactions), followed by calculating standardized residuals relative to controls. All structural MRI scans were processed using FreeSurfer v5.0 or higher with manual or automated multi-atlas brain segmentation masking. ANOVA with Tukey's HSD posthoc comparisons was applied to each phenotype to identify case-control differences.

**Results:** We selected 3 tiers of cognitive phenotypes for genetic analyses that have relatively high heritability according to meta-analysis (h^2 = 28-62%; average 43%): i) 8 individual cognitive tests (7 MATRICS battery tests and Block Design), ii) 7 cognitive domains (verbal learning, visuospatial learning, attention/vigilance, processing speed, verbal working memory, spatial working memory, problem solving), and iii) general cognitive ability "g". Eleven brain structural phenotypes were selected for genetic analyses: cortical grey matter, brain volume, volume or cortical thickness of superior temporal gyrus, inferior and middle frontal gyrus, anterior cingulate, inferior parietal lobule, insula, lateral ventricles, hippocampus, and amygdala. Cognitive and structural phenotypes were confirmed to significantly differ between SCZ and controls in this sample collection ($p < 0.05$). SCZ subjects performed significantly worse than controls for all individual cognitive tests, cognitive domains, and "g", with effect sizes (standardized mean difference) between -0.52 and -1.12, averaging -0.90 ($p < 0.001$). GHR subjects performed between SCZ and control subjects for Trails A, Category Fluency, and Word List Learning (effect size relative to controls: -0.34; -0.35; -0.88; $p < 0.05$), but were more similar to SCZ for Letter-Number Span and Continuous Performance Test, and more similar to controls for Symbol Coding, BVMT, and Block Design ($p > 0.05$). SNPs with prior SCZ GWAS evidence ($p<5x10-8$) and functionally related polygene sets are being tested for association by linear regression and meta-analysis across samples, as well as multivariate regression analysis to identify phenotypic profiles with common neural mechanisms.

**Conclusions:** Careful harmonization of cognitive and brain structure intermediate phenotypes across sites is essential to minimize noise in the data and thereby increase power to detect genetic associations. Association analyses of known SCZ risk variants from GWAS with cognitive and neuroanatomical traits in one of the largest sample collections of its kind are expected to help elucidate the function of genetic risk variants in neural processes underlying SCZ pathophysiology.

**Keywords:** Cognition, Structural MRI, psychiatric genetics, schizophrenia, GWAS

**Disclosures:** Nothing to disclose.

### M182. Optogenetic Assessment of Dynamic Input Integration in the Ventral Striatum

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**Background:** Striatal medium spiny neurons (MSN) serve as a site of convergence for multiple brain regions involved in goal-directed behavior, including the prefrontal cortex (PFC) and hippocampus (HP). These distinct excitatory inputs are believed to differentially influence striatal circuitry in an activity dependent manner. Electrophysiological recordings from anesthetized rats showed that robust PFC stimulation leads to a reduction in ongoing HP-evoked MSN responses, in part, through the recruitment of local inhibitory mechanisms within the ventral striatum (VS). These data indicate that burst-like cortical activity is capable of attenuating weaker, less salient excitatory input within the striatum. Here, we explored the heterosynaptic mechanisms involved in cortical suppression of competing excitatory synaptic inputs on ventral striatal MSNs.

**Methods:** Whole-cell current-clamp recordings were performed from rats receiving bilateral hippocampal injections of a viral vector (AAV) expressing channelrhodopsin 2 (ChR2) under the CamKinase II promoter. Input interactions were assed in VS MSNs through electrical stimulation of PFC fiber tracts and light pulse stimulation of HP inputs expressing ChR2.

**Results:** We have demonstrated that optogenetically evoked HP EPSPs are greatly attenuated after a short latency (50 ms) following burst-like corticostriatal stimulation (5 pulses, 20 Hz, 0.1-0.5 mA) but not a longer (500 ms) delay. Bath application of picrotoxin (100 uM), but not saclofen (2 uM), reduced the magnitude of suppression suggesting inhibitory GABAA, but not GABAb, receptor activation is likely to play a role. As the reduction is not complete, we assessed the role of two signaling molecules which are known to modulate striatal neurotransmission in a retrograde manner. In the VS, endocannabinoid activation of CB1 receptors reduces presynaptic glutamate and GABA release. In addition, subsets of VS MSNs contain dynorphin which, upon release, could decrease glutamate release through activation of presynaptic kappa opioid receptors (KOR). We found that bath application of the CB1 receptor antagonist AM251 (2 uM) enhanced cortical suppression of optically evoked HP responses suggesting the locus of action for AM251 is on inhibitory interneurons. Similar experiments are being conducted using the KOR antagonist nor-BNI.

**Conclusions:** These findings further substantiate the assertion that shifts in VS neuronal activity involve local suppression of competing afferent inputs converging on the same MSN. Furthermore, these data suggest local heterosyanptic suppression involves several signaling pathways.

**Keywords:** Ventral Striatum, Hippocampus, Medial Prefrontal Cortex

**Disclosures:** Employed by Pfizer Inc.

### M183. Shared Genetic Etiology does not Explain Differential Risk of Immune Diseases in Schizophrenia

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**Background:** Schizophrenia patients are at greater risk of developing autoimmune diseases compared to the general

population, and vice versa. The reasons for this phenomenon remain unknown, but overlapping genetic risk factors have been proposed. To clarify the underlying causes we evaluated whether there are shared genetic risk pathways between schizophrenia and autoimmune diseases.
**Methods:** We evaluated genetic overlap between schizophrenia and 18 autoimmune diseases, using genotype data from the Schizophrenia Working Group of the Psychiatric Genomics Consortium. First, we evaluated whether individual SNPs associated with immune diseases were also associated with schizophrenia. Second, we evaluated whether genetic risk scores comprised of genome-wide significant immune SNPs were associated with schizophrenia case status. Third, we evaluated whether polygenic risk scores comprised of SNPs associated with immune disease at subthreshold significance ($p > 5x10-8$) were associated with schizophrenia case status.
**Results:** (1) Among 563 non-HLA autoimmune risk SNPs, five were also associated with schizophrenia ($p < 8.0x10-5$). Within the HLA region, four of the 18 strongest associated risk variants for the autoimmune diseases were also associated with schizophrenia ($p < 8.0x10-5$). (2) Genetic risk scores for 18 different immune diseases were not significantly associated with schizophrenia case status when considering only non-HLA variants (all $p > 1x10-3$). We observed significant evidence of pleiotropy between schizophrenia and alopecia areata (OR = 1.02), celiac disease (OR = 0.94), primary sclerosing cholangitis (OR = 0.95), rheumatoid arthritis (1.04) and Sjogren's syndrome (OR = 0.94), but these findings were not robust to removing the top HLA variant. (3) Polygenic risk scores for primary biliary cirrhosis, type 1 diabetes, and ulcerative colitis were significantly associated with schizophrenia case status ($p < 1x10-3$). However, the effect size of the polygenic scores for these immune diseases on schizophrenia case status was modest ($R2 < 0.1\%$).
**Conclusions:** We found no robust evidence of genome-wide genetic overlap between schizophrenia and 18 immune diseases. Previous studies report modest genetic correlation between schizophrenia and Crohn's disease, type 1 diabetes, and rheumatoid arthritis with inconsistencies in magnitude and direction of effect. We may have lacked statistical power to detect modest pleiotropic effects in the present study. Nevertheless, this is the first comprehensive evaluation of genetic overlap between schizophrenia and immune diseases, undertaken in the largest schizophrenia GWAS dataset currently available. Our results suggest that shared genetic risk factors are not a major factor contributing to differential risk of immune diseases seen in schizophrenia. Instead, environmental factors such as medications, lifestyle, and previous infections may play a more significant role in predisposing patients with schizophrenia to immune diseases.
**Keywords:** schizophrenia genetics, schizophrenia, genetic, polygenic risk score, Immune
**Disclosures:** JL Kennedy has acted as a consultant to GlaxoSmithKline, Sanofi-Aventis and Dainippon-Sumitomo, received honoraria from Novartis and Roche, and is a member (unpaid) of the SAB for Assurex Health Corp. The remaining authors have no conflicts to disclose. The funding sources did not influence the study design, data analysis, or writing of this manuscript.

## M184. A Translational Approach to Differentiate a Novel PDE4 Inhibitor, from a PDE10 Inhibitor which Lacked Clinical Efficacy in Schizophrenia

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**Background:** The selective inhibitor of phosphodiesterase-10A (PDE10A), PF-02545920, was recently reported to be ineffective as a monotherapy in acute exacerbation of schizophrenia. In order to continue to work on the development of novel treatments for schizophrenia it has been imperative to investigate the limitations of translating our preclinical findings to the clinic. Deficits in sensory gating have been detected in a proportion of schizophrenia patients through measurement of electroencephalogram (EEG) event-related potentials (ERP). Here we have used behavioral and in vivo electrophysiological endpoints of gating to characterize and differentiate the preclinical antipsychotic profile of a novel PDE4 inhibitor from PF-02545920. The compounds were also compared to standard antipsychotics in genetically modified mice (Df1/+) carrying the murine equivalent of the human 22q11 genetic deletion, associated with a 20-fold increased risk for schizophrenia and accounting for almost 1% of schizophrenia cases.
**Methods:** The effects of PF-02545920 were compared to the second generation D2 receptor antagonist, risperidone and the novel PDE4 inhibitor, ABI-4. The effects of these compounds were assessed on endpoints related to schizophrenia in adult male mice. Sensorimotor gating was measured using prepulse inhibition (PPI) of startle. Auditory gating was assessed using EEG from a surface electrode over the frontal cortex in a paired auditory evoked potential paradigm in a cohort of freely moving mice. To capture the effects of these compounds in a disease-relevant model, C57Bl6/J and genetically modified mice carrying the murine equivalent to the human 22q11 genetic deletion (Df1/+) were used (Paylor et al. 2001).
**Results:** Df1/+ mice showed reduced prepulse inhibition of startle compared to wild type litter mates ($F1,14 = 10.84$; $P = 0.0053$). Acute administration of risperidone (1 mg/kg s.c.) or ABI-4 (0.1-0.3 mg/kg s.c.) restored levels of PPI, while PDE10 inhibition was ineffective. In contrast, all three mechanisms were active in the conditioned avoidance responding paradigm at the doses evaluated in PPI. The level of gating of the EEG response to pairs of auditory stimuli (S1 and S2; 75 dB intensity, separated by 0.5 s) was not different between Df1/+ ($S2/S1 = 0.40 \pm 0.03$, $n = 10$) and wildtype mice ($S2/S1 = 0.44 \pm 0.04$, $n = 10$). Risperidone (1-3 mg/kg s.c.) produced a moderate increase in gating through increasing the amplitude of the S1 response. ABI-4 (0.03-1 mg/kg s.c.) produced a greater increase in gating through increasing S1 and having a modest effect on S2 response amplitudes. In contrast, PF-02545920 (1-10 mg/kg s.c.) had no effect on auditory gating.
**Conclusions:** While the standard of care antipsychotic agent, risperidone, improved sensorimotor and auditory gating, the lack of effect of the PDE10 inhibitor in these assays is congruent with the lack of clinical efficacy in schizophrenia. The profile of the PDE4 inhibitor, which included a robust, dose-dependent increase in auditory

gating at low levels of target engagement, supports further development of this mechanism for the treatment of schizophrenia. The translatability of this EEG endpoint and the link to an endophenotype of schizophrenia make this an attractive measure to include in early clinical development to provide confidence in rationale to de-risk larger Phase 2 studies.
**Keywords:** PDE4, Schizophrenia-like Behavior, EEG, ERP, sensory gating
**Disclosures:** All authors are employees of Pfizer Inc.

### M185. Region-Specific Dendritic Spine Loss of Pyramidal Neurons in Dopamine Transporter Knockout Mice

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**Background:** Dopamine transporter (DAT) knockout (KO) mice show numerous behavioral alterations, including hyperlocomotion, cognitive deficits, impulsivity and impairment of prepulse inhibition of the startle reflex (PPI), phenotypes that may be relevant to frontostriatal disorders such as schizophrenia. Dendritic spine changes of pyramidal neurons in the dorsolateral prefrontal cortex (DLPFC) are among the most replicated of findings in postmortem studies of schizophrenia. The mechanisms that account for dendritic changes in the DLPFC in schizophrenia are unclear.
**Methods:** DAT KO mice were crossed with Thy1-GFP transgenic mice (line M). Neurons with well- defined dendrites in the plane of section were chosen for imaging and analysis. High-resolution 3-dimensional images were taken using a LSM5 PASCAL confocal microscope. Pyramidal shaped neurons were randomly selected from both the prelimbic and infralimbic areas of the mPFC, the motor cortex, the CA1 region of the dorsal hippocampus and the basolateral amygdala. Spine density (spines / 10 μm) was calculated for each neuron by dividing the total number of spines by the total length of all dendritic trees. After neurons were reconstructed and the average values for the size of soma and the dendritic parameters were calculated, we performed analysis of spine density as a function of distance from the soma using Neurolucida software. Mean spine density was obtained by averaging the values for all measured neurons in each experimental group (mPFC: $n = 34$ - 37 from 5 animals per genotype; motor cortex: $n = 22$ - 27 from 5 animals per genotype; CA1 region of the hippocampus: $n = 29$ - 34 from 5 animals per genotype; basolateral amygdala; $n = 26$ - 31 from 5 animals per genotype).
**Results:** Basal spine densities of pyramidal neurons in the medial prefrontal cortex (mPFC), the motor cortex, the CA1 region of the hippocampus, and the basolateral amygdala were studied in DAT KO mice. Pyramidal neurons were visualized using DAT KO mice crossbred with a Thy1-GFP transgenic mouse line. We observed a significant decrease in spine density of pyramidal neurons in the mPFC and the CA1 region of the hippocampus in DAT KO mice compared to that in WT mice. On the other hand, no difference was observed in spine density of pyramidal neurons in the motor cortex or the basolateral amygdala between DAT genotypes. Cell body size of pyramidal neurons in DAT KO mice was similar to that in WT mice in all brain regions.
**Conclusions:** These results suggest that spine density loss of pyramidal neurons in the mPFC and the CA1 region of the hippocampus in DAT KO mice might account for behavioral alterations observed in DAT KO mice via impairment of cognitive processing and executive functions. This might suggest that aberrant dopaminergic signaling may trigger dystrophic changes in dendrites of hippocampal and prefrontocortical pyramidal neurons in schizophrenia. Dendritic changes in frontal cortical pyramidal neurons, like those observed in DAT KO mice, are among the most replicated findings in post mortem studies of schizophrenia. Since DAT KO mice displayed loss of spine density in pyramidal neurons in the mPFC and the CA1 region of the hippocampus, DAT KO mice might be a useful model of some aspects of cognitive deficits in schizophrenia patients.
**Keywords:** transgenic mice, medial prefrontal cortex, pyramidal neuron
**Disclosures:** Nothing to disclose.

### M186. The Novel Atypical Antipsychotic Brexpiprazole, Alone and in Combination with Escitalopram, Facilitates Prefrontal Glutamatergic Transmission via a Dopamine D1 Receptor-Dependent Mechanism

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**Background:** Brexpiprazole (Rexulti®), a novel atypical antipsychotic (AAP), has recently been approved by the FDA for the treatment of adult schizophrenia with effect on both positive and negative symptoms and, also, as an adjunct therapy for adults with major depressive disorder (MDD). Brexpiprazole shows a broad receptor (R) binding profile and, in similarity with the third generation antipsychotic drug aripiprazole, acts as a partial agonist at the dopamine D2/3R, albeit with lower intrinsic activity. Brexpiprazole is also a partial agonist at the 5-HT1AR, where it binds with high affinity, and is a potent antagonist at the 5-HT2AR.
Given the well documented significance of NMDARs and D1Rs for optimal cognitive functioning, particularly working memory and executive ability, facilitation of NMDAR-mediated synaptic transmission in the prefrontal cortex (PFC) may significantly contribute to ameliorate cognitive impairments in both schizophrenia and in depression. Furthermore, solid clinical data show that low doses of antipsychotic drugs may augment the antidepressant effect of selective serotonin reuptake inhibitors (SSRIs) and reduce the time of onset of this effect. Preclinical studies indicate that the rapid and sustained antidepressant effect of e.g. ketamine and scopolamine is critically dependent on AMPAR activation in the rat medial PFC (mPFC) and results in a facilitation of glutamatergic synaptic transmission in the same brain region.

Here we investigated the effects of brexpiprazole, given alone and in combination with the SSRI escitalopram, on glutamatergic NMDAR- and AMPAR-induced transmission in the rat mPFC. The effect of brexpiprazole, alone and in combination with escitalopram, for its ability to potentiate electrically evoked excitatory postsynaptic potentials (EPSPs) was also examined.
**Methods:** Intracellular single-electrode technique was used to record pyramidal cells in layer V/VI of the mPFC in slice preparations from rat brain.
**Results:** Brexpiprazole facilitated NMDA-induced currents in pyramidal cells in an inverted U-shape mode, with a maximal effect at 10 nM. Furthermore, the combination of a low, by itself ineffective concentration of brexpiprazole (3 nM) and escitalopram (3 nM), significantly enhanced the NMDA-induced current. The enhanced effects on NMDA-induced currents induced by brexpiprazole 10 nM, as well as the combination of brexpiprazole and escitalopram, were mediated at least in part via D1Rs, since the effects were blocked by D1R antagonist SCH23390.
Brexpiprazole (3-100 nM) did not have any effect on AMPA-induced currents in mPFC pyramidal cells. However, in combination with a low, subeffective concentration of escitalopram (3 nM), a significantly enhanced AMPA-induced current was seen. This enhancement was also found to be mediated at least in part via D1Rs, since the effect was blocked by SCH23390.
Brexpiprazole (10 and 30 nM) facilitated the electrically evoked EPSP. The facilitated effect on EPSPs of brexpiprazole 10 nM was blocked by the selective NMDAR antagonist APV, which indicates that the effect of brexpiprazole on EPSP is indeed mediated via the NMDAR. Alone, neither brexpiprazole 3 nM nor escitalopram 3 nM had any effect on electrically evoked EPSPs. However, the combination of brexipiprazole and escitalopram enhanced the electrically evoked EPSPs.
**Conclusions:** Our results show that brexpiprazole indeed facilitates glutamatergic, NMDAR-mediated transmission in the rat mPFC. Given the PCP model of schizophrenia, this effect may contribute to ameliorate both positive and negative symptoms as well as cognitive impairments in the patients. These conclusions are further supported by animal studies showing a cognitive enhancing effect of brexpiprazole in animals treated with NMDAR-antagonists.
Moreover, brexpiprazole added to an SSRI synergistically potentiated AMPAR- as well as NMDAR-mediated transmission, and also electrically evoked EPSPs in the rat mPFC. These mechanisms may be significantly involved in the brexpiprazole augmentation of the antidepressant effect of e.g. SSRIs in poorly responding patients. The enhanced effect on AMPA-induced currents, in similarity to what has been shown with other drugs and drug combinations possessing a rapid and potent antidepressant effect, i.e. combinations of AAPs and SSRIs or ketamine, shows that brexpiprazole may also be used to produce this effect. Given the complex pharmacological profile of brexpiprazole, the specific mechanisms that mediate the facilitation of glutamatergic transmission in the mPFC remain to be completely understood. In all probability, D1R activation alone may not be sufficient to generate the facilitated AMPAR-mediated transmission in this brain region, as previous studies already indicate that also enhanced serotonergic activity and activation of 5-HT1A receptors may be needed to achieve this end.
In summary, the present study provides novel data that strongly support a cognitive-enhancing effect of brexpiprazole in both schizophrenia and depression, and provides novel experimental evidence for the utility of adjunctive treatment with brexpiprazole in MDD patients with inadequate response to SSRIs.
**Keywords:** AMPA receptor, NMDA Receptor, Atypical antipsychotics, SSRI, electrophysiology
**Disclosures:** This study was supported by Swedish Research Council (grant no. 4747), Karolinska Institutet, Torsten Söderbergs Stiftelse, Hjärnfonden, and a research grant from Lundbeck A/S, Denmark.

## M187. Effect of Brexpiprazole on Agitation and Hostility in Patients with Acute Schizophrenia

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**Background:** Patients diagnosed with schizophrenia can be at times hostile and aggressive and/or agitated. Violent or threatening behavior is a frequent reason for admission to a psychiatric inpatient facility. If such behaviors continue after admission they can prolong hospitalization and interfere with discharge. A significant treatment challenge is the management of agitation/aggression, and the reduction of the intensity and frequency of future episodes of agitation/aggression. Brexpiprazole is a serotonin-dopamine activity modulator that has demonstrated efficacy in the treatment of schizophrenia. We evaluated the efficacy of brexpiprazole compared with placebo for reducing hostility/agitation in patients hospitalized with acute exacerbation of schizophrenia and investigated the independence of reduction in hostility from the reduction in positive symptoms in general.
**Methods:** Analysis of pooled data was performed from 2 randomized, double-blind, placebo-controlled, 6 weeks studies of fixed dose once daily brexpiprazole (2 or 4 mg) conducted in patients with acute schizophrenia. Agitation and hostility were assessed by analysis of the Positive and Negative Syndrome Scale (PANSS) excited component (PANSS-EC) score [excitement (P4), (P7), tension (G4), uncooperativeness (G8), poor impulse control (G14)] and by analysis of the PANSS Marder Factor Uncontrolled Hostility/Excitement score [P4, P7, G8, G14]. To confirm a specific effect of brexpiprazole on hostility, the overall change from baseline to week 6 on the hostility item (P7) of the PANSS for patients with a baseline P7 score $\geq 2$ was analyzed. For the hostility analysis, treatment group differences were conducted using mixed model repeated measures (MMRM), including: 1. treatment, visit, and treatment-by-visit interaction as fixed effects, and positive symptoms [sum of delusions (P1), conceptual disorganization (P2), hallucinatory behavior (P3), grandiosity (P5), suspiciousness/persecution (P6), unusual thought content (G9)] as covariate; 2. the analysis was also conducted with adjusting for the presence of akathisia and 3. the analysis

was adjusted for the presence of akathisia and somnolence recorded as spontaneously reported adverse events.
**Results:** Analyses of pooled data supported efficacy of brexpiprazole 4 mg/day (n = 350; LSMD = -1.11, p = 0.0002) and 2 mg/day (n = 359, LSMD = -0.69, p = 0.0200) vs placebo (n = 358) in change in PANSS-EC score from baseline to Week 6, and of brexpiprazole 4 mg/day (LSMD = -1.18, p < 0.0001) and 2 mg/day (LSMD = -0.62, p = 0.0311) in change in PANSS Marder Factor Uncontrolled Hostility/Excitement score from baseline to Week 6. A total of 775 patients met the criteria for hostility at baseline (P7 ≥ 2); 256 and 268 patients were treated with brexpiprazole 2 mg and 4 mg respectively, and 251 were on placebo. Brexpiprazole 4 mg was superior to placebo in reducing the PANSS hostility item at week 6 (LSMD = -0.34, p = 0.0024) while brexpiprazole 2 mg showed numerical improvement (LS mean change from baseline of -0.63 vs -0.49); based on the used covariate analysis, these improvements were independent of improvements in positive symptoms. To control for the effect of adverse events of akathisia and somnolence, the analysis was repeated by introducing the presence of akathisia or akathisia and somnolence as additional covariates. In both analyses brexpiprazole 4 mg reduced the PANSS hostility score relative to placebo at week 6 (LSMD = -0.32, p = 0.0034; LSMD = -0.33 p = 0.0033, respectively) while brexpiprazole 2 mg showed numerical improvement (LS mean change from baseline of -0.63 vs -0.51 for placebo; LS mean change from baseline of-0.58, vs -0.46 for placebo, respectively). When these 3 analyses were performed with patients with greater hostility at baseline (P7 ≥ 3; brexpiprazole 2 mg = 170, brexpiprazole 4 mg = 196; placebo n = 180), brexpiprazole 4 mg was superior to placebo in reducing the PANSS hostility item at week 6 (LSMD -0.34, p = 0.0080; LSMD = -0.33, p = 0.01; LSMD = -0.33, p = 0.0098).
**Conclusions:** The results of these analysis showed that brexpiprazole 2 and 4 mg/day improved symptoms of agitation and hostility compared to placebo. Improvement in hostility was specific, i.e., it was shown by covariate analysis to be independent of change in other positive symptoms as well as akathisia or akathisia and somnolence, and the degree of improvement was greater at 4 mg/day.
**Keywords:** Hostility, Agitation, Brexpiprazole
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All Financial Involvement with a pharmaceutical or biotechnology company, a company providing clinical assessment, scientific, or medical products or companies doing business with or proposing to do business with ACNP over past 2 years: Actavis (Forest), Alkermes, AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Forum, Genentech, Janssen, Jazz, Lundbeck, Merck, Medivation, Mylan, Novartis, Noven, Otsuka, Pfizer, Reckitt Benckiser, Reviva, Shire, Sunovion, Takeda, Teva, and Valeant
Income Sources & Equity of $10,000 per year or greater:
In the last 2 years: Actavis (Forest), Alkermes, Bristol-Myers Squibb, Eli Lilly, Forum, Genentech, Janssen, Jazz, Lundbeck, Merck, Mylan, Novartis, Noven, Otsuka, Pfizer, Reviva, Shire, Sunovion, Takeda, Teva
Financial Involvement with a pharmaceutical or biotechnology company, a company providing clinical assessment, scientific, or medical products or companies doing business with or proposing to do business with ACNP which constitutes more than 5% of personal income:
In the last 2 years: Actavis (Forest), Alkermes, Janssen, Lundbeck, Merck, Otsuka, Shire, Sunovion, Takeda
Emmanuelle Weiller is an employee of H. Lundbeck A/S, Valby
John Ouyang, Ross A. Baker and Catherine Weiss are employees of Otsuka Pharmaceutical Development & Commercialization, Inc.

## M188. The Transcription Factor Nuclear Factor-KB is a Molecular Hub of Cortical Immune Activation in Schizophrenia

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**Background:** Convergent lines of evidence indicate a key role of immune- and inflammation-related abnormalities in the pathophysiology of schizophrenia, including higher mRNA levels for multiple cytokines and for the viral restriction factor interferon-induced transmembrane protein (IFITM) in the prefrontal cortex (PFC) (Volk et al, Am J Psychiatry 2015 in press). The transcription factor nuclear factor-κB (NF-κB) directly regulates the expression of the same cytokines and IFITM variants that are overexpressed in schizophrenia, and we recently reported higher mRNA levels for two NF-κB family members (i.e. NF-κB1 and NF-κB2) in the PFC in schizophrenia. Furthermore, NF-κB transcriptional activity is initiated through multiple canonical and non-canonical pathways that involve a diverse collection of receptors, DNA binding inhibitors, and disinhibiting kinases. In addition, evidence of immune disturbances has also been reported for bipolar disorder (BP) and major depressive disorder (MDD). Consequently, we determined whether other NF-κB family members and components of canonical and non-canonical pathways that activate NF-κB are similarly over-expressed in the PFC in schizophrenia, BP, and MDD.
**Methods:** Quantitative PCR was employed to measure mRNA levels for NF-κB-related markers in PFC area 9 from 62 schizophrenia subjects individually matched to healthy comparison subjects for sex and age and also from 19 triads of BP, MDD and healthy comparison subjects.
**Results:** In schizophrenia subjects, we found higher mRNA levels for 1) other NF-κB family members, including RelA (+27%) and c-Rel (+19%) but not RelB, 2) multiple receptors that initiate NF-κB signaling (e.g., TLR4 [+27%], IL-1R [+71%], LTβR [+43%], CD40 [+24%], RANK [+7%]), 3) inhibitors that bind to, interfere with, and are themselves induced by NF-κB (e.g., IκBα [+53%], IκBβ [+8], but not IκBε) and 4) kinases that phosphorylate and allow IκBs to be degraded (e.g., IKKα [+8%], IKKβ [+12%], IKKγ [+8%]). Transcript levels for NF-κB2 (+39%) and c-Rel (+12%), but not other NF-κB-related markers, were higher in BP, while mRNA levels of NF-κB-related markers were not altered in MDD.
**Conclusions:** The convergent findings of higher mRNA levels for 1) multiple NF-κB family members, 2) canonical and non-canonical pathway markers that initiate NF-κB signaling, and

3) immune markers that are reported to be induced by NF-κB (i.e. IκBs, cytokines, and IFITM) suggest that NF-κB transcriptional activity is elevated in schizophrenia. Given the central role of NF-κB as a transcriptional regulator of immune activation, higher NF-κB activity may represent a molecular hub that promotes greater transcription of a wide array of immune-related markers in the PFC in schizophrenia but not in mood disorders. Taken together, these studies support further investigation into the potential role of NF-κB-related markers as therapeutic targets in schizophrenia. Finally, NF-κB-related mRNAs are currently being quantified in the frontal cortex of mice exposed to immune stimulation prenatally or in adulthood to determine whether alterations in NF-κB-related markers reflect a long-lasting disturbance in response to an immune-related insult that occurred in utero or ongoing immune activation in adulthood.
**Keywords:** schizophrenia, immune, neuroinflammation, Bipolar Disorder, Major Depressive Disorder
**Disclosures:** Nothing to disclose.

### M189. Exercise-Induced Effects on Neurocognition in Schizophrenia

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**Background:** Physical fitness is integral to the maintenance of both physical and cognitive health across the lifespan. Aerobic exercise has been linked to improvements in cognitive performance and neuroplasticity in regions vital to cognitive functioning, such as the hippocampus. This exercise-induced enhancement is in part modulated by endogenous levels of brain-derived neurotrophic factor (BDNF), a key player in synaptic plasticity and neuroprotection. Cognitive impairment is one of the defining characteristics of schizophrenia (Sz) and is associated with the substantial social dysfunctions seen in the disorder. In this preliminary study, we asked whether exercise can improve cognitive systems impaired in Sz. Deficits in declarative memory and executive functioning in Sz have been linked to alterations in structure and function of the hippocampus and prefrontal cortical networks. Using clinical, behavioral and neuroimaging techniques, our primary aim was to study the effect of exercise on hippocampal-dependent memory systems, as well as the effects on structural and functional changes in the hippocampus and other functionally related regions. A secondary aim was to examine exercise-related effects across a broader age range than previously studied to determine whether age moderates the impact of exercise on neurocognition in this population.
**Methods:** Ten veterans (1 female) between the ages of 30 and 67 (median = 56.0) were enrolled in a 12-week exercise program (3 times a week) that comprised 30 min of cardio workout and 30 min of strength training. Participants met DSM-5 criteria for schizophrenia or schizoaffective disorder and received baseline cognitive, clinical, neuroimaging and fitness assessments that were re-administered after 6 and 12 weeks. To assess fitness level, physiological measures were collected and aerobic capacity (Metabolic Equivalent of Task, or MET) was calculated pre- and post-intervention. Cognitive measures included a spatial memory task (Erickson et al., 2011), pattern separation task (Bakker et al., 2008), and the Dot Pattern Expectancy task (DPX; Jones et al., 2010), a measure of executive functioning. To assess exercise-induced neuroplasticity, structural (T1-weighted MPRAGE) and resting state (rs-fMRI) images were acquired on a 3T Siemens Trio, pre- and post-intervention. Serum BDNF levels were also quantified at baseline and endpoint visits.
**Results:** Nine of the 10 participants completed the intervention with a group mean attendance rate of 73%. There was a significant increase in MET from baseline to endpoint ($p = 0.02$). Behaviorally, veterans showed improvements in spatial memory from baseline to endpoint (Session x Item interaction, $p < .05$, t-test on 3-item condition, $p < .05$); however, no improvements were seen with the pattern separation task. We found significant improvements in an element of contextual processing, as measured by the DPX task ($p < .05$). Age did not modulate the behavioral effects. Structurally, no changes were seen from baseline to endpoint in hippocampal volume ($p > .05$). However, there was a significant negative correlation between change in hippocampal volume and age ($r = -.70$, $p < .05$), suggesting age may modulate exercise-induced changes in hippocampal volume. Functionally, resting state findings suggest an increase in connectivity between the hippocampus and middle frontal gyrus, which parallels the increase in performance in executive functioning. Serum BDNF levels did not change between baseline and endpoint ($p > .05$); however, change in BDNF was correlated with change in aerobic capacity ($r = .85$, $p < .01$) such that greater change in MET was a significant predictor of change in BDNF. This association was independent of age.
**Conclusions:** These data support the feasibility of implementing an exercise regimen to improve fitness and cognition in Sz. We replicated findings that exercise increases aerobic capacity in Sz and showed that an increase in serum BDNF was correlated with increased aerobic capacity. We further showed that spatial memory and components of executive functioning improved from baseline to endpoint, and paralleled increases in fronto-temporal connectivity. Structural findings, however, suggest that hippocampal plasticity may be modulated by age in this population suggesting that greater benefit may be seen if exercise programs are implemented earlier in the course of the disorder. The physiological and neurocognitive enhancements seen here provide the foundation for further exploration into the mechanisms underlying exercise-induced neuroplasticity in Sz and across the age span.
**Keywords:** physical exercise, neural plasticity, schizophrenia, neurocognition
**Disclosures:** Nothing to disclose.

### M190. Are Antipsychotics Neurotoxic or Neuroprotective? A Long-Term Comparison of Two Treatment Strategies: Study Design of the Multicenter APIC Trial

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**Background:** Continuation of antipsychotic drug treatment for at least 12 months after remission of the first psychotic

episode represents the gold clinical standard, and it is recommended by all international treatment guidelines. Numerous studies have shown that the risk of relapse is significantly increased, if drug treatment is terminated prematurely. However, only a minority of patients achieve functional remission, even if they fully comply with treatment. Long-term adverse effects of the currently available drugs, specifically brain grey matter loss and development of supersensitivity psychosis, might outweigh their benefits. Thus, the current standard of long-term maintenance antipsychotic treatment, which has the primary goal of relapse prevention, has to be questioned. Here we hypothesise that intermittent antipsychotic treatment (targeted exclusively at positive symptoms) initiated after the first psychotic episode is associated with less regional and global brain (grey matter) volume loss over a period of two years than continuous antipsychotic treatment. Furthermore, we hypothesise that this targeted treatment approach is associated with better functional outcome (fewer negative symptoms, better cognitive performance, better quality of life) than continuous antipsychotic treatment, although the latter is initially associated with fewer relapses.

**Methods:** All patients with first-episode schizophrenia admitted to a hospital belonging to the consortium will undergo a volumetric MRI, ideally before initiation of treatment. If antipsychotic treatment has to be started immediately for clinical reasons, the MRI will be acquired within three days of initiation of antipsychotic treatment. Informed consent for the initial MRI and the treatment phase of the trial will be obtained separately. Patients will be treated according to clinical standards. After randomisation into one of the two treatment arms - maintenance treatment (control) or intermittent treatment (experimental) -, patients in the experimental arm will be tapered off medication. All patients will be regularly seen by the study personnel. Volumetric MRI studies will be repeated after 6 and 12 months (with 12 months being the primary endpoint). A long-term follow-up of all patients (beyond the present trial, 24 months minimum) is intended to obtain information about recovery rates and long-term outcome.
Patient recruitment started in June 2015.

**Results:** Various volumetric measures obtained with MRI have been used in patients with first-episode schizophrenia. These include total brain volume, total grey matter volume, grey matter volume in certain subregions of the brain (prefrontal cortex, hippocampus), white matter volume, and ventricular volumes (total, third, lateral ventricles). The most robust findings were obtained with total grey matter volume as primary endpoint. Grey matter volumes in brain regions with special importance for schizophrenia (prefrontal cortex, hippocampus) are secondary endpoints. The available literature suggests that functional outcome of schizophrenia is related to structural brain changes.
Other secondary endpoints chosen for this study are well-established variables for assessment of outcomes of schizophrenic psychoses. The Positive and Negative Syndrome Scale (PANSS) and the Clinical Global Impression Scale (CGI) belong to the most often used scales for the assessment of psychopathology in psychiatric research. The SF-36 is a short-form health survey that has been used in thousands of clinical trials including hundreds in psychiatric populations. The Brief Assessment of Cognition in Schizophrenia (BACS) scale is a comprehensive scale for the assessment of cognitive deficits associated with schizophrenia. It correlates with larger batteries such as the MATRICS and its German version has been validated.

**Conclusions:** Here the study design of the first prospective, randomized study, which compares the effects of maintenance with intermittent antipsychotic drug treatment on brain structure and functional outcome in schizophrenia, will be presented.

**Keywords:** Antipsychotic-naïve first-episode schizophrenia, Structural MRI, Antipsychotic Treatment

**Disclosures:** Dr. Gründer has served as a consultant for Cheplapharm (Greifswald, Germany), Eli Lilly (Indianapolis, Ind., USA), Lundbeck (Copenhagen, Denmark), Roche (Basel, Switzerland), Servier (Paris, France), and Takeda (Osaka, Japan). He has served on the speakers' bureau of Eli Lilly, Gedeon Richter (Budapest, Ungarn), Janssen Cliag (Neuss, Germany), Lundbeck, Roche, and Servier. He has received grant support from Boehringer Ingelheim (Ingelheim, Germany) and Roche. He is co-founder of Pharma Image GmbH (Düsseldorf, Germany) and Brainfoods UG (Selfkant, Germany).

### M191. White Matter Neurons Numbers and Densities Across the Human Lifespan, Including the Potential Role(s) of Antipsychotic Drugs

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**Background:** Increased numbers and densities of subcortical white matter neurons (WMN) have been reported for a subset of cases diagnosed with schizophrenia and in non-human primates chronically exposed to antipsychotic drugs (APD). The underlying cellular and molecular mechanisms remain unresolved: This alteration could reflect a fixed lesion of early brain development, but it is not known whether there is ongoing dynamic regulation of WMN numbers in the adult brain.

**Methods:** We determined the proportion of neuronal nuclei in frontal lobe white matter of 104 brains, including 65 controls from newborn to 93 years of age and 39 cases diagnosed with schizophrenia from 22 to 87 years of age. Based on the results – an overall decline in WMN over the lifespan but an increased proportion of WMN in a subset of patients with schizophrenia - we subsequently explored the effect of antipsychotic drugs.
26 adult macaque monkeys were exposed for six months to either clozapine, haloperidol or placebo, and measured by structural MRI frontal gray and white matter volumes before and after treatment, followed by observer-independent, flow cytometry-based quantification of neuronal and non-neuronal nuclei and molecular fingerprinting of with cell-type specific transcripts.

**Results:** In human subjects, normal aging was associated with a decline in the WMN ratio after early childhood. Interestingly, 15% (6/39) subjects with schizophrenia, exceeded the 99th percentile of controls. Normal aging was associated with a decline in the WMN ratio after early childhood whereas the excess WMN in schizophrenia showed no association with age.
After clozapine exposure in non-human primates, the WMN ratio increased in subcortical white matter, in conjunction with a subtler but not significant increase in overlying gray matter. Numbers and proportions of nuclei expressing the oligodendrocyte lineage marker, OLIG2, and cell-type specific RNA expression patterns, were maintained after APD exposure. Frontal lobe gray and white matter volumes remained indistinguishable between APD and control groups.
**Conclusions:** Frontal lobe WMN are subject to decline during the course of normal maturation. A subset of subjects with schizophrenia has increased WMN independent of age. In non-human primates, chronic clozapine exposure increases the proportion of WMN in frontal subcortical white matter pointing towards a modulatory effect of certain antipsychotics on prefrontal cell composition.
**Keywords:** Schizophrenia, White Matter, Antipsychotic Treatment, Clozapine
**Disclosures:** Nothing to disclose.

### M192. Odor Deficits in Chronic Schizophrenia: Relationship to Cognition and Symptoms

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**Background:** Deficits in Odor identification and discrimination have been found in previous studies of patients with schizophrenia (SZ), and structural and functional abnormalities in the olfactory system of schizophrenia are well documented. Some studies have reported relationship between odor deficits in SZ and negative symptoms. We investigated these questions in a new sample of chronic SZ and controls who are also participating in a study of epigenetic markers in the lymphocytes of SZ. Sequencing of activation and silencing of gene methylation by methylating enzymes (DNMT1 3a) and change in histone acetylation may be involved in epigenetic modifications of the development of the olfactory system.
**Methods:** In this initial group of sample we studied 34 patients with chronic SZ and 23 controls. Odor identification discrimination was assessed with Sniff n Sticks smell test battery for Odor Identification and Odor Discrimination. Current psychiatric symptoms were assessed with PANSS interview. Cognition was assessed by MATRCIS battery. A blood sample was drawn for measurement of mRNA of enzyme related to methylation of genes (DNMT, TETT 1)and genes products heavily regular by promoter methylation( BDNF, glucocorticoid receptor),although assays on these samples have not been completed at this time.
**Results:** SZ had significantly ($P<0.01$)lower scores than controls on the small identification and discrimination tests of the Sniff n Sticks battery. However, on the odor identification, but not on discrimination, there was a significant sex effect, with identification being deficient in male SZ but not females. There were no differences between male vs. female controls on the odor tests. There was a moderate statistically significant association between scores on odor discrimination and scores on the MATRICS battery (composite $r=0.39$ $P=0.02$, and working memory $r=0.44$ $P=0.01$), but this association was stronger in females than males. In controls there was only a significant association with higher odor identification with the scores on the MCCB domain of attention vigilance and no sex difference. In SZ patients poorer performance on odor discrimination was associated with higher scores on Negative symptoms ($r=0.51$, $P<0.01$), and higher scores on PANSS Total ($r=0.39$, $P=<0.03$) and no strong differences in effects in males vs. female SZ. Relationships to biological markers are being investigated.
**Conclusions:** This current research confirms the previously reported deficits in olfaction in schizophrenia, and the relationship of olfactory deficits to negative symptoms. It suggests that sex differences may be important in odor identification test, but not the odor discrimination test in SZ. This is the first study to show olfactory deficits related to performance on the MATRCIS battery and suggest that there may be a sex difference in the strength of this relationship.
**Keywords:** schizophrenia, olfactory, cognition
**Disclosures:** Nothing to disclose.

### M193. Switching Patients with Acute Schizophrenia to Brexpiprazole: Post-Hoc Analysis of a Double-Blind Randomized Maintenance Treatment Study

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**Background:** The management of schizophrenia requires effective and tolerable treatments to improve outcomes and reduce the risk of relapse. Brexpiprazole is a serotonin-dopamine activity modulator that is a partial agonist at 5-HT1A and dopamine D2 receptors at similar potency, and an antagonist at 5-HT2A and noradrenaline alpha1B/2C receptors. The efficacy and safety of brexpiprazole for the treatment of adults experiencing schizophrenia was demonstrated in both two 6-week and one maintenance phase 3 trials.
As patients were washed-out from prior antipsychotics as part of the protocol in the short-term pivotal brexpiprazole studies, there are no available data to guide switching to brexpiprazole for acutely ill patients. Here we report the rate of adverse events in a group of patients who were cross-titrated from a previous antipsychotic to brexpiprazole during the conversion phase of the maintenance treatment trial [NCT01668797].
**Methods:** Patients experiencing an acute exacerbation (PANSS total score $>80$) of schizophrenia were cross-titrated from current antipsychotic treatment(s) to brexpiprazole as part of the study protocol before entering a 12 to 36 weeks single-blind stabilization phase on brexpiprazole (1 to 4 mg). Brexpiprazole 1 mg/day was initiated, in

addition to other oral antipsychotic treatment(s), on day 1 of the conversion phase. Other oral antipsychotic treatment(s) were gradually reduced while brexpiprazole was increased in 1-mg increments at scheduled visits to a maximum of 4 mg/day. The cross-titration was recommended by protocol to be a minimum of 1 week and a maximum of 4 weeks at the discretion of the investigator. In order to enter the stabilization phase, subjects must have been able to tolerate a minimum dose of brexpiprazole 1 mg/day.

**Results:** A total of 406 patients entered the conversion. Four hundred and four patients received at least one dose of brexpiprazole and had a mean PANSS total score of 91.1 at the start of the conversion phase; 89% of patients completed the conversion phase (346/387, excluding 19 patients withdrawn by sponsor due to termination of study following positive interim analysis). Two percent (8/406) of patients discontinued the conversion phase due to adverse events. Fifty-two percent of patients (212/406) were cross-titrated over 4 weeks, while 13.3% (54/406), 10.3% (42/406), and 4.2% (17/406) of patients were cross-titrated over 3, 2, or 1 week, respectively. An additional 19.5% (79/406) were cross-titrated over a period greater than 4 weeks. During the 4 weeks of initial brexpiprazole exposure (including cross titration with prior antipsychotic in the conversion phase and/or titration of brexpiprazole alone during initial stabilization phase), the rates of treatment emergent adverse events were similar to what was observed in the 6-week pivotal studies. The average final dose of brexpiprazole after 14 weeks of treatment was 3.2 mg/day.

**Conclusions:** When given guidance that a 1 to 4 week cross-titration from previous antipsychotics should be followed prior to patients entering the stabilization phase of a long-term maintenance study, the vast majority of investigators chose to cross-titrate brexpiprazole over a period of at least 4 or more weeks. Despite the low numbers of patients who were cross-titrated over a 1 or 2 week period, the data provide an evidence base from which clinicians can choose a switching paradigm that best meets their patient's needs.

**Keywords:** Schizophrenia, Brexpiprazole, Antipsychotic switching, Tolerability

**Disclosures:** Catherine Weiss, John Ouyang and Ross A. Baker are employees of Otsuka Pharmaceutical Development & Commercialization, Inc. Ruth A. Duffy is an employee of Otsuka America Pharmaceuticals, Inc. Anna Eramo is an employee of Lundbeck LLC. Emmanuelle Weiller is an employee of H. Lundbeck A/S.

### M194. Oxidative Stress in the Pathophysiology of Psychiatric Disorders: Studies of Patient Biospecimens and Animal Models

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**Background:** Oxidative stress is implicated in the pathophysiology of psychiatric disorders from studies of patient biospecimens and animal models. However, it is unclear how changes in key mediators of oxidative stress are linked to clinical features such as symptom severity or neurocognitive function. We first examined human patients to determine whether neuronal circuits for cognition and negative symptoms are associated with oxidative stress in the pathophysiology of schizophrenia (SZ) and bipolar disorder (BP), particularly through superoxide dismutase-1 (SOD1) and glutathione (GSH). While human studies are essential for elucidating the pathophysiology of a human disease or condition, animal models are also useful for linking the pathophysiology to the pathological trajectory during neurodevelopment and identifying molecular changes associated with neuronal circuity disturbance and behavioral deficits. Thus, we examined stress-associated molecules affecting oxidative stress, the HPA axis, and the immune system in animal models of psychiatric disorders.

**Methods:** All participants in the Hopkins cohort completed a two-hour battery of neuropsychological tests to assess cognitive function. Peripheral whole blood and cerebrospinal fluid (CSF) samples were collected from each participant. SOD1 levels in CSF were assessed by western blot analysis. Total GSH [the sum of GSH and glutathione disulfide (GSSG)] was measured in plasma using modifications of the Tieze method. In the animal models, we performed a protein carbonyl assay using oxyblot protein oxidation detection kit to monitor the general level of oxidative stress in the prefrontal cortex (PFC) at 8 weeks of age. We also measured levels of other stress-related molecules such as corticosterone in serum and cytokines in the PFC at 8 weeks of age using an enzyme immune assay and mesoscale multiplex kits, respectively.

**Results:** We found a reduction of SOD1 in the CSF of patients with psychosis in comparison to healthy controls. Reduced SOD1 correlated with poor neurocognitive function. We also observed a reduction of GSH in plasma from patients with SZ and BP in comparison to healthy controls. In our analysis of animal models, protein carbonyls were increased in the PFC of disrupted-in-schizophrenia 1 (DISC1) and pericentriolar material 1 (Pcm1) mutant mice when compared to wild-type mice. We also observed increased levels of corticosterone in serum, and increased levels of interleukin-10 (IL-10) and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) in the PFC of DISC1 mutant mice in comparison to wild-type mice.

**Conclusions:** Our clinical and preclinical studies support the notion that intrinsic susceptibility to oxidative stress may underlie the pathophysiology of psychiatric disorders. With the current findings from patient biospecimens and animal models, we next need to examine mechanisms of how and when stress-associated cascades affect neuronal circuits that result in behavioral deficits after puberty.

We may develop novel strategies for the intervention and treatment of psychiatric disorders by targeting stress-associated cascades including oxidative stress.

**Keywords:** oxidative stress, psychiatric disorders, pathophysiology

**Disclosures:** A fund from Mitsubishi Tanabe Pharma Corporation was partly used for recruitment of 12 healthy

controls. Daisuke Fukudome is employed by Mitsubishi Tanabe Pharma Corporation.

### M195. Complex Genetic Overlap Between Schizophrenia Risk and Antipsychotic Response

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**Background:** Treatments for schizophrenia (SCZ) exist but do not alleviate symptoms for all patients and efficacy is limited by common, often severe side effects. Large-scale genetic studies of both rare and common variation have increased the number of genes and gene sets associated with SCZ risk. However, among the many important remaining questions is how these findings can inform and improve treatment. We hypothesize that genes implicated by genetic studies and those involved in therapeutic efficacy will overlap and by intersecting this information we can further our understanding of both the pathogenesis of the disorder and the manner in which to treat it.
**Methods:** We defined SCZ risk loci as genomic regions reaching genome-wide significance in the latest Psychiatric Genomics Consortium schizophrenia genome-wide association study (GWAS) and loss of function variants with only a single time among 5,079 individuals in an exome-sequencing study of 2,536 SCZ cases and 2,543 controls. Using two comprehensive and orthogonally created databases, we collated drug targets into 167 gene sets of pharmacologically similar drugs and examined enrichment of SCZ risk loci in these groups of drug targets. In addition, we assessed the contribution of rare variants to treatment response.
**Results:** We identified significant enrichment of SCZ risk loci from both common and rare variation in known targets of antipsychotics (70 genes, $p = 0.0078$), and novel predicted targets (277 genes, $p = 0.019$). Furthermore, treatment resistant patients had a significant excess of rare disruptive variants in gene targets of antipsychotics (347 genes, $p = 0.0067$) and in genes with evidence for a role in antipsychotic efficacy defined by PharmGKB (57 genes, $p = 0.0002$).
**Conclusions:** Our results support genetic overlap between SCZ pathogenesis and antipsychotic mechanism of action. This finding is consistent with treatment efficacy being polygenic in nature and not solely moderated by the dopamine and serotonin receptors thus implying that single target therapeutics may be insufficient. We further provide evidence of a role for rare functional variants in antipsychotic treatment response. We present this approach as a framework for enhancing both the understanding of treatment mechanism of action and disease pathology of complex disorders.
**Keywords:** pharmacogenomics, schizophrenia, treatment response
**Disclosures:** Nothing to disclose.

### M196. Dysregulation of the Actin Cytoskeleton Contributes to Dendritic Arbor Pathology in Schizophrenia and Bipolar Disorder

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**Background:** We recently reported decreased basilar dendritic spine density and dendrite length on deep layer III pyramidal cells in the dorsolateral prefrontal cortex (DLPFC) from subjects with schizophrenia and bipolar disorder. Since the actin cytoskeleton plays a key role in the regulation of dendritic spines and dendrite morphology, we assessed actin cytoskeletal regulatory proteins to identify those involved in the observed dendritic arbor pathology.
**Methods:** Based on our recent qRT-PCR study, previously obtained microarray data, and a review of the literature, we identified 13 candidate proteins namely: MARCKS, dysbindin-1, neurabin-I, spinophilin, CDC42, CDC42BPA, par-3, alpha-PIX, BDNF, trkB, kalirin, oligophrenin-1, and serine racemase. Using western blotting, protein expression was assessed in the DLPFC (BA 46) grey matter from subjects with schizophrenia ($n = 19$), bipolar disorder ($n = 17$) and unaffected control subjects ($n = 19$). Protein expression data were then correlated with dendritic parameter data obtained previously in many of the subjects.
**Results:** Relative to controls, the expression of the 75 kDa isoform of alpha-PIX (ARHGEF6) was increased in schizophrenia subjects ($p = 0.05$). Expression of the alpha-PIX 75 kDa isoform was inversely correlated with dendrite length ($r = -0.32$, $p = 0.04$) and the number of spines per dendrite ($r = -0.31$, $p = 0.05$). CDC42BPA expression was also increased in schizophrenia ($p = 0.02$), but did not correlate with dendritic parameters. Expression of the 1b isoform of dysbindin (DTNBP1) was increased in bipolar disorder subjects ($p = 0.03$) and was inversely correlated with dendrite length ($r = -0.31$, $p = 0.05$) and the number of spines per dendrite ($r = -0.31$, $p = 0.05$). Phospho-MARCKS expression was also increased in bipolar disorder ($p = 0.02$), but did not correlate with dendritic parameters. Expression of the other proteins did not differ significantly among groups.
**Conclusions:** The regulation of the actin cytoskeleton is altered in both schizophrenia and bipolar disorder. However, the source of the dysregulation differs between the two groups. alpha-PIX appears to be involved in dendritic arbor pathology in schizophrenia whereas, dysbindin is implicated in bipolar disorder. Elucidation of the similarities and difference between the neuropathology of schizophrenia and bipolar disorder, especially related to dendritic arbor pathology, could lead to the identification of novel biomarkers for diagnostics and therapeutics.
**Keywords:** dendrites, DLPFC, Postmortem Brain Tissue, molecular mechanisms
**Disclosures:** Nothing to disclose.

### M197. DNA Methylation as a Mechanism for Altered Glutamatergic Signaling in the Superior Temporal Gyrus of Individuals with Schizophrenia

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**Background:** Reduced dendritic spine density on glutamatergic neurons is among the most consistently observed findings in postmortem studies of individuals with schizophrenia (SCZ), affecting multiple brain regions including the superior temporal gyrus (STG). Impaired glutamatergic signaling is believed to underlie dendritic spine loss in the STG and auditory symptoms in individuals with SCZ. We recently showed that proteins that were differentially expressed in the STG of individuals SCZ were enriched in glutamate signaling pathway proteins. Studies in model systems have demonstrated that alterations in DNA methylation (DNAm) alter expression of glutamate signaling pathway proteins. Here, we explore the potential contribution of DNAm alterations to changes in expression of glutamate signaling pathway proteins observed in the STG of individuals with SCZ.
**Methods:** The Illumina Infinium HumanMethylation450 Beadchip Array was used to quantify genome-wide DNAm in the STG of postmortem brains from 22 individuals with SCZ and 22 age-matched individuals without DSM-IV psychopathology. The normalized Beta values for each CpG site (adjusted for age, sex, and PMI) of duplicate samples were averaged for all subjects within a group and a two-sample t-test between groups was performed and corrections for multiple comparisons made. All subjects used in this study have been extensively characterized with respect to dendritic spine density, synaptic protein expression profile, and polygenic risk for SCZ.
**Results:** Evidence for multiple sites of differential DNAm between individuals with SCZ and age-matched individuals without DSM-IV psychopathology was found. Among the most significantly altered CpG sites were those in genes that are part of the glutamatergic signaling pathway (e.g., GRIA1, CaMK2Beta). Additional regional analyses of differential DNAm are ongoing.
**Conclusions:** DNAm may be a mechanism for genome-wide SCZ-related changes in gene and protein expression. Future analysis will explore our a priori hypothesis that these regions of differential methylation are enriched among the genes of the glutamatergic signaling pathway and inversely correlated with expression of proteins in the glutamatergic signaling pathway.
**Keywords:** DNA Methylation, Postmortem Brain Tissue, glutamate receptor activity, Auditory cortex, schizophrenia
**Disclosures:** Nothing to disclose.

### M198. Prefrontal GABAergic and NMDA Glutamatergic Regulation of Working Memory During Delayed Responding: Modulation by D1 Receptor Stimulation

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**Background:** Cognitive dysfunction associated with schizophrenia has been proposed to result from deficiencies in GABA and/or NMDA receptor-mediated glutamate transmission within the frontal lobes. Preclinical work from our group and others has shown that antagonism of prefrontal GABA-A or NMDA receptors leads to cognitive abnormalities relevant to schizophrenia in rodents, including impairments in working memory and attention. Conversely, dopamine D1 receptor agonist drugs have been shown to enhance attention and working memory and may reverse disinhibitory effects of deficient GABA or NMDA receptor function by augmenting interneuron excitability. Delay-independent deficits in working memory have consistently been found in schizophrenic patients performing spatial delayed-response tasks. Thus, the goal of this study was first to address the nature of the impairment induced by diminished prefrontal GABA and NMDA receptor signaling in a delayed-response task in rats, and then to address whether these impairments could be rescued by stimulation of dopamine D1 receptors.
**Methods:** Separate cohorts of male Long Evans rats were trained in an operant delayed non-match to position (DNMTP) task. The tasks consisted of a sample phase in which one lever is extended, and a choice phase in which the subject must select the opposite lever, separate by a variable delay (1-24s). Well-trained animals were implanted with bilateral cannulae in the medial prefrontal cortex (PFC). On test-days, animals received counter-balanced intra-PFC infusions of either saline or the GABA-A receptor antagonist bicuculline (12.5-50 ng), saline or the NMDA receptor antagonist MK-801 (3-6 ug), or counter-balanced infusions of either saline or bicuculline (50 ng) and injections of either saline or the dopamine D1 receptor agonist SKF 81297 (0.05-0.3 mg/kg, i.p.).
**Results:** Prefrontal GABA-A antagonism induced a delay-independent deficit in task performance at both doses. Antagonism of prefrontal NMDA receptors also induced delay-independent deficits in performance, but only at a higher dose. Concomitant administration of a moderate dose of D1 agonist (0.1 mg/kg) appeared to reverse impairments in working memory induced by intra-PFC bicuculline.
**Conclusions:** In conclusion, these results demonstrate that prefrontal NMDA and GABA-A receptor antagonism leads to working memory impairments qualitatively similar to those observed in schizophrenia. Further, agents that enhance D1 receptor function appear to reverse these deficits, suggesting that enhancement of D1 receptor signaling may represent a novel treatment strategy for cognitive deficits observed in schizophrenia.
**Keywords:** schizophrenia, working memory, glutamate GABA, Dopamine
**Disclosures:** Nothing to disclose.

### M199. Brain Bioenergetics Measured by 31P Magnetization Transfer Spectroscopy in Unaffected First-Degree Relatives of Patients with Psychotic Disorders

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**Background:** Psychotic disorders have substantial inherited determinants, and some of these can be studied in

unaffected first-degree relatives. Of the determinants for the schizophrenias and bipolar disorders, several lines of evidence from in vivo, in vitro and postmortem studies indicate specific brain bioenergetic and mitochondrial abnormalities. The present study examined brain bioenergetics in unaffected siblings of patients with psychotic disorders using a technique previously successfully applied in probands with illness: 31P magnetic resonance spectroscopy magnetization transfer (31P-MT-MRS).
**Methods:** The forward reaction rate constant (kf) of the creatine kinase (CK) enzyme was measured using 31P-MT-MRS in the frontal lobe in unaffected siblings, first episode psychosis patients and healthy unrelated controls. Brain parenchymal pH and steady-state metabolite ratios of high energy phosphate-containing compounds (PCr and ATP), inorganic phosphate, and membrane phospholipids PDE and PME were also measured. Participants underwent clinical evaluation with the Structured Clinical Interview for DSM-IV-TR, the MATRICS Consensus Cognitive Battery, Wisconsin Schizotypy scales and Extrapyramidal Symptom Rating Scale. 31P-MT-MRS acquisitions were obtained with a 4T whole-body scanner interfaced with a Varian INOVA console, using a custom-designed prefrontal surface coil tuned to the 31P resonance frequency for collection of 31P-MT-MRS data and a quadrature 1H surface coil for imaging and shimming. All study procedures were approved by the McLean Hospital Institutional Review Board and participants provided written informed consent.
**Results:** 19 unaffected siblings, 31 first episode psychosis patients and 8 healthy controls have participated thus far, including 5 male and 14 female unaffected siblings, 27 males and 4 females with first episode psychosis, and 4 male and 4 female healthy controls. The mean age is 24.47 (SD = 5.75) for unaffected siblings, 22.29 (SD = 2.83) for first episode psychosis patients and 24.62 (SD = 3.29) for controls. In a previous study of patients with schizophrenia (n = 26), we found a significant reduction of 22% of CK kf and a reduction in intracellular pH, compared to healthy individuals (n = 26). In the present study, based on the multifactorial inheritance of psychiatric disorders, unaffected siblings are expected to show a significant reduction in brain CK kf compared to controls, but these reductions in CK kf are expected to be of lesser magnitude than those seen in patients. Exploratory analyses will be carried out on other available imaging measures (pH, PCr, and steady state ratios of HEP-containing metabolites), and on the relationship of imaging measures to functional status, psychosis predisposition measures and symptom measures. Relatives with higher scores on psychosis predisposition measures, lower functional status and cognitive dysfunction are expected to have significantly greater reduction in brain CK kf.
**Conclusions:** Our study was designed to provide insights into familial abnormal brain bioenergetics in individuals at risk for psychotic disorders, using a novel 31P-MT-MRS approach. As the brain is a high user of energy, deficits in energy production and use are one factor that appears to underlie risk for many brain disorders, including psychotic disorders.
**Keywords:** Bioenergetics, schizophrenia, bipolar, relatives, at-risk

**Disclosures:** Dost Ongur (Scientific Advisory Board for Lilly in 2013); Guy Chouinard (1 Invited guest lecture by Otsuka Pharmaceutical in 2014).

## M200. Human Factor Evaluation of a Novel Digital Health Feedback System in Psychiatry

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**Background:** Poor adherence to medication is a well-established problem for psychiatric patients and has been consistently associated with poor health outcomes. Despite poor outcomes, deviations from adherence remain very difficult to detect in practice. The Digital Health Feedback System (DHFS) is a new class of combination drug-devices developed for patients with psychiatric disorders that objectively measures and reports a patient's medication ingestion to detect non-adherence to oral anti-psychotics. The DHFS consists of a medication embedded with an ingestible sensor, wearable sensor, and software applications that enable the secure collection and sharing of objective medication adherence information using a patient mobile interface (i.e., patient app) and corresponding web-based interface (dashboard) for healthcare professionals (HCPs) and caregivers. In the process of developing this technology it is important to minimize use-related hazards and risks, and demonstrate that the 3 intended groups of users (patients, HCPs, and caregivers) can properly use the DHFS. This is particularly critical for psychiatric patients since these illnesses are often characterized by patient deficits in the domains of cognition and functional skills. Such impairments in schizophrenia (SCZ), major depressive disorder (MDD) and bipolar disorder (BPD) may compromise safe and effective use of the device. The objective of the Human Factors (HF) testing was to assess the safe and correct use of the system by the intended users for the intended uses following the method recommended in the FDA guidance for developing a safe and effective drug-device system.
**Methods:** The FDA requires both objective and subjective qualitative data to prove safe and effective use. Unlike clinical trials, HF studies are based on qualitative data. Per FDA and Human Factors guidance all tasks considered critical to safety or essential for effective use were tested with the intended users (patients, HCPs, and caregivers). Risks to the users were identified. The results of these iterative (or formative) studies identified areas for improvement which were then used to implement design modifications to the patient app and electronic instructions for first use. The final system was then validated through simulated use testing with the intended users on both the patient-facing and HCP/caregiver-facing portions of the final product. The validation goal for each task is to demonstrate that the risks have been reduced, effectiveness achieved and to show that if any risks remain, they are outweighed by the benefits.

**Results:** During the summary validation studies for the patient portion of the system 32 tasks were tested. Remaining risks were analyzed in accordance with FDA Guidance (Applying HF&UE to Optimize Medical Device Design, 22 Jun 2011) and Human Factors standards (ANSI/AAMI HE75:2009). The residual risks were found to be low and outweighed by the benefits of the overall system. The portion of the system accessed by the healthcare professional and caregivers tested 11 tasks. Analysis found the residual risk, based on HF standards and guidance, to be minimal and outweighed by the benefits of the system.
**Conclusions:** An analysis of the validation study results and residual risks suggests that the DHFS is safe and effective when used by its intended population of patients with schizophrenia, bipolar 1 disorder, and major depressive disorder, their caregivers, and their healthcare professionals. The final design reflects a development methodology informed by the intended user populations, and includes design modifications to address limited cognitive functioning present in some of the patients in these diagnostic categories through design simplicity, limiting alternatives to completing a task, and directing attention to relevant information. As a result, the DHFS represents a novel approach to objectively determine and provide adherence information to the patients, HCPs and caregivers.
**Keywords:** Digital Health Feedback System, Human Factors Testing, Serious Mental Illness
**Disclosures:** Timothy Peters-Strickland, Ainslie Hatch, Benjamin Bartfeld, Linda Pestrich, Shashank Rohatagi, Felicia Forma and John Docherty are employees of Otsuka. Jane Lea Smith is an employee of GfK contracted by Otsuka for this work and Praveen Raja is an employee of Proteus.

## M201. Missense Mutations in Four Genes Underlie Phenotypically Distinct Subtypes of Psychosis, Accounting for >30% of Cases in an Ethnically Diverse Research Sample

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**Background:** GWAS studies in schizophrenia have not yielded targets for person-specific interventions. Alternatively, studies can focus on genes that were initially identified as harboring disruptive de novo mutations in sporadic cases. We examined the impact of four such genes on illness phenotypes.
**Methods:** Structured interviews (DIGS), cognition (WAIS III), symptoms (PANSS) were examined in 48 genotyped cases finding that over 30% of the sample carried a rare/missense mutations in any of 4 genes. Gene carrier groups were compared to cases without any of these mutations and healthy controls.
**Results:** Carriers of disrupted genes showed significant differences, as follows: SLC39A13 (zinc transporter) (n = 4) had the greatest psychopathology and severe cognitive deficits; TGM5 (n = 4) had fewer symptoms but slower processing speed; PTPRG (n = 5) had prematurity, childhood psychosis and good cognition except poor working memory; ARMS/KIDINS220 (n = 5) had comparable severe pathology in all symptom factors and cognitive scores, though degeneration is suggested in light of their early accomplishments. Individual case vignettes highlighted familial psychosis, learning disorders, substance abuse, traumatic brain injuries and medical comorbidity in all 4 subgroups.
**Conclusions:** The results suggest that genes prone to de novo mutations in sporadic cases may provide missing leverage to resolve the complexity of schizophrenia. A differential focus on working memory, processing speed, neuroprotection and zinc treatment should be pursued for these newly identified conditions. Other findings are that ethnicity may not limit genetic research when the focus is on gene function rather than particular sequence variations, and that premorbid exposures may sometimes reflect pleiotrophic effects of psychosis vulnerability genes rather than exposures producing nongenetic phenocopies. This novel approach may be applicable to other complex disorders.
**Keywords:** de novo mutation, schizophrenia, Phenotypes
**Disclosures:** Nothing to disclose.

## M202. Altered Temporal Patterns of Prefrontal BDNF During Decision Making Shifts

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**Background:** Brain-derived neurotrophic factor (BDNF) exerts neuromodulatory effects on synaptic transmission, and is essential for actively regulating learning, memory, and motivational processes. Recent evidence suggests that intrastriatal BDNF infusion into the dorsal striatum regulates cognitive flexibility by altering cortiostriatal glutamatergic transmission (D'Amore et al., 2013). However, the role of endogenous BDNF signaling in flexible decision-making remains unknown. Activity-dependent alterations in BDNF expression is considered to be a key event in synaptic plasticity and cognition. As frontostriatal circuits involving discrete regions of the prefrontal cortex (medial: mPFC and orbital: oPFC) and striatum, are critical in maintaining different forms of cognitive flexibility such as extradimensional shifting and reversal learning, we hypothesized that shifting to new strategies would produce neuronal activity-dependent alterations in BDNF expression in these circuits.
**Methods:** Male C57BL/6J mice were trained in an operant task that required the animals to shift a visual cue-based strategy to an egocentric spatial response-based strategy. Another cohort of mice was trained on reversal learning where they initially acquired a response-based discrimination and were then required to flexibly adapt to the reversal of stimulus-response contingencies. Animals assigned to the behavioral control groups were exposed only to the initial discrimination stage of the task. Brains were removed either during the initial learning (early phase) or after complete acquisition (late phase) of the new strategy to conduct quantitative immunohistochemical examination of BDNF and c-fos (a marker of neuronal activation) expression in

the cortical regions of interest. BDNF expression in the striatal synaptosomes was examined using immunoblotting.
**Results:** BDNF-immunopositive cell counts increased in all subdivisions of the oPFC (ventral, medial and lateral regions) and mPFC (anterior cingulate, prelimbic and infralimbic regions) following the strategy as well as reversal shifts (main effect: all $F(2,42) > 35.37$, $p < 0.001$). Moreover, a significant time x behavioral shift interaction was observed (all oPFC/mPFC regions: $p < 0.006$). During the early phase, an overall increase in BDNF-positive cells was noted in all regions irrespective of the shift type. Additionally, synaptic BDNF levels also increased in the striatum (ventral: $F(2,14) = 3.64$, $p < 0.05$; dorsal: $F(2,15) = 6.30$, $p < 0.05$) indicating elevated overflow of prefrontal BDNF to target striatal regions. However, BDNF effects were dissociated based on the shift type following task acquisition. Reversal learning was mostly associated with higher BDNF in the oPFC while medial prefrontal regions exhibited elevated BDNF expression during strategy shifting. Double immunostaining revealed performance-related increases in BDNF/c-fos co-labelling in the oPFC and mPFC regions, respectively (all $p < 0.001$).
**Conclusions:** Flexible decision-making produces temporal alterations in the frontostriatal BDNF expression. Moreover, the dissociation between cortical region-specific neuronal activity and BDNF levels based on higher-order and lower-order behavioral shift becomes apparent only after the strategy for optimal performance is acquired. As BDNF regulates synaptic transmission, prefrontal BDNF alterations may play a critical role in modulating corticostriatal activity to maintain shifts in strategies with changing environmental demands. Deficits in frontoexecutive processes such as cognitive flexibility are associated with major neuropsychiatric disorders such as schizophrenia, addiction and depression. Therefore, BDNF may serve as a neurobiological substrate for a neurocognitive endophenotype common to multiple psychiatric conditions.
**Keywords:** Decision Making, Major Psychiatric Disorders, BDNF, Prefrontal cortex, Mice
**Disclosures:** Nothing to disclose.

### M203. Hyperactivation of Salience Network during Eye Gaze Perception in Schizophrenia

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**Background:** The ability to process social information accurately and effectively is disrupted in schizophrenia (SZ), severely affecting social functioning. Abnormal processing of eye gaze direction—a ubiquitous social cue—significantly accounts for deficits in broader social functioning in SZ. Few studies have examined the neural mechanisms underlying altered gaze perception in SZ. This study aims to identify altered neural circuitry of gaze perception in SZ, which may provide useful treatment targets for future therapeutic strategies.
**Methods:** Twenty-one individuals with SZ (age: 31.9 ± 10.4; 10M/11F) and 20 healthy controls (HC) (age: 32.2 ± 14.0; 10M/10F) completed the study. Gaze perception was probed with a psychophysics eye-contact perception task during BOLD fMRI. The task was presented in a blocked-event-related design, with stimuli of faces with 9 varying gaze directions (from averted to direct in gradual increments). Participants had to indicate by pressing a button whether they feel the face is looking at them (eyes: yes/no), or the gender of the face (gender: male/female). Trial types (eyes, gender) were modeled as regressors to identify the brain regions recruited in gaze processing. All p values for fMRI results were FWE-corrected.
**Results:** HC and SZ were well matched for age, sex, and parental education. The two groups performed similarly on gender identification, $t(39) = 1.10$, $p = .28$. Participants endorsed eye contact in a linear fashion respective to eye-contact signal strength: 12%, 14%, 21%, 32%, 44%, 52%, 66%, 80%, and 89%, $F(4.6, 181.1) = 180.0$, $p < .001$. No significant group effect, $F(1,39) = 1.86$, $p = .18$, or Group X Signal Strength interaction, $F(4.6, 181.1) = 1.43$, $p = .22$, was observed.
Eyes trials, relative to gender trials, elicited increased BOLD signals in numerous brain regions, most prominently in posterior medial frontal cortex (pMFC), bilateral inferior frontal gyrus/anterior insula, supramarginal gyrus/superior temporal gyrus, precentral gyrus, precuneus, and superior/mid/inferior occipital gyrus, all p's < .001.
For eyes trials, SZ (relative to HC) showed increased activation in pMFC (x, y, z = [-9, 8, 52], $k = 3104$, $p < .001$) and right insula (x, y, z = [39, 5, 4], $k = 457$, $p = .005$). For gender trials, SZ (relative to HC) showed increased activation in left pre- and post-central gyrus ($k = 656$, $p = .001$). Group comparison for the Eyes-Gender contrast yielded marginally higher activation in pMFC (x, y, z = [0, 2, 64], $k = 282$, $p = .091$) in SZ compared with HC.
**Conclusions:** Gaze perception, relative to gender identification, activated distributed brain regions associated with salience processing. Hyperactivation was observed in pMFC and insula in SZ during gaze perception, supporting aberrant affective processing of social information in the disorder. Future analyses will compare the SZ and HC for brain activation as well as functional and effective connectivities to identify specific neural mechanisms underlying altered social cognition in schizophrenia.
**Keywords:** psychosis, social cognition, functional neuroimaging
**Disclosures:** Nothing to disclose.

### M204. Cariprazine for Negative Symptoms of Schizophrenia: Pooled Post Hoc Analysis of 2 Randomized, Double-Blind, Placebo- and Active-Controlled Trials

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**Background:** Schizophrenia is a complex neuropsychiatric syndrome characterized by positive symptoms (e.g., delusions, hallucinations), negative symptoms (e.g., anhedonia), and several domains of cognitive impairment. Antipsychotics

have efficacy on positive symptoms, but the treatment of negative symptoms remains a clinical challenge. Cariprazine is an orally active and potent dopamine D3 and D2 receptor partial agonist antipsychotic with preferential binding to D3 receptors; it is currently in development for the treatment of schizophrenia, bipolar mania, bipolar depression, and as an adjunctive treatment for major depressive disorder. A pooled post hoc analysis of 2 randomized controlled Phase II/III cariprazine trials (NCT00694707, NCT01104766) was conducted to investigate the effects of cariprazine on the negative symptoms of schizophrenia in a subset of patients with acute exacerbation of schizophrenia and predominant negative symptoms.

**Methods:** The constituent studies were 6-week, international, randomized, fixed-dose, double-blind, placebo- and active controlled studies in adults with acute exacerbation of schizophrenia. In RGH-MD-16 (n = 732), cariprazine 1.5, 3, and 4.5 mg/d and risperidone 4 mg/d were investigated; in RGH-MD-04 (n = 617), cariprazine 3 and 6 mg/d and aripiprazole 10 mg/d were investigated. The primary efficacy measure in both studies was change from baseline to Week 6 on the Positive and Negative Syndrome Scale (PANSS) total score. Cariprazine doses of 1.5-3.0 and 4.5-6.0 mg/d were pooled for post hoc analyses. Patients with predominant negative symptoms were identified based on a model defining 8 states of schizophrenia (Lenert et al, Schizophr Res. 2004; 71:155-65); the selected schizophrenia health status for the patient subset being investigated (n = 285/1349) was State 6 at baseline (ie, PANSS factor score for negative symptoms $\geq$24, PANSS factor score for positive symptoms $\leq$19, and PANSS factor score for cognitive impairment $\geq$27). Patients in State 6 are clinically considered as having severe negative symptoms, mild to moderate positive symptoms, and severe cognitive dysfunction. Mean change from baseline in PANSS factor scores for negative symptoms (items N1-N4, N6, G7, G16) was analyzed using a mixed-effects model for repeated measures ($\alpha = 0.05$, 2-sided, without adjustments for multiple comparisons) in the patient subset with predominant negative symptoms; effects sizes were calculated by dividing the mean difference versus placebo by the pooled standard deviation.

**Results:** In the pooled subset of patients with predominant negative symptoms (n = 285), mean (SD) baseline PANSS factor scores for negative symptoms were 27.5 (2.9) for placebo (n = 67), 27.8 (3.4) for cariprazine 1.5-3 mg/d (n = 85), 27.5 (3.0) for cariprazine 4.5-6 mg/day (n = 64), 27.6 (3.7) for risperidone 4 mg/d (n = 34), and 28.2 (3.2) for aripiprazole 10 mg/d (n = 35). The least squares mean difference (LSMD [95% CI]) in change from baseline to Week 6 on the PANSS factor score for negative symptoms was statistically significant versus placebo for cariprazine 1.5-3 mg/d (-2.5 [-4.2, -0.8], P = .0038; effect size = 0.54), cariprazine 4.5-6 mg/d (-3.7 [-5.5, -1.9], P < .0001; effect size = 0.79), and risperidone (-2.5 [-4.7, -0.3], P = .0258; effect size = 0.54); however, the LSMD for aripiprazole versus placebo was not statistically significant (-1.0 [-3.1, 1.2], P = .3661; effect size = 0.21). Significant effect versus placebo was observed by Week 2 for cariprazine 1.5-3 and 4.5-6 mg/d and by Week 3 for risperidone.

**Conclusions:** After 6 weeks of treatment, significantly greater statistical improvement versus placebo on the PANSS factor score for negative symptoms was observed for cariprazine and for risperidone, but not for aripiprazole. This pooled post hoc analysis suggests that cariprazine may have an effect on negative symptoms in patients with schizophrenia and predominantly negative symptoms. Since patients in the constituent studies and subsequent post hoc analysis had acute exacerbation of schizophrenia, cariprazine trials in which patients are prospectively selected for primary negative symptoms are merited.

**Keywords:** schizophrenia, Negative Symptoms, cariprazine
**Disclosures:** Supported by funding from Forest Laboratories, LLC, an affiliate of Actavis, Inc., and Gedeon Richter Plc. I, Willie R Earley, am an employee and shock shareholder of Forest Research Institute, an affiliate of Actavis, Inc.

## M205. Effects of the Rapidly-Acting Antipsychotic Agent Sodium Nitroprusside (SNP) on Synaptic Spine Function and Morphology in Medial Prefrontal Cortex

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**Background:** In clinical trials, a single dose of the nitric oxide (NO) donor SNP has been reported to rapidly induce a prolonged reduction in both positive and negative symptoms in schizophrenic patients that starts within 24 hours and lasts for a week or more (1). The clinical trials were paralleled by preclinical studies showing a single injection of SNP produces a prolonged block of the acute psychotomimetic effects of phencyclidine or ketamine lasting at least one week (2). As the antipsychotic effect of SNP considerably outlasts its presence in the body, we hypothesized that SNP was inducing persisting structural changes in brain synaptic morphology.

**Methods:** Whole cell patch clamp recordings were made from layer V pyramidal cells in normal adult rat mPFC brain slices; during recording cells were labeled passively with Neurobiotin. EPSCs responses were recorded in response to bath application of transmitters that activate two different inputs to layer V cells: serotonin (5-HT), via the 5-HT2A receptor-activates a subset of cortico-cortical inputs and hypocretin/orexin (hcrt), which activates midline/intralaminar thalamic inputs. The EPSC responses were tested in brain slices 24 hours following in vivo injection of SNP (3 mg/kg, s.c.). Slices were fixed and treated with Strepavidin-594 to amplify fluorescence signals. Imaging of the apical dendritic field was by 2-photon laser or confocal microscopy; images were subsequently analyzed for spine density and spine morphology.

**Results:** In the SNP-treated group there was an increase in both frequency and amplitude of 5-HT induced EPSCs. The SNP-induced increase in 5-HT responses was blocked by ODQ (10 mg/kg, i.p.)—a selective inhibitor of the NO receptive site guanylyl cyclase—injected 30 minutes prior to SNP. In the case of hcrt, while there was an increase in amplitude, frequency did not increase, indicating thalamic inputs do not participate fully under our low-activity conditions. The EPSC changes were associated with an

increase in spine diameter, but surprisingly not spine density, indicating that there had been enhanced functional synaptic inputs in the absence of an increased number of spines under our low activity conditions. It remains to be seen if there would a NO-dependent increase in spine density under high activity conditions as has been seen in slice cultures or after prior exposure to enriched environments (3).

**Conclusions:** Our results are in accord with the hypothesis that a single exposure to the NO donor SNP, by inducing persisting enhancements in EPSC responses and spine morphology may explain its prolonged antipsychotic effect. The increase in amplitude of EPSCs in response to both thalamic (hcrt-activated) and cortico-cortical (5-HT activated) responses matches with an increase in diameter of dendritic spines. However, 5-HT also produced a large increase in EPSC frequency without a corresponding increase in spine density as we had expected based on previous studies with activity-dependent drugs synaptogenic drugs such as ketamine. This apparent discrepancy is possibly explained by studies showing that activating the NO signaling pathway increases the formation of population of large, complex spines tentatively identified as MISs (spines with multiple synaptic inputs) (4); the latter studies demonstrate that there can be an increase in synaptic connections without a change in spine density. Interestingly, MISs can be induced in a NO-dependent manner by overexpression of the major postsynaptic scaffolding protein PSD-95. Given previous studies showing that the trafficking of 5-HT2A receptors to the postsynaptic density is dependent on its association with PSD-95 (5), we have initiated studies on whether SNP upregulates PSD-95 as well as other players involved in synaptomorphogenesis (e.g., BDNF, mTOR, p70S6K, ARC, GluR1).

**Keywords:** schizophrenia, nitric oxide donor, synaptic spine, medial prefrontal cortex, guanylyl cyclase

**Disclosures:** Nothing to disclose.

**References:**

(1) Hallak et al., AMA Psychiatr., 2013.
(2) Maia-de-Oliveira et al., Schizophr. Res., 2015.
(3) Niconenko et al., PNAS, 2013.
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(5) Abbas et al., J. Neurosci., 2009.

## M206. Cariprazine as Monotherapy for the Treatment of Predominant Negative Symptoms of Patients with Schizophrenia: A Double-Blind, Active Comparator-Controlled Trial

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**Background:** Cariprazine is an orally active and potent dopamine D3 and D2 receptor partial agonist with preferential binding to D3 receptors. Cariprazine has demonstrated efficacy for the treatment of schizophrenia in three 6-week, randomized, double-blind, placebo-controlled, phase II/III clinical trials in patients with acute psychotic exacerbations and also in chronic stabilized patients in a relapse prevention clinical trial. Post hoc analysis of the 6-week efficacy trials on a subset of patients with high levels of negative symptoms demonstrated significantly greater improvement relative to placebo [Debelle et al., Eur Neuropsychopharm 2014; 24(Suppl 1): S534; Debelle et al., Eur Psychiatry 2015, 30(Suppl l): 242]. Persistent and predominant negative symptoms of schizophrenia are burdensome and disabling for schizophrenic patients while no real treatment options exist at the moment. Therefore, the objective of this clinical trial was to evaluate the efficacy, safety, and tolerability of cariprazine relative to another antipsychotic in an adequate and well-conducted clinical trial in patients with predominant negative symptoms of schizophrenia.

**Methods:** This study was a multinational, randomized, double-blind, risperidone-controlled, parallel group clinical trial in adult patients with predominant, negative symptoms of schizophrenia. To be enrolled in the clinical trial and randomized to study treatment, patients had to have predominant negative symptoms, defined as PANSS factor score for negative symptoms (PANSS-FSNS) $\geq$ 24 and at least 2 of the 3 core negative symptoms scored at least 4; PANSS factor score for positive symptoms (PANSS-FSPS) $\leq$ 19; no clinically relevant depressive symptoms and no or limited extrapyramidal symptoms; assessed as stabilized with predominant negative symptoms for a retrospective 6-month period prior to screening, and for a prospective 4-week period prior to randomization. Following 2 weeks of cross-titration and discontinuation of previously taken antipsychotic(s), patients were treated with either cariprazine target dose 4.5 mg/d, or with risperidone target dose 4 mg/d for 24 weeks. The primary efficacy parameter was the improvement in negative symptoms, defined as change from baseline (CfB) to endpoint in PANSS-FSNS. The secondary efficacy parameter was functional improvement, defined as CfB to endpoint in Personal and Social Performance Scale (PSP) total score.

**Results:** 461 patients were randomized 1:1 to double-blind risperidone (n = 231) or cariprazine (n = 230) treatment. PANSS-FSNS (27.5 in risperidone, 27.7 in cariprazine treatment groups, respectively), PSP total score (48.1, 48.8 respectively) and PANSS-FSPS (8.6, 8.8 respectively) were similar at baseline in the two treatment groups. 77.4% of the patients completed the 26-week study treatment duration, in both groups. CfB at week 26 in the primary parameter, PANSS-FSNS, was significantly larger in the cariprazine treatment group than in the risperidone treatment group (LSMD = -1.47; 95% CI: [-2.39, -0.53]; p = 0.002; MMRM, ITT). The mean CfB in the PANSS-FSNS always favored cariprazine at each follow-up with statistically significant differences from Week 14 onward. CfB at week 26 in the secondary parameter, PSP total score, showed similarly a significantly greater improvement with cariprazine when compared to risperidone (LSMD = 4.63; 95% CI: [2.71, 6.56]; p < 0.001; MMRM, ITT). The CfB in the PSP score always favored cariprazine at each follow-up visit with statistically significant differences from Week 10 onward. Statistically significant differences in favor of cariprazine over risperidone at Week 26 were shown in the CfB PSP self-care area score ($P = 0.004$), in the PSP socially useful activities area score ($P < 0.001$), and in the PSP personal

and social relationships area score ($P < 0.001$). The difference in CfB in the PSP disturbing and aggressive area score between cariprazine over risperidone at Week 26 was not statistically significant. Patients tolerated the study treatment well, as reflected by low discontinuation rates due to adverse events (AEs). The most common AEs ($\geq 10\%$) during study treatment were insomnia (10.0%), and headache (10.4%), both in the risperidone treatment group.

**Conclusions:** 26-week cariprazine treatment, given as antipsychotic monotherapy, was significantly more effective on negative symptoms and on functioning than risperidone in patients with predominant negative symptoms of schizophrenia. The side effect profiles of cariprazine and risperidone were similar in this study.

**Keywords:** schizophrenia, cariprazine, Antipsychotic monotherapy

**Disclosures:** Nothing to disclose.

### M207. Abnormal Fucosylation-Associated Enzyme Expression in Schizophrenia

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**Background:** The role of posttranslational protein modifications in the pathophysiology of schizophrenia (SCZ) has become a recent target of investigation toward understanding the complex mechanisms at play in this devastating neuropsychiatric illness. One modification, glycosylation, has come under study due to the role glycan adornment plays in modulating a wide variety of inter- and intracellular processes. Our lab has reported altered N-glycosylation of neurotransmitter transporters and receptor subunits and recently identified abnormal mRNA expression of 36 glycosylation-associated enzymes in SCZ cortex. A subset of these enzymes, fucosyltransferases (FucTases) and fucosidases, catalyze the attachment or cleavage of the deoxyhexose fucose on oligosaccharide, glycoprotein, and glycolipid substrates.

FUT11 is one of several α-1,3-FucTases expressed in brain; however, unlike other α-1,3-FucTases, FUT11 does not localize to the Golgi and is posited to play a unique role in cell biology as it does not fucosylate proteins which are common substrates of other α-1,3-FucTases. The α-1,6-FucTase FUT8 is responsible for the core fucosylation of the most proximal GlcNAc residue of N-linked glycans. GDP-fucose protein O-FucTase 1 and 2 (POFUT1 and POFUT2) mediate O-fucosylation of epidermal growth factor and thrombospondin-like repeat (TSR) domains, respectively. Substrates of POFUT1 include the NOTCH family of proteins, important in neuritogenesis and dendritic spine formation in post-mitotic neurons. POFUT2 substrates include the ADAMTS family of proteins, which influence the extracellular matrix (ECM) to permit synaptic remodeling when they are O-glycosylated with a Glc-β-1,3-Fuc disaccharide. Plasma α-L-fucosidase (FUCA2) is a pH-dependant fucosidase found primarily in the lysosome and extracellular space playing a role in both glycoprotein degradation and extracellular molecular interactions with the cell membrane. In order to elaborate on the potential dysregulation of fucosylation in SCZ we measured protein expression of these fucosylation enzymes in the superior temporal gyrus (STG) of SCZ and comparison subjects.

**Methods:** Samples of gray matter from the full cortical thickness of the left STG (Brodmann area 22) of 16 SCZ and 14 comparison subjects were obtained from the Mount Sinai Medical Center brain collection. Prepared samples of total homogenate were loaded in 4-12% Bis-Tris gels and run using standard SDS-PAGE and semi-dry transfer methods. Membranes were then probed with antibodies against FUT8, FUT11, POFUT1, POFUT2, FUCA2, and valosin-containing protein (VCP) as a loading control. The relative abundance of proteins was determined by measuring the signal intensity of each target normalized to the signal intensity of VCP.

**Results:** Protein expression of POFUT2 and FUT8 are altered in schizophrenia. POFUT2 fucosylates protein TSR domains and demonstrates a 30% increase in expression relative to comparison subjects in SCZ brain ($t(28) = 2.46$, $p = 0.020$). FUT8 is the only known α-1,6-FucTase in mammals and protein expression of this enzyme was found to be 35% less in SCZ STG relative to comparison subjects ($t(28) = 2.09$, $p = 0.046$). FUT11, POFUT1, and FUCA2 expression was not found different between diagnostic groups in STG.

**Conclusions:** This study demonstrates that protein levels of POFUT2 and FUT8 are abnormal in SCZ, suggesting that altered fucosylation may contribute to SCZ pathophysiology. Interestingly, these enzymes both play important roles in neuritogenesis and dendritic spine formation via their modification of substrates essential for these processes. For example, secretion of some ADAMTS protein isoforms requires fucosylation by POFUT2 and thus the increased expression of POFUT2 may facilitate greater transport of these lectican-cleaving molecules into the extracellular space. Cleavage of lecticans allows the ECM to reform permitting the cytoskeletal architecture within the cell to push the cell membrane forward during synapse formation and neuritogenesis. Abnormal ECM remodeling could contribute to abnormal dendritic spine morphology, which has been reported in SCZ brain.

The finding of decreased FUT8 expression is particularly noteworthy given recent evidence from fut8 inhibition in cell culture and fut8 knock-out mice, which demonstrate functional effects on neurite and synapse formation and a behavioral phenotype characterized as "schizophrenia-like." This has been purported to arise from the dual role of α-1,6-fucosylation-mediated inhibition of the activin signaling pathway and enhancement of the TGF-β signaling pathway. TGFβ and activin signaling cascades overlap intracellularly and the contrasting effects of α-1,6-fucosylation of these substrates has been suggested to mediate neurite formation in a spatiotemporal manner.

Together, these data suggest that decreased FUT8 expression and increased POFUT2 expression may affect proper neuritogenesis, dendritic spine morphology, and synaptic remodeling, all of which are known to be altered in SCZ. Abnormalities of glycosylation in SCZ have been well-established previously and these current findings suggest abnormal expression of glycosylation-associated enzymes may represent an underlying mechanism contributing to

alterations of multiple intra- and intercellular signaling pathways in SCZ. Disruptions to these essential mediators of cellular function may contribute to the diverse and variable molecular, cellular, and behavioral abnormalities evident in SCZ pathophysiology.
**Keywords:** postmortem human brain, glycosylation, neuritogenesis
**Disclosures:** Nothing to disclose.

### M208. Ketamine Induced NMDA-Receptor Blockade and Hippocampal Glutamate in Healthy Volunteers

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**Background:** Aberrant hippocampal glutamatergic signaling has been postulated as a disease mechanism in schizophrenia. Magnetic Resonance Spectroscopy (MRS) studies found elevated Glx (glutamate + glutamine) in unmedicated patients with schizophrenia in vivo in different areas of the brain, including the hippocampus, which may be secondary to N-methyl-d-aspartate receptor (NMDAR) hypofunction. To test the hypothesis that NMDAR blockage would result in increased hippocampal Glx, we measured ketamine induced Glx changes in healthy volunteers.
**Methods:** We conducted a Magnetic Resonance Spectroscopy (MRS) study to evaluate changes in hippocampal Glx during a ketamine challenge (0.27mg/kg over 10 minutes, then 0.25mg/kg/hour for 50 minutes, 0.01ml/s) in a group of 19 healthy volunteers. Psychotomimetic effects were assessed with the Brief Psychiatric Rating Scale (BPRS) and the Clinician Administered Dissociative States Scale (CADSS). Imaging was performed on a 3T head-only scanner (Magnetom Allegra, Siemens Medical Solutions, Erlangen, Germany), equipped with a circularly polarized transmit/receive head coil. MRS data were collected from a voxel in the left hippocampus (2.7x 1.5x 1cm). A series of sagittal, coronal, and axial T1-weighted anatomical scans (gradient-recalled echo sequence, TR/TE = 250/ 3.48ms, flip angle = 70°, 5mm slice thickness, 1.5mm gap, 512x 512 matrix) were acquired for voxel placement. Following manual shimming, water-suppressed spectra were acquired using the point-resolved spectroscopy sequence (PRESS; TR/TE = 2000/ 80ms; 1200 Hz spectral bandwidth; 1024 points; number of averages = 640 (21min 20s). MRS data were analyzed in jMRUI. Spectra were quantified with respect to creatine in the time domain using the AMARES algorithm. For statistical analyses, we used a mixed repeated measures design with neurometabolites as dependent variables, experimental condition as fixed factor, and voxel grey matter fraction as covariate.
**Results**: Subjects reported a significant increase in both BPRS and CADSS scores during the ketamine challenge. We found an increase in Glx with ketamine compared to saline (saline: 0.62 +/- 0.13; ketamine: 0.69 +/-0.08; $F = 3.756$; $p = 0.04$), even after excluding statistical outliers ($F = 9.408$; $p < 0.01$). We found no correlations between clinical symptoms and Glx (all $p > .05$).
**Conclusions:** Here, we describe an increase of hippocampal Glx during a ketamine challenge in healthy volunteers that is similar in extent to our previous report of elevated hippocampal Glx in unmedicated patients with schizophrenia. This is consistent with a study revealing hippocampal hypermetabolism and structural deficits in patients transitioning from a prodromal state to syndromal psychosis, and reporting that ketamine causes increased extracellular glutamate, hippocampal hypermetabolism, and atrophy in a mouse model, suggesting that glutamate acts as driver of hippocampal pathology. Because hypermetabolism and glutamate excess may antedate structural changes, development of drugs designed to modulate NMDAR function could be promising in the quest of arresting disease progression in schizophrenia or even in efforts of preventing the emergence of the full clinical picture.
**Keywords:** Ketamine, schizophrenia, magnetic resonance spectroscopy
**Disclosures:** Nothing to disclose.

### M209. Role of Locus Coeruleus-Ventral Tegmental Area Circuit in Mediating the Resilience to Social Stress

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**Background:** Rather than a simple lack of pathological alterations in the brain, resilience is an active stress-coping process. Understanding the mechanisms underlying resilience could provide us with novel therapeutic targets for the treatment of depression, among other stress-related psychiatric disorders. Emerging evidence has implicated the role of locus coeruleus-norepinephrine (LC-NE) system in stress resilience. It is widely known that the LC-NE nucleus globally primes neurons in the brain to be responsive to stimuli. However, knowledge regarding circuit-specific LC-NE mechanisms of resilience is lacking. Taking advantage of circuit-probing electrophysiological, optogenetic, and molecular profiling approaches, we explore the function of LC-NE neurons projecting to the ventral tegmental area (VTA), a key nucleus involved in the segregation of resilient and susceptible phenotypes (Krishnan V et al., Cell 2007; Chaudhury D., Nature 2013).
**Methods:** In this study, we employed a 10-day chronic social defeat stress (CSDS) paradigm to segregate resilient and susceptible behavioral phenotypes in C57BL/6J mice. After the 10-day CSDS procedure, mice were divided into susceptible or resilient phenotypes based on social interaction scores. Utilizing a retrograde lumaflour, we visualized LC neurons projecting to the VTA (LC-VTA neurons), and recorded their firing activity in the LC slice preparations from stress-naïve control, susceptible, and resilient mice. To investigate the relationship between firing alterations of LC-VTA neurons and behavioral outcomes, we utilized a combination of viral and optogenetic techniques.

Specifically, we injected retrograde AAV2/5-Cre in the VTA and AAV5-DIO-ChR2 in the LC to selectively express ChR2. We then delivered optical stimulation to LC-VTA neuron bodies of test animals (20 minutes per day for 10 days). To investigate the postsynaptic adrenoceptors involved in the VTA, we focused on the VTA dopamine neurons projecting to the nucleus accumbens (VTA-NAc), a subpopulation of VTA dopamine neurons known to display active neuroadaptations in resilient animals (Friedman AK et al., Science 2014). Utilizing a circuit-mapping molecular profiling approach (Ekstrand MI et al., Cell 2014), we injected CAV-GFP into the NAc and AAV-FLEX-NBL10 into the VTA of DAT-IRES-Cre or TH-Cre mice and screened potential adrenoceptor subtypes in VTA-NAc dopamine neurons with immunoprecipitation.
**Results:** Our electrophysiological recordings from lumaflour+ LC-VTA neurons showed that the baseline firing activity of these neurons was significantly elevated in resilient mice, when compared to control and susceptible mice. In contrast, we found these neurons to have comparable firing rates in susceptible and stress-naïve mice. Next, to determine whether increasing the firing activity of the LC-VTA circuit promotes resilient behaviors in previously defined susceptible mice, we selectively expressed ChR2 in LC-VTA neurons, as stated above. We found that 10-day repeated optogenetic activation (20 minutes/day) of LC-VTA neurons in susceptible mice reversed social avoidance behaviors. Surprisingly, the same 10-day repeated optical stimulation of LC-VTA neurons in susceptible mice normalized the pathophysiological hyperactivity and induced a new homeostatic balance between excitatory hyperpolarization-activated cation channel current (Ih) and inhibitory potassium (K+) currents, an active adaptation signature seen in the VTA-NAc dopamine neurons of resilient mice. Using a circuit-mapping molecular profiling technique, we screened the molecular targets of LC noradrenergic inputs on VTA-NAc dopamine neurons and found a higher expression of α1b and β3 adrenoceptors in VTA-NAc dopamine neurons, when compared to overall VTA dopamine neurons. To further confirm these receptors' role in mediating resilience mechanisms, we repeatedly infused a cocktail of methoxamine hydrochloride (α1 agonist) and CL316243 (β3 agonist) into the VTA for 10 days and found a potent reversal of social avoidance behaviors, which is consistent with the 10-day optical stimulation-induced effects.
**Conclusions:** These circuit-specific investigations support the notion that LC-VTA neurons play an important role in mediating the resilience mechanisms. Specifically, the electrophysiological and optogenetic studies demonstrate that activation of LC-VTA neurons promotes the active ion channel and cellular homeostasis in VTA-NAc dopamine neurons. Moreover, the molecular profiling and pharmacological examinations reveal that α1b and β3 receptors mediate interactions between the LC-NE system and the VTA reward circuit. Taken together, our findings not only elucidate a novel resilient neural circuit in the brain, but provide a new molecular target for developing treatments for depression.
**Keywords:** Locus coeruleus, Ventral Tegmental Area, resilience, neural circuits, molecular mechanisms
**Disclosures:** Nothing to disclose.

## M210. HPA Axis Dysregulation in Men with Hypersexual Disorder

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**Background:** Hypersexual disorder, integrating pathophysiological aspects such as sexual desire deregulation, sexual addiction, compulsivity and impulsivity was suggested as a diagnosis for the DSM-5. The neurobiology behind this disorder is for the moment not known. A dysregulation of the hypothalamic pituitary adrenal (HPA) axis has been shown in many psychiatric disorders but has not been investigated in hypersexual disorder. The aim of this study was to assess the function of the HPA axis in men with hypersexual disorder.
**Methods:** The study includes 67 male patients with hypersexual disorder and 39 healthy male volunteers. Basal morning plasma levels of cortisol and ACTH were assessed and low dose (0.5 mg) dexamethasone suppression test was performed with cortisol and ACTH measured post dexamethasone administration. Non-suppression status was defined with DST-cortisol levels $\geq$138 nmol/l. The Sexual Compulsive scale (SCS), Hypersexual disorder current assessment scale (HD:CAS), Montgomery-Åsberg Depression Scale-self rating (MADRS-S) and Childhood trauma questionnaire (CTQ), were used for assessing hypersexual behavior, depression severity and early life adversity.
**Results:** Patients with hypersexual disorder were significantly more often DST non-suppressors and had significantly higher DST-ACTH levels compared to healthy volunteers. The patients reported significantly more childhood trauma and depression symptoms compared to healthy volunteers. CTQ scores showed a significant negative correlation with DST-ACTH whereas SCS and HD: CAS scores showed a negative correlation with baseline cortisol in patients. The diagnosis of hypersexual disorder was significantly associated with DST non-suppression and higher plasma DST-ACTH even when adjusted for childhood trauma.
**Conclusions:** The results suggest HPA axis dysregulation in male patients with hypersexual disorder.
**Keywords:** HPA axis, hypersexual disorder, Childhood trauma
**Disclosures:** Nothing to disclose.

## M211. The Amygdala Functionally Drives the Ventral-to-Dorsal Striatal Shift in the Development and Maintenance of a Cocaine-Seeking Habit

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**Background:** The transition from goal-directed to habitual drug seeking has been shown to rely on a functional shift in dominance of dopamine control of drug-seeking behavior from the ventral to the dorsal striatum. Well-established habitual drug seeking that is maintained at high rates by response-contingent presentations of drug-associated

conditioned stimuli (CSs) depends upon dorsolateral striatal (DLS) dopamine transmission recruited by the ventral striatum. Since Pavlovian stimulus-drug associations, including their influence on conditioned reinforcement, depend upon processing in the amygdala, we have investigated the involvement of the amygdala in recruiting the DLS, a structure to which it has no direct projections. The amygdala has two possible routes of influence over DLS dopamine (DA) function: (i) via projections from the basolateral amygdala (BLA) to the nucleus accumbens core (NAcC), through which it can engage the serial connections with midbrain DA neurons and hence the nigral innervation of the DLS; (ii) via direct projections from the central nucleus of the amygdala (CeN) to substantia nigra (SNc) dopamine neurons that directly innervate the DLS. Whereas the BLA is required for learning Pavlovian CS-US (drug) associations, the CeN – SNc—DLS circuitry may be more involved in stimulus-response habit processes. Electrophysiological recordings were used to investigate whether the physiological influence of the BLA on the DLS is dependent upon glutamatergic mechanisms in the NAcC. Furthermore, we hypothesized that behaviorally, BLA, but not CeN, inactivation would influence goal-directed cocaine seeking, and that the CeN would become progressively more important as the behavior undergoes the transition to DLS dopamine-dependence.

**Methods:** Functional connectivity between the BLA and the DLS was assessed electrophysiologically in anesthetized rats by measuring the influence of electrical stimulation of the BLA at various intervals (from 1 ms to 1000 ms) on the response probability of ipsilateral DLS medium spiny neurons to stimulation of their direct input from the motor cortex (M1) both with and without ipsilateral NAcC glutamate receptor blockade. For the behavioral assessments, a separate group of rats were implanted with two sets of bilateral cannulae targeting the amygdala (either the BLA or the CeN) and DLS followed by an indwelling intravenous catheter. They were then trained to self-administer cocaine (0.25 mg/infusion) under a FR1 schedule with infusions occurring in the presence of a 20-sec light CS. Following acquisition, the amygdala was bilaterally inactivated via intracranial infusions of baclofen + muscimol (0.3/0.03 nmol/side) immediately prior to 15-min cocaine seeking test sessions in which each lever press was only reinforced by a 1-sec presentation of the CS [FI15(FR1:S)]. The response requirement was then gradually increased across sessions to a FI15(FR10:S) second-order schedule in which cocaine seeking was maintained over 15-min delays by 1-sec CS presentations on every tenth lever press. Cocaine seeking tests were again conducted. According to a latin-square design, rats were challenged with the following sets of infusions: bilateral amygdala baclofen + muscimol or vehicle; bilateral DLS α-flupenthixol (15 μg/side) or vehicle; unilateral amygdala baclofen + muscimol combined with contralateral (unilateral) DLS α-flupenthixol (0, 10, and 15 μg/side); and unilateral amygdala baclofen + muscimol combined with ipsilateral (unilateral) DLS α-flupenthixol (15 μg/side). All procedures were conducted in accordance with the United Kingdom 1986 Animals (Scientific Procedures) Act.

**Results:** BLA stimulation induced a marked change in spike probability of up- or down-regulated neurons in the DLS, but only when this stimulation occurred 100, 200, or 300 ms before M1 stimulation, showing that the BLA cannot directly regulate the activity of DLS neurons, but can modulate their activity through a polysynaptic route. This polysynaptic pathway was then shown to involve antecedent glutamatergic mechanisms in the NAcC as glutamate receptor blockade at this site eliminated the BLA-dependent up- and down-regulation of medium spiny neuron activity in the DLS. In the behavioral experiments, bilateral BLA, but not CeN, inactivation disrupted cocaine-seeking at the early-stage tests. Following extended training on the FI15(FR10:S) second order schedule bilateral CeN, but not BLA, inactivation disrupted cocaine-seeking to a level similar to that following bilateral DLS dopamine receptor blockade. Disconnection of the CeN and DLS achieved by unilateral inactivation of the CeN combined with DA receptor blockade in the contralateral DLS, resulted in a dose-dependent decrease in cocaine seeking.

**Conclusions:** The present study confirmed that the BLA is functionally connected to the DLS via the NAcC and demonstrated that BLA processing is required in the early acquisition of cocaine seeking (when goal-directed). The role of the CeN in regulating cocaine seeking only emerged when seeking behaviour was well established (habitual) and behavior is maintained by contingent presentations of the cocaine-associated conditioned reinforcer. Thus, through parallel circuitries, a balance between the BLA and the CeN is required to promote and support the acquisition and performance of habitual cocaine seeking.

**Keywords:** cocaine seeking, amygdala, nucleus accumbens, dorsolateral striatum

**Disclosures:** Nothing to disclose.

### M212. NMDA Receptor GluN2D Subunit is Indispensable in Phencyclidine (PCP) Effects

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**Background:** Phencyclidine (PCP), a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, increases locomotor activity in rodents and causes schizophrenia-like symptoms in humans. Although activation of the dopamine (DA) pathway is hypothesized to mediate these effects of PCP, the precise mechanisms by which PCP induces its effects remain to be elucidated.

**Methods:** We investigated the effect of PCP on extracellular levels of DA (DAex) in the striatum and prefrontal cortex (PFC) using in vivo microdialysis and locomotor activity in mice lacking the NMDA receptor channel GluN2A or GluN2D subunit. Gene expression changes in the brains were analyzed by using two different DNA arrays, array originally prepared in Kazusa DNA Research Institute and Illumina array.

**Results:** PCP significantly increased DAex in wildtype and GluN2A knockout mice, but not in GluN2D knockout mice, in the striatum and PFC. Acute and repeated administration of PCP did not increase locomotor activity in GluN2D knockout mice. Furthermore, DNA array experiments

revealed that PCP-induced fos expression was abolished in GluN2D knockout mice.
**Conclusions:** These results suggest that PCP enhances dopaminergic transmission, increases locomotor activity, and induces fos expression by acting at GluN2D. GluN2D may be a new target for pharmacotherapy of PCP dependence and schizophrenia.
**Keywords:** NMDA Receptor, Dopamine, c-Fos, psychosis, NMDA channel blocker
**Disclosures:** Nothing to disclose.

### M213. Chronic Cocaine Exposure Alters Decision Making Through a D1 Medium Spiny Neuron Mechanism to Promote Relapse

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**Background:** Learned associations between salient experiences and the environments within which they occur are the basis of decision-making and guide behavior towards advantageous outcomes. These associative learning processes play a critical role in survival, and are essential for animals to successfully navigate their environment. The nucleus accumbens (NAc) plays an integral role in learning, selecting and executing goal-oriented behaviors, and is a key neural substrate of cue-reward associations. Associative learning about environmental stimuli and rewards is dysregulated in addiction, and an inability to extinguish previously formed associations is thought to contribute to pathological drug seeking as well as relapse after periods of abstinence. Thus, identifying the specific neuronal ensembles/subpopulations that drive these behaviors, and determining therapeutic strategies to manipulate this cell population to promote extinction of these associations, would be advantageous to improving treatment outcomes in drug addicted individuals. Drugs of abuse mimic reward-related learning signals in large part by increasing dopaminergic transmission to the NAc and thereby altering activity of D1- and D2-expressing medium spiny neurons (MSNs). However, dopamine exerts opposing actions at these MSN subtypes, resulting in divergent effects on motivation and decision-making, as well as diverse adaptations in downstream circuits. Thus, to elucidate neuronal encoding of cue-reward associations, it is critical to determine cell-type specific contributions to associative learning.
**Methods:** To examine real-time activity of NAc neurons during associative learning for drug rewards, we injected AAV-DIO-GCaMP6f into the NAc of mice expressing Cre-recombinase in D1 or D2 MSNs to induce GCaMP6f expression specifically in each cell type and recorded calcium transients through an optic fiber while mice performed conditioned place preference (CPP). Animals were injected with 10 mg/kg IP cocaine and confined to one side of a three-chamber CPP apparatus for 25 minutes. In alternating sessions, the other chamber was paired with saline. In a choice test following the final pairing, mice were allowed to freely explore both chambers in the absence of drug. Photometry was used to record temporally specific neural activity around entries into the paired and unpaired chamber. Subsequently, the effects of prior chronic exposure on cocaine reward and concomitant D1 and D2-MSN activity were assessed. Animals were injected for seven days with 10 mg/kg IP cocaine in their home cage followed by 7 days of withdrawal and then MSN activity was recorded during CPP and the choice test. Further, animals were placed back in the chamber and MSN activity was recorded during extinction and cocaine-induced reinstatement. Finally, to confirm the causal role of D1 or D2 MSNs in associative learning, we expressed the inhibitory DREADD, hM4Di, selectively in D1 or D2 MSNs and administered clozapine-N-oxide (CNO; 5mg/kg) prior to cocaine choice testing or to cocaine pairing to inhibit activity.
**Results:** Acute cocaine administration enhances D1 and suppresses D2 MSN activity in the NAc of awake, behaving mice, and that cocaine-induced facilitation of D1 MSN activity is required for formation of cocaine-context associations. Further, temporally precise, cell-type specific signaling encodes contextual information about cocaine experiences such that increased D1 activity precedes entry into a drug-paired context, with decreased D2 activity occurring after entry. Further, chronic cocaine exposure impairs extinction of contextual associations by preventing the extinction of D1 MSN signaling that precedes drug-paired context entry. Chronic cocaine also enhances reinstatement by selectively augmenting the effects of cocaine on MSN signaling only when animals are in the drug-paired context. Manipulating this D1 signal by DREADD-induced D1 inhibition was able to block the expression of conditioned preference, confirming that D1 signaling is the critical mediator of drug-context learning. More importantly, reducing the activity of the D1 populations is sufficient to block/extinguish the expression of preference, an effect which persists for weeks.
**Conclusions:** Together, these data elucidate the cell-type specific engrams that encode cocaine reward learning, and the D1-mediated mechanism by which prior cocaine exposure retards extinction of drug-cue associations to promote relapse. Further, these processes have been localized to a single cell population in the nucleus accumbens that can be easily manipulated to attenuate the strength of these associations and potentially prevent relapse in drug addicted individuals.
**Keywords:** cocaine, calcium imaging, Medium Spiny Neuron, Nucleus Accumbens, associative learning
**Disclosures:** There are no disclosures to report.

### M214. Paternal Cocaine Exposure Elicits Learning Deficits in Male Progeny

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**Background:** Illicit drug use remains a significant public health concern and constitutes a substantial economic and societal burden worldwide. Cocaine taking is often

associated with global cognitive impairments, including deficits in attention and declarative memory. Additionally, paternal environmental perturbations such as chronic stress, a high-fat diet or drugs of abuse can produce profound effects on the physiology and behavior of offspring via epigenetic changes in sperm. Our recent studies demonstrated that cocaine exposure in sires reprogramed the germline epigenome and produced alterations in mood and addiction-like behaviors in progeny. However, it remains unclear whether paternal cocaine exposure is associated with cognitive impairments in their progeny. We hypothesized that paternal cocaine taking might reduce cognitive function in subsequent generations.
**Methods:** Behavioral, electrophysiological and molecular biology techniques were combined to examine the influence of paternal cocaine self-administration on memory formation and synaptic plasticity in offspring. Male rats self-administered cocaine daily for 60 days, the duration of spermatogenesis, and controls received yoked saline infusions. Sires were then bred with drug-naïve females resulting in cocaine-sired and saline-sired first generation (F1) offspring. Memory formation was examined in both male and female adult (60 days and older) drug-naïve offspring. We used a hippocampus-dependent object location memory task, where animals were exposed to two identical objects. Following a long (24 hours) or short (30 minutes) delay, animals were returned to the training arena in which one of the objects was in a novel location. Time spent exploring each object was recorded during all sessions. Memories are encoded by changes in synaptic strengths via cellular mechanisms such as long-term potentiation (LTP). We measured synaptic plasticity in a subset of the animals that were tested for spatial memory performance no less than two weeks after the behavioral tests, when rats no longer show any trace of the memory. Hippocampus slices were collected and theta burst-induced LTP was measured in the Schaffer collateral pathway. Glutamate signaling through N-methyl-D-aspartate receptors (NMDARs) in the hippocampus is critically important for memory formation and synaptic plasticity. Hippocampus tissue was collected from a separate cohort of naïve adult F1 offspring to measure levels of glutamate, the endogenous NMDAR co-agonist D-serine, as well as the expression of other molecules critical for glutamatergic signaling and memory formation.
**Results:** Male offspring of saline-treated sires spent more time exploring the displaced object during the 24-hour object location memory test, indicating intact long-term spatial memory. In contrast, cocaine-sired male progeny showed impaired long-term memory and explored both objects equally during the 24-hour test. Following the acquisition phase of memory formation, short-term labile memories are converted to longer-lasting traces through a gene- and protein synthesis-dependent consolidation process. Short-term memory was assessed 30 minutes after training to determine which phase of memory formation was affected in cocaine-sired progeny. The offspring of cocaine-exposed sires had impaired short-term memory, suggesting that paternal cocaine taking impairs the ability of male offspring to form hippocampus-dependent spatial memories. These learning deficits were sex specific in that object location memory was intact in all female offspring. Theta bursts-induced LTP in the Schaffer collateral pathway was impaired in cocaine-sired offspring compared to controls. Interestingly, there was an overall positive correlation between spatial memory performance and LTP induction ($R2 = 0.4737$, $p = 0.0192$), suggesting that learning and synaptic plasticity impairments in progeny may be caused by similar underlying mechanisms. Levels of the endogenous NMDAR co-agonist D-serine, which is critically involved in memory formation and synaptic plasticity, were diminished in the hippocampus of cocaine-sired offspring compared to controls. Bath application of D-serine restored LTP in hippocampal slices from cocaine-sired rats. In addition, hippocampal micro-injections of D-serine prior to training on the object location task restored memory in the male offspring of cocaine-exposed sires.
**Conclusions:** Our results demonstrate that paternal cocaine exposure elicits learning and synaptic plasticity impairments and that these effects are associated with reduced D-serine levels in the hippocampus of male progeny.
**Keywords:** Epigenetics, cocaine, Memory and Learning, novel object recognition, D-serine
**Disclosures:** Nothing to disclose.

## M215. A Selective Role for Mesolimbic Circuitry in Cognitive Deficits Following Adolescent Alcohol Use

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**Background:** Adolescent alcohol use remains a major public health concern due in part to well-established findings implicating the age of alcohol use onset with the development of alcohol use disorders and persistent decision-making deficits in adults. However, therapeutic treatment options remain limited. We have demonstrated with a preclinical model that alcohol exposure during adolescence promotes maladaptive, risky choice behavior in adulthood. This impairment represents a unique vulnerability of the developing brain as we have also shown that adults given identical exposure do not show this deficit. In addition, we have established neural correlates of risk bias within the ventral striatum where phasic dopamine release in response to risky options is increased by adolescent alcohol exposure and is positively correlated with risk preference. These findings suggest that changes in striatal dopamine transmission, as a consequence of adolescent alcohol exposure, could bias choice by assigning greater value to risky options, but the underlying circuits and mechanisms involved remain unknown. Therefore, we hypothesized that midbrain circuitry, including specific inputs to the mesolimbic dopamine system, may be corrupted by early life alcohol exposure.
**Methods:** Male rats were given voluntary access to an alcohol or control gelatin for twenty days throughout the adolescent period (post-natal days 30-50). Separate groups of animals were prepared in adulthood for 1) electrochemical detection of stimulated phasic dopamine transmission by fast-scan cyclic voltammetry in the ventral striatum, 2) detection of tonic dopamine levels by microdialysis and ultra-high performance liquid chromatography tandem

mass spectrometry, 3) slice electrophysiology for measurement of IPSCs and EPSCs in dopamine neurons, and 4) in vivo behavioral pharmacology. To examine the hypothesis that alcohol exposure promotes a selective perturbation to mesolimbic circuitry and to isolate the ventral tegmental area (VTA), dopamine measurements were made in an anesthetized preparation after electrical stimulation of the pendunculopontine nucleus (PPT) and the medial forebrain bundle (MFB). Next, to examine the hypothesis that altered dopamine transmission is due specifically to changes in VTA dopamine neuronal activity, electrophysiological measurements were made in slices obtained from adult animals with and without a history of adolescent alcohol. In the VTA, both dopamine and GABA neurons express GABAA receptors however, specific pharmacological manipulation is made possible by that fact that the subunit composition differs between the two. Therefore, we used a subunit selective GABAA allosteric agonist (L-838,417) to assess the differential consequences of applying this "brake" on dopamine signaling in animals with and without a history of adolescent alcohol use. Finally, we examined the ability of L-838,417 to mitigate increased risk preference in alcohol-exposed animals with in vivo pharmacology during a probabilistic decision-making task.

**Results:** Here we isolate a specific circuit including the PPT that promotes significantly elevated phasic dopamine transmission after adolescent alcohol use. Conversely, stimulation of the MFB did not elicit differential dopamine release between groups, suggesting that the effects of adolescent alcohol intake on phasic dopamine transmission originate in the VTA and not in the dopamine terminal regions of the striatum. Unexpectedly, VTA dopamine neurons recorded in adult brain slices from animals exposed to alcohol during adolescence exhibited enhanced IPSCs but not EPSCs relative to controls, indicative of increased inhibitory drive onto dopamine neurons. Supporting this surprising electrophysiological finding, we show that adolescent alcohol intake promotes a relative decrease in tonic dopamine levels in the ventral striatum of adult animals compared to controls, with a close negative correlation between tonic dopamine levels and risk-taking behavior. These data support previous work positing a complex interplay between tonic and phasic dopamine where chronic drug/alcohol use may persistently decrease tonic dopamine levels in the striatum while in parallel increasing phasic dopamine responses to salient stimuli including cues related to drug and alcohol use. Finally, we demonstrate that pharmacological normalization of phasic dopamine transmission following adolescent alcohol use with L-838,417, which has no behavioral effects in control animals, mitigates maladaptive risk-taking in previously alcohol-exposed animals.

**Conclusions:** Here, we identify a selective perturbation to mesolimbic circuitry that promotes maladaptive decision making after adolescent alcohol use and demonstrate its pharmacological reversal in adulthood. Together these results provide unique insight into the underlying mechanisms involved in heightened risk-taking behavior and highlight a potential new therapeutic target for further investigation.

**Keywords:** Alcohol, Dopamine, Decision Making

**Disclosures:** Nothing to disclose.

## M216. Dopamine D2/3 Receptor Availability Associated with Simulated Drug Choice in Methamphetamine Addiction

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**Background:** Individuals with methamphetamine use disorder choose to take methamphetamine over engaging in other rewarding activities. This phenomenon is perhaps best modeled in laboratory paradigms in which non-human animals or actively-using humans can choose between receiving a drug infusion or an alternative reward (e.g., money). Because ethical constraints typically prohibit the administration of active drugs to individuals who are in treatment or abstaining, this drug-choice approach is only appropriate for active users. In recognition of these challenges, members of our team have developed a set of tasks to capture "simulated" drug choice (i.e., rather than receiving actual drugs and rewards, individuals make choices to view drug-related and non-drug-related pictures). On these tasks, participants with cocaine use disorder choose to view more cocaine-related images (e.g., people smoking crack) and fewer pleasant images unrelated to drug use (e.g., smiling babies, erotic scenes) than healthy controls. This differential (drug > pleasant) choice, which mirrors the drug-choice paradigms deployed in animal models and actively-using humans, was associated with a more severe addiction, worse drug-related outcomes, and/or abnormalities in brain function as measured with functional magnetic resonance imaging (fMRI). In the current study, we probed for a potential correlation between dopamine D2-type receptors with this differential drug > pleasant choice using positron emission tomography (PET) while extending results to another stimulant use disorder (methamphetamine).

**Methods:** Ten individuals with methamphetamine use disorder (METH) and 12 healthy controls (HC) completed two tasks (an "explicit" and a "probabilistic" picture viewing task), and were scanned with PET to assess D2-type receptor availability. On the "explicit" choice task, participants were instructed to choose, via continued button pressing, which of two fully visible side-by-side images they preferred to view. Images were sampled from four picture categories (pleasant, unpleasant, neutral, and meth). On the "probabilistic" choice task, participants were instructed to sample 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. Dopamine D2-type receptor availability, measured as BPND, was assayed using [18F]fallypride, a high-affinity radioligand for dopamine D2/D3 receptors. PET scanning was performed on a Philips GEMINI TF PET/CT scanner (PET data were not collected from one METH participant).

**Results:** On both choice tasks, results of mixed ANOVAs revealed image category (pleasant, unpleasant, neutral, meth) x study group (METH, HC) interactions [explicit: $F(2.0,39.5) = 8.49$, $p = 0.001$; probabilistic: $F(2.1,41.3) = 5.07$, $p = 0.010$].

Post hoc comparisons showed that, compared with HC, METH participants chose fewer pleasant images (both tasks) and more methamphetamine images (explicit task only, but with a similar direction of effects on the probabilistic task). Indeed, for both tasks the meth > pleasant choice-difference scores differed between the groups [explicit: $t(20) = 4.08$, $p = 0.001$; probabilistic: $t(20) = 2.73$, $p = 0.013$]. Of even greater interest, in METH but not HC, meth > pleasant choice differences scores on the explicit task were negatively correlated with D2-like receptor availability in the nucleus accumbens ($r = -0.88$, $p = 0.002$), and in the caudate ($r = -0.82$, $p = 0.007$) and putamen ($r = -0.86$, $p = 0.003$) (the higher the drug-related choice, the lower the receptor availability). Using Fisher's r-to-z transformation, the correlations were shown to differ between METH and HC in the nucleus accumbens ($z = 2.99$, $p = 0.003$) and putamen ($z = 1.98$, $p = 0.048$). There were no significant correlations on the probabilistic task.

**Conclusions:** Similarly to cocaine users, METH participants chose to view more drug-related images and fewer pleasant images compared with HC. This differential meth > pleasant choice was negatively correlated with D2-type receptor availability in the striatum, a core region comprising the dopamine reward circuit. These results provide a novel neurochemical correlate, and possible mechanism, for a laboratory paradigm of drug-seeking that can be administered even in treatment settings and that has established construct validity with respect to real-world clinical outcomes. Our work can potentially inform treatment strategies for methamphetamine and other stimulant addictions. For example, although many medications aiming to reduce the reinforcing effects of drugs have produced negative or mixed results, an alternative treatment approach could involve increasing the value of alternative reinforcers. Indeed, the most effective treatments should not only reduce the reinforcing effects of drugs, but also increase allocation of behavior to non-drug alternatives, a framework captured by these choice tasks. More broadly, our results contribute to basic translational neuroscience research by addressing the central and enduring question in addiction research of whether dopamine deficits contribute to drug-biased decision-making.

**Keywords:** Positron emission tomography, Dopamine (D2, D3) receptors, choice, human laboratory, Human Neuroimaging

**Disclosures:** Nothing to disclose.

## M217. Whole Brain Mapping of Monosynaptic CB1 Receptor Inputs into Dopamine Neurons of the Ventral Tegmental Area

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**Background:** The mesolimbic dopamine (DA) pathway is critically involved in reward-seeking and appetitive behaviors. It originates in the ventral tegmental area (VTA) of the midbrain and projects rostrally to provide profuse DA innervation to terminal regions, in particular the nucleus accumbens. Impaired DA release within the mesolimbic system is a hallmark of schizophrenia, depression and drug abuse. The activity of DAergic cells in the VTA is potently modulated by the endocannabinoid system (ECS). The ECS influences neuronal activity through presynaptic inhibition of neurotransmitter release. The cannabinoid type 1 receptor (CB1R) is the main receptor involved in this signaling pathway. Here, we identify afferents that control firing of DAergic cells in the VTA by means of signaling at CB1Rs.

**Methods:** Using DAT::Cre transgenic mice, we first injected two AAV viruses into the VTA. The viruses infected DA cells with an avian retroviral receptor, the mammalian rabies glycoprotein and a fluorescent tag. Mice were later transduced with a pseudorabies virus in which the rabies glycoprotein has been removed and replaced with a fluorescent protein. The pseudorabies binds specifically to DA cells expressing the avian retroviral receptor and utilizes the rabies glycoprotein in DA cells to infect cells that form monosynaptic, classical synapses with DA neurons. Once we identified monosynaptic inputs to DA neurons in the VTA, we performed a combination radioactive in-situ hybridization and immunohistochemistry experiment.

**Results:** We were able to visualize cells that were infected by the pseudorabies virus and cells that express CB1R mRNA. For each brain region in which we found rabies infected cells, we performed a cell count of the pseudorabies infected neurons and the pseudorabies neurons expressing CB1R mRNA to determine the ratio of afferents to DA neurons that are and are not under the control of the ECS. Our results identify previously unseen discreet populations of VTA DA neuron afferents that have the potential to signal through the ECS.

**Conclusions:** Our results confirm that VTA DA neurons are under the control of multiple input regions, which can engage ECS signaling to change activity patterns of dopamine neurons in an activity-dependent negative feedback fashion. These findings are timely and relevant in light of the renewed interest in marijuana and cannabinoid-based therapies for a number of psychiatric conditions.

**Keywords:** cannabinoid, Dopamine, Ventral tegmental area (VTA), Rabies tracing

**Disclosures:** Nothing to disclose.

## M218. Functional Heterogeneity among Midbrain Dopamine Neurons in the Control of Cue Attraction, Conditioned Locomotion, and Reinforcement

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**Background:** The specific nature of dopamine's (DA) role in motivated behavior is a matter of debate. This is due in part to the anatomical complexity of the system, where neurons in the more medial ventral tegmental area (VTA) project largely to the prefrontal cortex and nucleus accumbens, while neurons in the more lateral substantia nigra (SN) project to the dorsal striatum. Emerging research suggests that these populations also vary in their genetic makeup, physiology, and neurotransmitter release profiles.

**Methods:** To parse the contribution of these distinct subpopulations to different facets of appetitive motivation and learning, we targeted expression of channelrhodopsin to dopaminergic neurons in TH::cre rats. Experiment 1) Pavlovian Conditioning: TH::Cre + /- rats first received Pavlovian training, in which blue laser was delivered unilaterally to either the VTA or SN, independent of behavior. For PAIRED rats, this stimulation was predicted by the presentation of external cues, while for UNPAIRED rats cues and stimulation were unassociated. Conditioned Reinforcement: Rats were next given the opportunity to lever press for cues in the absence of laser delivery and responded robustly for VTA, but not SN paired cues. Intracranial Self Stimulation: Finally, rats were allowed to nose poke for brief laser pulses. Experiment 2) We followed up on this experiment by using a retrograde AAV approach to target dopamine neurons projecting specifically to the nucleus accumbens core or dorsal striatum.
**Results:** Experiment 1) We found that, across training, different conditioned responses to the Pavlovian cues emerged for VTA and SN stimulation. PAIRED, but not UNPAIRED, rats receiving the most medial VTA stimulation developed cue-light approach behavior, suggesting the cue had become attractive. Cues predictive of SN laser delivery elicited locomotion in response to the cue, expressed as rotation contralateral to the hemisphere of laser delivery, but no approach. Importantly, UNPAIRED rats did not develop these conditioned responses, even though they received the same number of laser stimulations. This self-stimulation behavior was robust regardless of laser target site in the VTA and SN. Experiment 2) Pavlovian cues paired with optogenetic stimulation of core-, but not dorsal striatum-projecting neurons elicited approach behavior and supported conditioned reinforcement, but stimulation of both projections was reinforcing. We are currently exploring similar functional dissociations among midbrain GABA neurons, as well applying these methods to dissect midbrain control of cocaine-seeking behavior.
**Conclusions:** Together, these results demonstrate that anatomically distinct DA neuron subpopulations control the attribution of motivational value to neutral cues to spur attraction, psychomotor activation, and support conditioned reinforcement, while the ability of DA neurons to directly reinforce actions is relatively consistent throughout the midbrain. This points to circuit-level "rules" by which DA neurons contribute to a diverse set of motivational processes.
**Keywords:** Dopamine, Ventral tegmental area (VTA), optogenetics, incentive motivation, Pavlovian
**Disclosures:** Nothing to disclose.

### M219. Genome-Wide Mapping of Ethanol Sensitivity in the Diversity Outbred Mouse Population

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**Background:** A strong predictor for the development of alcohol use disorders (AUDs) is altered sensitivity to the intoxicating effects of alcohol. Individual differences in the initial sensitivity to alcohol are controlled at least in part by genetic factors, yet finding the specific genes that underlie these differences has proven difficult. Mice offer a powerful tool for elucidating the genetic basis of behavioral and physiological traits relevant to AUDs; yet conventional experimental crosses have only been able to identify large chromosomal regions rather than specific genes. Genetically diverse, highly recombinant mouse populations allow for the opportunity to observe a wider range of phenotypic variation, offer greater mapping precision, and thus increase the potential for efficient gene identification.
**Methods:** We have taken advantage of the newly developed Diversity Outbred (DO) mouse population to identify and map narrow quantitative trait loci (QTL) associated with ethanol sensitivity. We phenotyped 608 JAX Diversity Outbred mice (DO) for three measures of ethanol sensitivity: ataxia, hypothermia, and loss of the righting response (LORR). We genotyped a subset of these mice at $\sim$78k markers across the genome and performed high precision QTL mapping using the R program DOQTL.
**Results:** A paired samples t-test indicated that on average, there was a significant and robust decrease in pre-ethanol performance as compared to post-ethanol performance on the Rotarod latency to fall, $t(600) = 25.53$, $p < 0.0001$, $d = 1.04$. A repeated-measures ANOVA indicated that following ethanol administration, subjects showed significant changes in body temperature over time, $F(2.956, 1747.087) = 797.788$, $p < 0.0001$, $\eta\_p\hat{}2 = 0.574$. During LORR testing, the majority of subjects (85.5%) both lost and regained the righting reflex during the testing period, with duration of LORR ranging from 3 minutes to the cut-off time of 180 minutes ($M = 87.7$, $SD = 47.2$). Importantly, we observed tremendous variation in all three traits which enables genetic mapping of naturally occurring genetic variation that is associated with trait variation. We identified 10 suggestive QTLs associated with ethanol sensitivity on chromosomes 6, 7, 8, 9, 12, 15, 16 (LODs $> 5$; $p < 0.05$) and one significant QTL located on chromosome 11 (LOD $> 8$; $p < 0.05$).
**Conclusions:** With increased sample size to improve mapping power and resolution, and the inclusion of RNA-Seq and other molecular profiling we will be able to apply a systems genetic strategy to construct the network of correlations that exist between DNA sequence, gene expression values and ethanol-related phenotypes. This information can in turn be used to identify alleles that contribute to AUDs in humans, elucidate causative biological mechanisms, or assist in the development of putative treatment strategies.
**Keywords:** QTL, alcohol use disorder, genetics
**Disclosures:** Nothing to disclose.

### M220. How Does Cocaine Interfere with Brain Development?

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**Background:** Studies in humans and non-human primates (Lidow et al. Brain Res Dev Brain Res. 2001) suggest that

cocaine exposure during the second trimester of pregnancy is particularly damaging to the developing fetus. Examination of the developmental effects of cocaine in human subjects is extraordinarily complex because of varying levels and times of exposure, confounding environmental factors, and unknown genetic variables. Nevertheless, surveys of humans after prenatal cocaine exposure show persistent developmental effects, including increased susceptibility to adolescent substance abuse (Min et al., J. Adolesc. 2014). It has been suggested that a principal effect of cocaine on cortical development involves interference with progenitor cell proliferation (Lidow et al. 2001). More specifically, cocaine inhibited proliferation of a rodent neural progenitor cell line, AF5, via oxidative endoplasmic reticulum stress (Lee et al., PLoS Medicine, 2008). N-oxidative metabolism of cocaine caused phosphorylation of PERK and eIF-2a, increases in ATF4, decreasing cyclin A2 and cell proliferation via G1/S phase arrest. Cocaine also down-regulated cyclin A in primary human neural and A2B5 progenitor cells and in fetal rat brains. Reversing cyclin A2 down-regulation by gene transfer counteracted the inhibition of proliferation by cocaine. The effects of cocaine on neural progenitor proliferation were found to be mediated by oxidative metabolism of cocaine via cytochrome P450 3A.
Due to the substantial species differences in cytochrome P450-mediated metabolism of drugs, a human model system is required to further elucidate the mechanisms involved in cocaine's interference in brain development.
**Methods:** In the present study, we employed human embryonic stem cells to develop two- and three-dimensional in vitro models that replicate many significant features of human neocortical development. These models allow for the staged expression of markers characteristic of various features of neocortical development, as well as formation of upper- and lower-layer cortical neurons, glutamatergic and GABAergic neurons, and radial glia.
**Results:** Exposure to 3 micromolar cocaine for 1 hour every other day during in vitro cortical neurogenesis caused increased generation of reactive oxygen species (ROS), inhibition of proliferation of neural progenitor cells, and accelerated neurogenesis as indicated by outward migration of BrdU + TUJ1 neurons. Overall neocortical development, as indicated by TUJ1 + expression, was impaired. Cimetidine, an inhibitor of the cytochrome P450 3A (CYP3A) subtype, prevented each of these effects of cocaine.
Only two isoforms of CYP3A, CYP3A5 and CYP3A43, are present in differentiating neural cells. Additional experiments employed lentiviral vectors to deliver shRNA to examine whether CYP3A5 or CYP3A43 is responsible for the effects of cocaine. It will be shown that knockdown of CYP3A5, but not CYP3A43 reverses the effects of cocaine on ROS generation, proliferation, premature neuronal differentiation, and overall neocortical development. Finally, human embryonic stem cell lines which were identified as expressing mutant, non-functional CYP3A5 will be used to show that the effects of cocaine on ROS generation, proliferation, premature neuronal differentiation, and overall neocortical development are dependent on the presence of a functional CYP3A5 enzyme.
**Conclusions:** Notably, functionality of the CYP3A5 enzyme varies by ethnicity. While the majority of individuals of African descent express functional CYP3A5, the majority of Western Europeans have mutations in at least one allele. Other ethnic groups show intermediate levels of CYP3A5 functionality. Individuals lacking CYP3A5 would show a decreased susceptibility to cocaine-induced interference with cerebral cortical development. This may explain conflicting results of studies that have examined cocaine-induced developmental damage in human subjects. Therefore, this factor should be accounted for when interpreting previous studies and when screening for subjects in future experiments. In addition, it is possible that CYP3A5 functionality is related to additional neurotoxic and developmentally toxic effects of cocaine.
**Keywords:** cocaine-related disorders, neurodevelopment, pharmacogenetics, cocaine
**Disclosures:** A provisional patent application has been filed for one of the in vitro models used in this study.

## M221. Adolescent Cannabinoid Self-Administration in Rats: Effects on PFC-Dependent Working Memory, Protein Expression, and Indicators of Abuse Liability

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**Background:** Adolescence is characterized by risky behaviors like drug-taking, and marijuana (Cannabis sativa) use is widespread. Importantly, the neurobiological consequences of chronic cannabis use are not yet fully characterized, and there is debate amongst the public at large about the risks vs. benefits of cannabis use. Adolescents may be particularly vulnerable to the effects of cannabis because of ongoing neurodevelopment in the prefrontal cortex (PFC), a key regulator of cognition and inhibitory control, and thus there is potential for adolescent cannabis use to cause long-term effects on cognitive function. Though some rodent and primate research does show evidence of cognitive deficits after experimenter-administered cannabinoids during adolescence, it remains unknown whether similar deficits occur in a model of drug use and addiction – cannabinoid self-administration. In order to understand what effects cannabinoid self-administration has on the development of cognitive processes, expression of neural signaling molecules, and measures of abuse liability we developed an adolescent cannabinoid self-administration paradigm and assessed effects on working memory and propensity for reinstatement.
**Methods:** We first identified the critical period for the ontogeny of working memory performance in adolescent rats. The ontogeny of working memory was assessed by comparing performance in adolescent (starting on postnatal day 28; p28; n = 12) and adult (> p70; n = 8) male rats on a delayed-match-to-sample working memory task with equivalent training history. We then established intravenous self-administration of the synthetic cannabinoid agonist WIN55,212-2 (WIN) in a separate cohort of male rats and examined the long-term effects of cannabinoid self-administration during adolescence on working memory performance. Separate groups of rats were trained to self-administer WIN (n = 24) or sucrose (controls; n = 23) in

daily 2- or 6-hr sessions during our identified critical period for working memory development in adolescence (p38-52). Working memory performance was determined in adulthood, and tests of cue-induced reinstatement on days 1 and 21 of abstinence were assessed. At the end of behavioral testing, the brains from rats with a history of 2-hr WIN or sucrose self-administration in adolescence were taken. The PFC was dissected and underwent subcellular fractionation to determine levels of expression of proteins regulating GABAergic and glutamatergic signaling by Western blot. All animal procedures were approved by our Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.
**Results:** Daily comparison of working memory performance found that adolescents performed consistently worse than adults (lower accuracy) when the delay between sample and response was long. A significant difference by age was observed up to p46, and adolescent performance did not reliably overlap adults until late adolescence (p51). There was no effect of age on acquisition of nose poke responding or on performance when there was zero delay between sample and response. Rats readily acquired self-administration of both WIN and sucrose and exhibited significant cue-induced reinstatement to both reinforcers after extinction. The WIN group also demonstrated an "incubation of craving" effect with significantly greater cue-induced responding on day 21 vs. day 1 of abstinence. Surprisingly, both short- and long-access WIN self-administration in adolescence produced significant improvements in adult working memory performance. In addition, we found significant differences in the expression of GABAergic and glutamatergic signaling proteins in the mPFC of the short access WIN self-administration group, including increased vmPFC membrane expression of the GABA transporter GAT1 and GABAB receptor 2 subunit, indicating that self-administered WIN can produce long-term consequences on cortical development.
**Conclusions:** Our findings suggest that working memory becomes adult-like in late-adolescence; that adolescent cannabinoid self-administration does produce effects indicative of abuse liability, but that a self-administered cannabinoid does not produce long-lasting working memory deficits.
**Keywords:** cannabinoids, Self-Administration, working memory, prefrontal cortex, cue reinstatement
**Disclosures:** Nothing to disclose.

### M222. Voluntary Consumption of Alcohol in Combination with Cocaine Alters the Neurobiology Underlying Relapse to Cocaine-Seeking

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**Background:** One of the difficulties in successful treatment of cocaine addiction is reducing the risk of relapse that exists even after long periods of abstinence. Relapse is modeled in animals using the extinction-reinstatement paradigm. Previous research has established the role of nucleus accumbens glutamate transmission in the reinstatement of cocaine-seeking and has shown that the antibiotic ceftriaxone prevents relapse to cocaine seeking in rats. However, it is estimated that 60% to 90% of cocaine addicts use alcohol in combination with cocaine. The combination of alcohol and cocaine potentially produces unique neuroadaptations that differ from those produced by either drug alone.
**Methods:** We developed a model of poly-drug addiction in which rats self-administered cocaine for two hours in an operant chamber and immediately afterwards were presented with unsweetened alcohol (20% v/v) in the home cage for 6 hours. Following two weeks of drug consumption, animals underwent extinction training for a minimum of two weeks. Animals were treated with IP ceftriaxone (100 or 200 mg/kg) or vehicle for 6 days prior to being tested for cue- and/or cocaine-primed reinstatement. A second group of animals were probed with microdialysis cannulae in the nucleus accumbens to measure glutamate levels during relapse to cocaine-seeking.
**Results:** We found that neither dose of ceftriaxone attenuated the reinstatement of cocaine-seeking. In agreement with previous work by our group and others, an increase in glutamate was found during the reinstatement to cocaine-seeking in rats that did not consume alcohol. However, in animals that self-administered both cocaine and alcohol, the reinstatement of the operant response that previously delivered cocaine was not accompanied by an increase in glutamate in the nucleus accumbens.
**Conclusions:** Glutamate transmission in the nucleus accumbens core does not mediate relapse to cocaine-seeking in animals that consume ethanol with cocaine. These findings indicate that medications targeting glutamate, such as ceftriaxone, may not be effective therapies for preventing relapse in humans that drink alcohol with their cocaine.
**Keywords:** Glutamate, cocaine, alcohol, addiction
**Disclosures:** Nothing to disclose.

### M223. Reward Processing Across Addictive Disorders

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**Background:** Given new conceptualizations of addictive disorders including both substance-related and non-substance-related disorders, an important research direction entails comparing reward processing across populations characterized by poor self-control. Individuals with cocaine dependence (CD) and pathological gambling (PG) demonstrate alterations in striatal and prefrontal brain areas during reward processing. Alterations in cortico-striatal areas are similarly noted in binge eating disorder (BED), a condition also characterized by impulse control problems. The current study directly compares neural responses during reward processing across these groups.
**Methods:** During functional magnetic resonance imaging (fMRI), participants performed the Monetary Incentive Delay Task (MIDT). The MIDT allows for separate assessment of anticipatory and outcome processing related

to reward and loss. Comparisons of regional activations were investigated in PG, CD and BED groups during prospect, anticipation and notification of reward or loss. Groups were matched on age, gender and smoking status.
**Results:** A conjunction analysis performed across PG, CD and BED groups demonstrated reduced activity in the ventral striatum during anticipation of win/loss and decreased activity in the insular cortex during outcome processing. During anticipatory phases the PG group showed decreased inferior frontal gyrus and ventromedial prefrontal cortex activity relative to the CD group. During anticipatory phases, few fronto-striatal activation differences were noted between PG and BED groups.
**Conclusions:** Both similarities and differences in reward processing are evident in disorders of self-regulation. These results will be discussed in the framework of current knowledge of the neurobiology of reward processing and in the context of brain structural alterations noted in these groups. Examining reward processing across disorders of impulse control provides additional insight into a neurobiological framework that might best conceptualize each disorder. These findings have implications in clarifying the etiology of these disorders and developing effective therapies.
**Keywords:** addiction, pathological gambling, Binge-eating disorder, reward processing, cocaine addiction
**Disclosures:** All authors report that they have no financial conflicts of interest with respect to the content of this submission.

### M224. Getting Over It: A Longitudinal Neuroimaging Study Demonstrating the Emergence of Executive Control Circuits in Treatment-Engaged Cocaine Users and Alcoholics

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**Background:** Emerging literature suggests that substance use in adolescence and young adulthood may alter frontal-striatal neural circuits that govern cognitive control, potentially fostering continued habitual use of the drug. We do not completely understand, however, what happens to these circuits following extended abstinence from the drugs.
**Methods:** In this study we investigated the natural evolution of frontal-striatal connectivity in treatment-engaged cocaine users and alcohol dependent individuals. Baseline functional connectivity data was acquired during the first week of a 28 day outpatient treatment program ($n = 16$; 10 alcohol, 6 cocaine), at the end of the program ($n = 8$; 4 alcohol, 6 cocaine), 1 month later ($n = 7$; 4 alcohol, 3 cocaine), and 2 months later ($n = 5$; 3 alcohol 2 cocaine).
**Results:** Among the alcohol dependent individuals, the baseline activity in several nodes of the executive control network (ECN, dorsolateral prefrontal cortex, lateral parietal cortex) was significantly higher in the individuals that remained enrolled in treatment, relative to those that dropped out of treatment. Among the cocaine dependent individuals there was lower baseline connectivity in the ECN than the alcohol users, but over time the strength of connectivity in this network appears to grow in the successfully abstinence cocaine users.
**Conclusions:** These preliminary data suggest that there is variance in baseline functional connectivity between drug classes, but that these networks have the ability to evolve in a developmentally healthy trajectory. This may provide a critical foundation from which we can develop evidence-based brain stimulation treatment strategies for individuals suffering with substance abuse disorders.
**Keywords:** addiction, Treatment, fMRI, Connectivity, executive function
**Disclosures:** Nothing to disclose.

### M225. PKMζ Knockout Enhances Cocaine-Taking and Cocaine Seeking

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**Background:** The atypical PKC, PKMζ has been proposed to be the molecular mark for memory. However, recent evidence has demonstrated that that it is not necessary for multiple forms of memory, nor long-term potentiation. Despite this, there is some evidence that PKMζ may play a more specific role in addiction.
**Methods:** The current study utilized PKMζ knockout mice and examined cocaine self-administration, extinction and reinstatement of drug seeking.
Results PKMζ knockout led to an increase in food and cocaine taking during the self-administration phase. Additionally, PKMζ knockout mice exhibit increased cocaine seeking during cue-induced reinstatement. This increase is not due to an increase in cocaine-induced locomotor activity nor a more generalized increased response to novelty. Furthermore, in contrast to the sex-specific effects seen in measures of anxiety, both male and female PKMζ knockout mice exhibit these increases in cocaine taking and seeking. Current studies are underway to determine whether similar effects are seen with a site-specific knockout of PKMζ within the nucleus accumbens.
**Conclusions:** Consistent with these findings, others have seen increased alcohol drinking in PKMζ knockout mice. Taken together, this suggests a specific role for PKMζ in dampening reward systems. Gaining a better understanding of this mechanism could provide further insight into potential therapeutic targets for drug development.
**Keywords:** cocaine addiction, cue reinstatement, PKMzeta
**Disclosures:** Nothing to disclose.

### M226. Involvement of Striatal Dopamine D2/D3 Receptors in the Modulation of Visual Attention During Rested Wakefulness and Sleep Deprivation

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**Background:** Sleep deprivation (SD) impairs brain activation and cognitive performance including attention. Using

imaging we have provided evidence that the impairment might reflect in part SD-induced reductions in dopamine D2 and D3 receptors (D2/D3R) levels in the striatum, which would attenuate dopamine signaling through striato-cortical pathways. Indeed medications (methylphenidate amphetamine) that enhance DA signaling improve attention either when misused by students preparing for an exam the night prior to the test or when used therapeutically for the treatment of attention deficit hyperactivity disorders (ADHD). Using PET we have shown that SD reduced D2/D3R in the striatum and that it impairs brain activation and performance during functional magnetic resonance imaging (fMRI) with cognitive tasks. However, the association between striatal D2/D3R and brain activation, and the potential effects of SD on this association are largely unknown. Here we hypothesize that DA signaling through D2/D3 receptors in striatum is implicated in the proper coordination of brain networks involved with attention while performing a visual attention (VA) task. Specifically, we propose that DA neurotransmission in the limbic (ventral striatum) and executive (caudate) basal ganglia loops modulate differentially fMRI activation during VA.

**Methods:** We evaluated striatal D2/D3R with [11C]raclopride PET and fMRI activation during a ball-tracking task with parametric increases of VA load in 14 healthy right-handed men (age 32 ± 8 years, education: 16 ± 2 years) twice on two different days, during rested wakefulness (RW; after a good night sleep) and during SD (30-35 hours, supervised by team member). SPM8 was used for standard image preprocessing and for fMRI signal estimation, independently for the 2-, 3- and 4-ball tracking conditions of the VA task. The average values of the non-displaceable binding potential (BPND) in each voxel computed from normalized PET images were averaged within three regions-of-interest, caudate (CD), putamen (PU) and ventral striatum (VS), based on the Automated Anatomical Labeling digital atlas. SPM8 simple and multiple linear regression analyses were used to assess the association between fMRI signals in the brain and D2/D3R in CD, PU and VS. The statistical significance of the regression slopes that quantify the neurovascular coupling was set as PFWE $< 0.05$, corrected for multiple comparisons at the cluster level.

**Results:** Performance accuracy during the VA task was lower during SD than during RW ($P < 0.05$). BPND values in CD, PU and VS were lower for SD than for RW ($P < 0.05$, paired t-test). fMRI activation was higher in the thalamus and lower in SPC and PFC for SD than for RW (PFWE $<$ 0.05). Brain activation responses in SPC decreased in proportion to D2/D3R in VS and increased in proportion to D2/D3R in CD, both during RW and during SD (PFWE $<$ 0.001). For the PFC, brain activation responses in anterior cingulate cortex (ACC) or the supplementary motor area (SMA) increased in proportion to D2/D3R in VS and decreased in proportion to D2/D3R in PU, both during RW (ACC) and during SD (SMA)(PFWE $<$ 0.001). Brain activation responses in thalamus increased with D2/D3R in CD, VS and PU (PFWE $< 0.003$). The slopes of the linear associations between fMRI signals in anterior thalamus and D2/D3R in CD, and between fMRI signals in posterior thalamus and D2/D3R in VS were significantly steeper for RW than for SD (PFWE $< 0.02$).

**Conclusions:** Here we demonstrate distinct involvements of D2/D3R in different striatal regions in the modulation of the VA activation in thalamus, SPC and PFC. Specifically for the thalamus, the SD-related reduction in the availability of D2/D3R in the striatum was associated with decreased DAergic modulation and enhanced activation during SD; for the SPC, the balanced CD-to-VS D2/D3R modulation was stronger for RW than for SD and was associated with hypo activation during SD; and for the PFC, the balanced PU-to-VS D2/D3R modulation was predominantly associated with stronger deactivation during RW than during SD. Findings are consistent with a robust DAergic modulation of cortical activation, which counterbalance D2/D3R in dorsal versus ventral striatum and was not sensitive to SD. Findings are also consistent with a more fragile modulation of activation in the thalamus, which was mediated by D2/D3R in dorsal and ventral regions of the striatum and was impaired by SD.

**Keywords:** Dopamine, Attention, sleep disturbance, fMRI, PET

**Disclosures:** Nothing to disclose.

## M227. PET Imaging of TSPO Expression in Alcohol Dependent Subjects During Acute Abstinence: Comparison with Healthy Control Subjects

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**Background:** Neuroinflammatory processes are closely associated with behavioral changes that occur in chronic alcohol dependence. For example, dysregulated neuroimmune function may be responsible for hallmark behaviors of alcohol dependence such as impaired cognition and intensified withdrawal symptoms. Microglia are key CNS cells that become 'activated' in the presence of neuroinflammatory insult. The positron emission tomography (PET) radioligand [11C] PBR28 binds with high affinity to the 18 kDa translocator protein (TSPO), a mitochondrial protein which is overexpressed in activated microglia. The goal of our study was to measure TSPO levels with [11C] PBR28 PET imaging in alcohol dependent subjects during acute abstinence and healthy controls, preliminarily assessing neuroimmune function in alcohol dependence.

**Methods:** Eleven alcohol dependent subjects (8 M, 3F; 40 ± 7 years) were recruited for a single [11C] PBR28 PET scan, which occurred 3 ± 1 days after their last drink. Subjects were genotyped before imaging to identify their single nucleotide polymorphism rs6971 phenotype, which corresponds to relative binding affinity of [11C] PBR28 for TSPO. Low affinity binders were excluded from the study, while mixed affinity binders (MABs) and high affinity binders (HABs) were included. Eleven healthy controls, matched for sex, age, and binding status, were also imaged with [11C] PBR28 PET. Scans began with bolus injection of 475 ± 210 MBq, and PET data were acquired for 120 min on a high resolution research tomograph (HRRT), featuring arterial blood sampling for measurement of the input function.

Additional blood samples were acquired to measure peripheral cytokine levels. PET data were corrected for partial volume effects to account for gray matter atrophy. TSPO levels were quantified with total distribution volume (VT), calculated with the multilinear analysis method, in the cerebellum, occipital cortex, frontal cortex, and hippocampus.
**Results:** Alcohol dependent subjects averaged $7 \pm 2$ drinks/day with 4-7 drinking days/week over at least 9 heavy drinking years. There were 6 HABs and 5 MABs for each group. Across the four regions examined, [11C] PBR28 VT was 13% lower in alcohol dependent subjects compared to healthy controls. The VT values (units: mL/cm3) for alcohol dependent subjects vs. healthy controls in each region and group were: Cerebellum {MABs($2.8 \pm 0.3$ vs $3.3 \pm 0.4$); HABs ($4.5 \pm 0.5$ vs $5.3 \pm 0.2$)}; Occipital Cortex {MABs($3.6 \pm 0.4$ vs $3.9 \pm 0.4$); HABs ($5.2 \pm 0.5$ vs $6.1 \pm 0.4$)}; Frontal Cortex {MABs ($3.4 \pm 0.4$ vs $3.8 \pm 0.4$); HABs($5.2 \pm 0.5$ vs $6.2 \pm 0.5$)}; Hippocampus {MABs ($2.8 \pm 0.4$ vs $3.0 \pm 0.4$); HABs ($4.0 \pm 0.5$ vs $4.8 \pm 0.3$)}.
**Conclusions:** These preliminary findings suggest that alcohol dependent subjects in acute abstinence may have lower TSPO levels compared to healthy controls. We thus hypothesize that this may indicate compromised neuroimmune function in alcohol dependence. Future work will incorporate measures of peripheral cytokines, a class of neuroimmune signaling proteins with both pro- and anti-inflammatory effects, with the reported brain data to provide a comprehensive view of neuroimmune function in alcohol dependence.
**Keywords:** TSPO and [11C] PBR-28 PET, Alcohol dependence, PET study
**Disclosures:** Nothing to disclose.

### M228. Social Isolated DISC1 Mutant Mice Displayed High Sensitivity to Chronic Cocaine Exposure and Rolipram Treatment

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**Background:** Drug abuse and addiction are influenced by both genetic and environmental risk factors. However, the effect of gene-environment interaction in drug addiction is largely unknown. We recently reported that social isolation stress imposed during adolescence in the presence of a genetic risk led to neurochemical and behavioral changes in adulthood (Niwa et al, 2013). In the present study, we validated our animal model in the pathophysiology of cocaine addiction. We also examined the molecular changes of phosphodiesterases (PDEs) and checked whether a PDE4 inhibitor can ameliorate addictive behaviors to cocaine.
**Methods:** Male DISC1 dominant-negative transgenic mice under control of the prion protein promoter (DISC1-DN-Tg-PrP) and their male littermate wild-type mice were group-housed or isolated from 5 weeks of age continuously with free access to food and water. All animal handling procedures were approved by the animal research committees of Kyoto University and Kurume University. Daily cocaine exposure-induced locomotor sensitization and conditioned place preference (CPP) were assessed after 8 weeks of age. PDE enzyme activity and the phosphorylation of GluA1 and DARPP-32 at PKA sites were assessed in the nucleus accumbens (NAc) and cortex from brain slices.
**Results:** We studied cocaine-related behaviors of four groups of mice: wild-type mice without isolation (control); wild-type mice with isolation (E group); DISC1-DN-Tg-PrP without isolation (G group); and DISC1-DN-Tg-PrP with social isolation stress (GXE group). Acute and chronic cocaine exposure induced significantly higher locomotion in GXE group than in other groups. In CPP test, GXE group mice exhibited a significant place preference conditioned with repeated exposures of cocaine than other groups. We also found increased enzyme activity of PDE4 and enhanced effects of a PDE4 inhibitor rolipram on the phosphorylation of GluA1 and DARPP-32 at PKA sites only in the NAc of GXE model mice. When we injected rolipram before place conditioning of mice with cocaine in the CPP test, rolipram completely inhibited CPP induced by chronic cocaine exposure in GXE groups.
**Conclusions:** These results indicate that the gene-environment interaction enhanced sensitivity to chronic cocaine exposure and lead to development of cocaine addictive behaviors, which can be reversed by the treatment of a PDE4 inhibitor rolipram. Our GXE mouse model mice may be important in analyzing in the pathophysiology of drug addiction including intracellular signaling.
**Keywords:** addiction, DISC1, Gene environment interaction, PDE4
**Disclosures:** Nothing to disclose.

### M229. Hnrnph1 is a Quantitative Trait Gene for Methamphetamine Sensitivity

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**Background:** Psychostimulant addiction is a heritable substance use disorder; however its genetic basis is unknown. Quantitative trait locus (QTL) mapping in mice offers a complementary approach to genome-wide association studies in humans and can facilitate environment control and statistical power as well as novel gene and mechanistic discovery. Here, we focused on the genetic basis of sensitivity to the locomotor stimulant response to methamphetamine (MA) in mice - a behavior that is associated with dopamine release and activation of the brain reward circuitry in addiction. We focused on fine mapping a QTL on chromosome 11 whereby inheritance of the DBA/2J allele caused a reduced MA sensitivity relative to the C57BL/6J allele (Palmer et al., 2005).
**Methods:** All procedures were approved by the Boston University and University of Chicago Institutional Animal Care and Use Committees and were conducted in accordance with National Institutes of Health guidelines for the

care and use of laboratory animals. To fine map the QTL, congenic lines possessing various chromosome 11 intervals from the DBA/2J strain on a C57BL/6J background were backcrossed to C57BL/6J. We introduced and monitored new recombination events that were causally associated with reduced MA sensitivity (2 mg/kg, i.p.). Behavior was assessed in 5-min bins in an open field using video recording and tracking (AnyMaze). Repeated measures ANOVA identified genotype x time interactions and post-hoc analysis identified their source. To gain mechanistic insight into the effect of the QTL on brain function and behavior, transcriptome analysis via RNA-seq was performed on striatum punches (N = 8) from congenic mice showing reduced MA sensitivity (50-60 Mb). Single-end reads (50 bp, Illumina) were prepared and sequenced at the University of Chicago Genomics Facility. EdgeR was used to calculate differential gene expression (FDR < 5%) and Ingenuity Pathway Analysis (IPA) was used to identify canonical pathways and gene networks. Finally, Transcription activator-like effector nucleases (TALENs) were used to introduce double-stranded DNA breaks and frameshift deletions into the first coding exon of each of the two protein-coding genes within the 206 kb QTL interval (Rufy1 and Hnrnph1; Cellectis Bioresearch). TALENs mRNAs were injected into C57BL/6J embryos at the Transgenic and Genome Engineering Core Facility at BUSM and deletions were identified using a restriction digest-based PCR assay followed by cloning and Sanger sequencing. MA-induced conditioned place preference (MA-CPP) was performed as described (Kirkpatrick and Bryant, 2015).

**Results:** We positionally cloned a 206 kb QTL in congenic mice (chr. 11: 50,185,512-50,391,845 bp; mm9) that caused reduced MA sensitivity. This chromosomal region contained only two protein coding genes - heterogeneous nuclear ribonucleoprotein, H1 (Hnrnph1) and RUN and FYVE domain-containing 1 (Rufy1). Transcriptome analysis in the striatum of congenic mice implicated a neurobiological mechanism of the QTL that involved reduced mesolimbic innervation and striatal neurotransmission. As an example, Nr4a2 (nuclear receptor subfamily 4, group A, member 2; a.k.a. Nurr1), a transcription factor crucial for midbrain dopaminergic neuron development, exhibited a 2.1-fold decrease in expression (p 4.2 x 10 -15). Furthermore, two replicate TALENs lines possessing frameshift deletions in Hnrnph1, but not Rufy1, recapitulated the reduced MA sensitivity observed with the congenic QTL, thus identifying Hnrnph1 as a quantitative trait gene for methamphetamine sensitivity. Lastly, Hnrnph1 +/- mice also demonstrated less sensitivity to the addictive properties of MA as measured via MA-CPP.

**Conclusions:** These results define a novel contribution of Hnrnph1 to the stimulant and addictive properties of MA. Hnrnph1 codes for an RNA binding protein that did not have any previously known function in psychostimulant behavior. Interestingly, Hnrnph1 can regulate the expression and splicing of Oprm1 (mu opioid receptor gene) and a human intronic SNP in OPRM1 (rs9479757) was recently associated with decreased binding affinity of hnRNP H1, exon 2 skipping, and the severity of heroin dependence (Xu et al., 2014). Alternative splicing is a common genomic feature of RNA binding proteins and neuropsychiatric disorders and several HNRNPH1 binding sites are modulated by SNPs within genes that exhibited genome-wide significant associations with psychiatric disorders (Glatt et al., 2011). Our results provide a compelling rationale for a new line of investigation into the role of Hnrnph1 in neural development and plasticity associated with the addictions and other psychiatric disorders.

**Keywords:** psychiatric genetics, RNA binding protein, genome editing, QTL, QTG
**Disclosures:** Nothing to disclose.

## M230. Physical and Emotional Stress Alter Voluntary Morphine Consumption and Ventral Tegmental Area TORC2 Signaling

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**Background:** There is significant co-morbidity of mood disorders and drug dependence, but the mechanisms contributing to this co-morbidity are not well understood. Preclinical models of mood disorders typically employ chronic stress to elicit depressive-like behaviors, but these usually involve physical trauma, complicating study of pain-relieving opiate drugs. Here, we used physical chronic social defeat stress (CSDS), as well as a modified version, emotional CSDS, to investigate changes in morphine reward and to identify potential molecular mediators of stress susceptibility and reward. One promising candidate is mammalian target of rapamycin complex 2 (TORC2) given that changes in TORC2 signaling in the ventral tegmental area (VTA) have been observed in response to both chronic morphine and stress. TORC2 is ideally poised given its responsiveness to stimuli including cellular stress and it has effectors important for both neuronal activity and cytoskeletal remodeling. Here, we present evidence that chronic stress induces a bidirectional change in TORC2 signaling that contributes to morphine reward valence.

**Methods:** We subjected male c57Bl6 mice to physical or emotional CSDS and assessed social interaction (SI) on day 11 per standard protocols (Golden et al., 2013; Warren et al., 2013). Briefly, physical stress (PS) mice were subjected to a 5-10 min social defeat episode with a novel CD1 mouse daily for 10 days, and for emotional stress (ES), a second c57 mouse witnessed this encounter via a perforated Plexiglass partition. Following each defeat, the PS and ES mice were co-housed with CD-1 mice, physically separated but in sensory contact. After the tenth defeat, mice were singly housed and SI testing was performed 24 hours later. To assess morphine consumption, we used a two-bottle choice paradigm. Following acclimation to bottles filled with just water, singly-housed mice were given bottles filled with 0.2% sucrose and 0.3 mg/ml morphine sulfate or 0.06 mg/ml quinine sulfate, which were measured daily. Morphine intake was assessed at multiple time-points following CSDS, as well as during ES. To determine whether alteration of TORC2 activity affected morphine preference and susceptibility to stress, we utilized genetic and viral approaches. To increase TORC2 signaling, c57Bl6 mice received intra-VTA infusion of either HSV-GFP or HSV-GFP-Rictor. To decrease TORC2 signaling, floxed-Rictor mice received intra-VTA infusions of AAV-GFP or AAV-Cre, and progeny from

floxed-Rictor/TH-Cre crosses, which specifically lack TORC2 activity in catecholaminergic neurons, were also examined.
**Results:** Both physical and emotional CSDS decrease SI score on day 11, with physical stress eliciting more robust social avoidance than emotional stress. However, by day 39, physical and emotional stress mice exhibit similar levels of avoidance. Physical and emotional CSDS also significantly increase morphine preference and there is a significant negative correlation between SI score and morphine preference on day 11. Given that CSDS induces long-lasting changes in SI, we examined whether changes in morphine reward persist. We observed a similar significant negative correlation between SI score and morphine consumption 14 days after the last defeat. Next, we determined whether morphine preference was also altered during emotional CSDS. Contrary to results following stress, mice undergoing emotional CSDS showed decrease morphine consumption compared to controls. Finally, we wanted to determine whether individual differences in morphine preference could predict susceptibility to CSDS. We found that morphine preference (determined 14 days prior to stress) did not predict susceptibility to CSDS. Combined, these data suggest that CSDS differentially affects morphine reward, depending on when consumption is measured. Given the importance of the ventral tegmental area (VTA) in both CSDS and drug reward, we are currently investigating drug- and CSDS-induced changes in VTA signaling as potential mediators of these behavioral effects. One promising candidate is TORC2, which we have previously shown is a critical mediator of morphine-induced changes in VTA DA neuron structure and function. Interestingly, we observe bidirectional changes in TORC2 signaling induced by CSDS, where TORC2 signaling is initially decreased, then increased following CSDS, correlating with changes in morphine reward. We are now determining whether VTA TORC2 signaling plays a causative role in stress-induced phenotypes through genetic and viral approaches. Our preliminary data indicate that increasing TORC2 signaling prior to stress prevents development of susceptibility.
**Conclusions:** Interestingly, we observe bidirectional changes in TORC2 signaling induced by CSDS. Changes in TORC2 signaling appear to predict reward valence, as morphine intake is increased at time-points when TORC2 signaling is increased, and decreased when TORC2 signaling is depressed. Combined, these data suggest that TORC2 may play a critical role in stress-induced changes in morphine reward and consumption, supporting further study of TORC2 as a novel node for therapeutic intervention.
**Keywords:** morphine, Social defeat stress, Ventral tegmental area (VTA), mTOR, mouse behavior
**Disclosures:** Nothing to disclose.

### M231. Dissociating Appetitive and Consummatory Behavior in Drug Use Prone and Resistant Animals

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**Background:** Individual differences in an animal's locomotor response to an inescapable novel environment is considered a model of sensation-seeking in humans. Variability in the response to novelty predicts behavioral responses to acute cocaine administration. For example, animals with higher response to novel environment (HR) show greater locomotor activity to acute cocaine administration compared to animals with a lower response to novel environment (LR). However, results with respect to cocaine self-administration (SA) in HR and LR animals are mixed. First, it is well documented that higher response to novelty is associated with faster acquisition of cocaine SA suggesting this phenotype confers vulnerability to initial drug use. More recent evidence suggests, however, that this phenotype does not predict the transition from recreational drug use to characteristics of drug addiction such as heightened motivation to obtain cocaine.
It has been proposed that models of cocaine SA in rats often conflate consummatory behaviors and motivation because the lever that is used to assess motivation is also used by the animal to consume cocaine. This conflation calls into question the face validity of these rodent models since drug addiction is predominantly characterized by a motivation to obtain the drug of choice (e.g., seeking supply) which is temporally and characteristically distinct from consuming the drug of choice (e.g., injecting needle).
The goal of the current study was to separate the consummatory and motivational aspects of cocaine SA by requiring both HR and LR rats to press a lever for cocaine under a progressive ratio schedule of reinforcement (PR lever) in order to obtain access to a second, spatially and temporally distinct lever that could be used by the animal to consume cocaine under a hold-down procedure (HD lever). Our rationale was that this experimental design would assess the degree to which an animal's response to novelty would independently predict the motivation to obtain drug access (PR lever) and/or the amount in which the animals will consume (HD lever), thereby testing contrasting results in the literature within a single experimental design.
**Methods:** Animals were first prescreened for their locomotor response to an inescapable novel environment in a 90 minute session and then implanted with a jugular catheter for intravenous SA. Following recovery, animals were trained to perform progressively increasing number of lever presses on one lever (PR lever) in order to gain access to a second lever that when pressed would deliver cocaine intravenously using a hold-down procedure (HD lever). The hold down procedure allowed animals to press and hold down the HD lever in order to control the length of time the cocaine infusion pump would remain on. Thus, pressing and holding the lever turned on the pump at a consistent rate until the rat decided to disengage the lever and turn the pump off. Ultimately, animals were allowed 30 second access to the HD lever after fulfilling the progressively increasing demands of the PR lever. Five concentrations of cocaine were tested in each animal, with one concentration tested per day.
**Results:** Regression analysis was first used to examine the relationship between cocaine intake on the HD lever and breakpoints on the PR lever regardless of locomotor activity. For each concentration of cocaine, breakpoint and intake was significantly and linearly correlated such that higher intake was associated with greater breakpoints. As cocaine concentration increased, the variability in intake increased while variability in breakpoint decreased. Next we examined whether the

locomotor response to novel environment predicted behavior on either the PR or HD lever. For each concentration of cocaine, the response to novelty was positively correlated with intake, with relatively small and nonsignificant relationships the lowest concentrations that was progressively augmented and became significant as concentration increased. For the PR lever, the response to novelty was positively correlated with breakpoint and characterized by a robust positive relationship at the lowest concentrations that was progressively diminished and became nonsignificant as concentration increased. In other words, locomotor response to novelty strongly predicts breakpoints only when cocaine concentration remains low while predicting intake only when cocaine concentration is high. More detailed analyses demonstrated that these shifting relationships are due to the fact that the breakpoints for HR animals are insensitive to cocaine concentration and intake and are augmented relative to LR animals across all concentrations. LR animals, on the other hand, exhibit substantial shifts in breakpoints across increasing concentrations of cocaine with relatively low amounts of intake across all concentrations relative to HR animals.

**Conclusions:** Our data clarify and revise the current thinking regarding models of sensation seeking as predictors of vulnerability to addiction by showing that HR animals are highly motivated to take even small amounts of cocaine while motivation in LR animals is robustly sensitive to cocaine availability. This work also makes the point that vulnerability to addiction (as measured by PR) is explained by preexisting phenotypes only in a subset of animals (HR animals), and equal vulnerability to cocaine can be conferred to all phenotypes, but only under conditions where exogenous factors are primed to elicit motivation for cocaine.

**Keywords:** cocaine, Self-Administration, Vulnerability, motivated drug taking, Behavioral Pharmacology

**Disclosures:** Nothing to disclose.

### M232. Dopamine Release and Cocaine Sensitivity Differ Between Striosome and Matrix Compartments of the Striatum

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**Background:** The striatum is the major entry point for cortical input to the basal ganglia. As such it is involved in a number of neural functions including learning and action control. Two major output pathways from the striatum have been characterized: the direct and indirect pathways, which consist of D1 and D2 dopamine receptor-expressing neurons, respectively. The striatum can also be classified into striosome and matrix compartments, based on the differential expression of several proteins, including acetylcholinesterase, mu opioid receptor, dopamine transporter (DAT), and the nuclear receptor subfamily 4, group A, member 1 (Nr4a1). A number of functional differences between the striosome and matrix compartments have been implicated in psychiatric and neurological disorders including substance abuse, Parkinson's disease, and Huntington's disease. Given the importance of dopamine signaling in these conditions and the differences in expression of various dopamine transmission-related proteins between compartments, we hypothesized that dopamine release would differ between striosome and matrix compartments across the striatum.

**Methods:** To test this hypothesis, we used Nr4a1-eGFP mice to delineate striosome and matrix compartments and fast scan cyclic voltammetry to measure electrically evoked dopamine overflow from adjacent striosome and matrix compartment pairs. We then explored potential compartmental differences in neurobiological mechanisms known to contribute to dopamine release using pharmacological tools.

**Results:** We found that electrically evoked dopamine overflow in striosomes in the dorsal striatum was reduced approximately 35% compared to the matrix compartment. We further found that in the ventral striatum, this pattern was reversed, such that evoked dopamine overflow in striosomes was approximately 64% greater than in the corresponding matrix compartment. We then examined three common neurobiological mechanisms involved in modulating striatal dopamine release. We started by examining the effect of quinpirole, a D2 dopamine receptor agonist, on D2 autoreceptor-mediated inhibition of dopamine release and found no compartment differences in either the dorsal or ventral striatum. To examine the contribution of nicotinic acetylcholine receptors, we treated slices with DHBE and found that antagonism of nicotinic acetylcholine receptors inhibited dopamine overflow to a similar degree in both striosome and matrix compartments. We further found that in the presence of DHBE, the compartmental differences in the dorsal striatum remained intact. We then examined the role of DAT in the differences in dopamine release between compartments and found that cocaine enhanced dopamine overflow in striosomes to a greater degree than in the matrix at several concentrations. Furthermore, modeling of the dopamine overflow kinetics showed that cocaine inhibited dopamine uptake in the matrix to a greater degree than in striosomes. These difference in cocaine sensitivity, however, were limited to the dorsal striatum.

**Conclusions:** Together these findings demonstrate a previously undescribed strict regulation of striosomal dopamine relative to the matrix. These findings have interesting implications for normal striatal dopamine function, as well as dopamine and striatum-related neurological and psychiatric disorders, including addiction.

**Keywords:** voltammetry, Substance abuse, Basal Ganglia, dopamine transporter

**Disclosures:** Nothing to disclose.

### M233. Selective Loss of BDNF-TrkB-PLCγ Signaling in Accumbens Shell Neurons Attenuates Cocaine-Induced Dendritic Spine Formation, but Increases the Motivation for Cocaine

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**Background:** Chronic cocaine induces dendritic spine growth in accumbens shell (NACsh) neurons, but this effect

has not been shown to directly enhance addictive behavior. Spine formation has been functionally linked to BDNF-TrkB signaling in other brain regions, but whether this mechanism underlies cocaine-induced spine formation is unknown. We previously found that cocaine induces BDNF activation of TrkB signaling through phospholipase C gamma-1 (PLCγ-1), but TrkB can also activate other pathways including the extracellular regulated kinase (ERK) pathway. In this study, we tested the necessity of BDNF-TrkB-PLCγ signaling on dendritic spine formation in NACsh neurons, and compared effects with modulation of cocaine self-administration behavior.

**Methods:** Novel herpes simplex virus (HSV) vectors were constructed for this study that express the wild-type receptor (HSV-TrkB-WT), a kinase-dead dominant negative mutant (HSV-TrkB-KD), or a mutant TrkB that selectively blocks either TrkB signaling through PLCγ-1 but not ERK (HSV-TrkBY816F). All vectors co-expressed GFP and HSV-GFP only was used as a negative control. The ability of these vectors to selectively block certain BDNF-TrkB signaling pathways was assessed with infected HEK cell cultures at either basal or BDNF-stimulated conditions. For analysis of cocaine self-administration (SA) behavior, rats were implanted with bilateral NACsh cannulae and trained to SA cocaine on a fixed ratio (FR) schedule for 3-4 weeks; a dose-response for cocaine SA was assessed before, during, and after transient HSV expression of the TrkB mutants. A second HSV infusion was given prior to assessment of motivation for cocaine on a progressive ratio reinforcement schedule (PR). For morphological analysis, separate cohorts engaged in cocaine or saline SA for 3 weeks, and HSVs were infused into the NACsh followed by 2 more days of SA and 24 h withdrawal prior to brain perfusion. Dendritic spine densities were quantified from confocal images of GFP-labeled neurons using Volocity 3D analysis. All experimental protocols in animal studies were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Results:** BDNF increased both pERK/ERK and pPLC/PLC in HEK cells infected with TrkB-WT compared to HSV-GFP controls, while HSV-TrkB-KD infected cells failed to show BDNF-induced ERK or PLCγ phosphorylation. HSV-TrkBY816F allowed increases in pERK without concomitant increases in pPLC as predicted. Loss of TrkB-PLCγ signaling with TrkBY816F expression in NACsh neurons caused a transient leftward shift in the threshold dose necessary to maintain cocaine SA compared with GFP controls, indicating increased sensitivity to cocaine reinforcement. TrkBY816F expression also increased motivation for cocaine on the PR schedule of cocaine reinforcement. In contrast, loss of TrkB- PLCγ signaling with TrkBY816F expression during SA reversed cocaine-induced increases in spine density without affecting basal spine density in saline SA animals.

**Conclusions:** These results are the first to implicate local BDNF-TrkB activity in cocaine-induced morphological changes in the NACsh, and suggest that the TrkB-PLCγ signaling pathway is important for this effect. Since inhibiting this TrkB-PLCγ pathway also enhances the motivation for cocaine, cocaine-induced dendritic spine formation may represent a counter-adaptive process that reduces rather than promotes addictive behavior.

**Keywords:** Dendritic Spine, TrkB, addiction, cocaine

**Disclosures:** Nothing to disclose.

### M234. Changes in Cortico-Striatal Neuroplasticity Following Chronic Self-Administered Methamphetamine

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**Background:** Methamphetamine (meth) abuse induces changes in the prefrontal cortex (PFC) and nucleus accumbens (NAc), thereby control over drug seeking is dysregulated. Hence, meth-induced functional impairment of these two regions may increase abuse vulnerability. Here, we describe changes in cortico-accumbal neuroplasticity following a contingent meth administration protocol.

**Methods:** Meth was delivered IV on an FR1 schedule at a volume of 20 μg/50 μl infusion. Rats received one-hour meth self-administration (SA) sessions for 7 days, followed by 14 days of six-hour long access sessions and yoked saline controls. Rats were sacrificed 7 days after discontinuation of meth and whole-cell voltage clamp recordings were performed in the dorsomedial PFC (dmPFC) and NAc core (NAcc) of the same animals, field potentials were also recorded in the NAcc.

**Results:** In the dmPFC, the AMPA/NMDA ratio (calculated at +40mV as: IAMPA / ITotal – IAMPA) was reduced in meth SA rats compared to controls indicating a change in synaptic strength of deep-layer PFC pyramidal neurons. Specifically, we detected a trend towards an increase in the NMDA receptor-mediated currents in the meth-treated group. Whether this change is due to increased receptor expression or changes in receptor subunit composition remains to be determined. In the NAcc we observed a significant paired pulse depression (50 ms interpulse intervals) in meth SA rats compared to controls suggestive of an increase in the probability of neurotransmitter release. This increase in release probability was consistent between whole cell and field potential (fEPSP) recordings. In support, we found higher input/output fEPSP amplitudes in meth rats. Moreover, AMPA/NMDA ratio and long-term depression (LTD, 5Hz, 15 min) were unaffected in the NAcc.

**Conclusions:** Combined, our findings indicate changes in synaptic neurotransmission in both the dmPFC and NAcc following extended access meth. The relationship between meth pharmacology alone or in combination with behavior output has yet to be defined. As such, future endeavors will determine how these maladaptive consequences impact glutamate release, reuptake, and relapse to meth-seeking behavior.

**Keywords:** methamphetamine, self-administration, cortico-striatal plasticity

**Disclosures:** Nothing to disclose.

## M235. Effects of Maintenance Varenicline on Relapse in Those With and Without Schizophrenia Spectrum and Bipolar Disorders

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**Background:** Despite effective pharmacotherapeutic cessation aids, relapse to smoking is common after initial abstinence, and effective relapse prevention interventions have not been identified. Relapse following discontinuation of pharmacotherapy is particularly prevalent and rapid among smokers with serious mental illness (SMI).
Aim: To compare effectiveness of maintenance pharmacotherapy for relapse prevention in recently abstinent smokers with and without SMI.
**Methods:** To conduct a pooled analysis of two randomized, double-blind, placebo-controlled trials of maintenance varenicline and behavioral therapy for relapse prevention in smokers with and without SMI.
**Results:** There were significant effects of diagnosis, treatment, and a diagnosis by treatment interaction on point-prevalence abstinence at week 24. Those with SMI had reduced likelihood of abstinence; those on varenicline had increased likelihood of abstinence, and the impact of SMI diagnosis on abstinence differed by treatment. On varenicline, the odds of abstinence at week 24 did not differ between those with and without SMI ($87.2 \pm 0.8\%$ vs $81.9 \pm 0.2\%$, OR: 1.68, 95%CI:0.53,5.32, $p = 0.38$). On placebo, the week-24 abstinence rate in those with SMI was less than half that for those without SMI ($29.4 \pm 1.1\%$ vs. $61.8 \pm 0.4\%$, OR:0.26, 95%CI:0.13,0.52, $p = 0.0002$). There were significant differences in time to first lapse ($X23df = 94.52$, $p < 0.0001$; all pairwise comparisons $p < 0.05$) between the diagnosis by treatment strata (SMI vs. non-SMI by placebo vs. varenicline). Time to first lapse was shortest in participants with SMI on placebo ($Q1 = 12$ days, 95%CI:4,16), followed by participants without SMI on placebo ($Q1 = 17$ days, 95%CI:17,29), then those without SMI on varenicline ($Q1 = 88$, 95%CI:58,91, and lastly participants with SMI on varenicline ($Q1 > 112$, 95%CI:-non-est.)
**Conclusions:** Among those assigned to maintenance pharmacotherapy, there was no difference between those with and without SMI in week-24 abstinence or time to first lapse. Conversely, among those assigned to maintenance placebo, those with SMI were more than twice as likely to relapse and relapsed more rapidly than smokers without SMI. Maintenance varenicline appeared to normalize the relapse rate of smokers with SMI, such that it was similar to that of smokers without SMI undergoing the same treatment. Maintenance varenicline may help smokers with and without SMI to maintain long-term abstinence.
**Keywords:** schizophrenia, nicotine, varenicline, maintenance treatment, relapse
**Disclosures:** Pfizer provided data for this analysis. Dr. Evins has received research grant support from Pfizer in the past 3 years. No other authors have financial interests to disclose.

## M236. Disrupted Relationship of Conscientiousness to BOLD Activation During Error Monitoring and Resting State Functional Connectivity in Cocaine-Dependent and Healthy Control Subjects

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**Background:** High levels of impulsivity are associated with low treatment retention and predict relapse in substance use disorders. A growing literature suggests that indices of impulsiveness are associated with striatal-limbic alterations in cocaine-dependent patients and that related task-associated BOLD responses and resting state functional connectivity (rsFC) measures may be predictive of relapse. However, this literature remains variable and highly dependent on the particular measures of impulsivity and fMRI tasks utilized. To further explore these relationships using more global measures of functioning, we assessed 22 healthy controls and 50 2-4 week abstinent, treatment-seeking cocaine-dependent participants with an array of impulsivity measures (including neurocognitive, self-rating, and personality measures), two fMRI tasks assessing error monitoring, and rsFC fMRI.
**Methods:** Personality and self-rating, relative to neurocognitive, measures may best differentiate control and cocaine-dependent groups (Lobue et al. 2014). Our impulsivity measures were, therefore, limited to two personality domains (NEO-PR-I Conscientiousness; TCI Self-directedness) and three self-rating domains [(BIS-11 Motor and Nonplanning; Frontal Systems Behavior Scale - Disinhibition]. fMRI BOLD was assessed during two tasks that assess error monitoring: the Stop Signal Task (SST), a measure of inhibitory control, and the Response Reversal Task (RRT), a set-shifting task. To explore a more generalized neural response to error monitoring, a conjunction test was performed to identify voxels activated during error trials in both tasks ($Z > 6$; cluster threshold $p < .05$). Using the three striatal-limbic clusters identified in the conjunction analysis (described below), the combined SST and RRT BOLD response for each participant was extracted from a center of gravity 5mm seed in each cluster. Interaction effects of the experimental group with impulsivity measures were assessed using GLM, adjusting for gender and age, to determine its influence on average BOLD activity in the seed regions. rsFC analysis was then conducted in both groups (20 controls, 44 cocaine-dependent) using the identified conjunct striatal-limbic seed regions to generate individual corresponding rsFC maps. Voxel-wise analysis using a mixed-effects GLM (covariates: years of education, gender, motion) was performed to assess group main effect of whole-brain rsFC connection with the error-monitoring activated regions, main effect between rsFC maps and the impulsivity measures, and interaction between group $\times$ impulsivity measures ($p_{corrected} < 0.05$; $p_{uncorrected} < 0.02$ combined with cluster threshold $> 2646$ mm3).
**Results:** Conjunction analyses of the SST and RRT identified three striatal-limbic regions that were jointly activated during error monitoring tasks: left and right anterior insula and the pre-supplementary motor area/dorsal anterior cingulate cortex

(preSMA/dACC). The bilateral anterior insula (averaged from above left/right finding) showed a significant (uncorrected) group interaction with a single measure of impulsivity, Conscientiousness [$t(65) = 2.3$, $p < 0.023$]. This interaction was driven by a positive correlation in healthy controls ($r = 0.54$) but no correlation in cocaine-dependent subjects ($r = 0.004$). Subsequent rsFC interactions with impulsivity measures were therefore limited to Conscientiousness. The preSMA/dACC and the combined bilateral anterior insula were used as two seed regions to calculate rsFC maps. A significant interaction was observed between the combined anterior insula seed and the rostral anterior cingulate cortex (rACC)/medial prefrontal cortex (mPFC), driven by a positive relationship between anterior insula:rACC/mPFC connectivity strength in the healthy control group with the Conscientiousness score ($r = 0.64$) and, again, no relationship in the cocaine-dependent group ($r = -0.14$). Compared with the control group, the cocaine-dependent group showed a decrease in the rsFC strength between the anterior insula and right middle temporal gyrus.
**Conclusions:** Our findings suggest brain-behavior relationships relevant to impulsivity are disrupted in cocaine-dependent patients. The combined use of two fMRI tasks allowed a more global assessment of error processing and the use of personality and self-rating measures provided a more global assessment of impulsivity than typically provided by neurocognitive measures. The three identified conjunct striatal-limbic clusters are consistent with expected activation of the Salience Network (thought to orient and allocate attention to external stimuli) during error monitoring. The weakened relationship of anterior insula:rACC/mPFC FC strength to Conscientiousness in cocaine-dependent participants is of particular importance, as the rACC/mPFC is a key region of error monitoring that, together with the salience processing anterior insula, is responsible for self-control, self-monitoring of performance, and affect regulation. A disruption in the relationship of Conscientiousness, a measure of motivation, persistence, and control in goal-oriented behavior, with neural activation and inter-regional connectivity of error monitoring networks may therefore reflect disturbances relevant to the maintenance of abstinence in cocaine-dependent patients.
Supported by DA023203 (BA), ULT1R000451 (NIH CTSA/UT-STAR), and NIDA-IRP.
**Keywords:** cocaine addiction, neuroticism, Human Neuroimaging, Resting State Functional Connectivity, BOLD imaging
**Disclosures:** BA: Speaker's honoraria from American Academy of Addiction Psychiatry.
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### M237. Temporal-Medial Prefrontal Circuitry Identified in Non-Treatment Seeking Cocaine Users Predicts Relapse in an Independent Cohort of Treated Cocaine Dependent Individuals

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**Background:** Both clinical and preclinical data support differences in brain structure and functional connectivity following chronic cocaine use; however, results have not been consistent between studies. To date, few studies have explored the relationship between structural differences and functional network differences in chronic cocaine users, which might improve reliability of results. Further, differences found in cocaine users are not often investigated as predictors of treatment outcome, and have never, to the best of our knowledge, been applied to an independent sample. In this study, we identify brain areas where cortical thickness differs between non-treatment seeking cocaine users (NTSCU) and healthy controls (HC). We then use these areas of difference as seeds in resting connectivity analyses to identify network alterations that relate to the structural differences. Finally, we apply the circuits found to differ between NTSCU and HC to an independent cohort of treated cocaine dependent (TxCD) individuals scanned in the final week of a residential treatment program and followed for six months.
**Methods:** 64 NTSCU and 67 HC matched on age, gender, IQ and education underwent an MPRAGE structural scan and a 6 minute resting state fMRI scan. Cortical thickness (Freesurfer) was compared between groups using a general linear model, controlling for nicotine dependence, which differed between the groups. Regions of cortical thickness difference were used as seed regions for resting state functional connectivity (rsFC) analyses. Time courses from each voxel in the seed region were averaged to create a reference time courses, which were then correlated with the time course of every other voxel in the brain for each participant. rsFC maps were generated separately for NTSCU and HC and compared using a general linear model controlling for nicotine dependence. The relationship between seed region cortical thickness and rsFC in the regions where connectivity differed between NTSCU and HC was examined with a regression analysis. Regression analyses were also performed between cortical thickness/rsFC differences and cocaine use characteristics (years of use and intensity of current use). We then extracted rsFC strength of the resulting 16 circuits from a TxCD cohort of 45 individuals, 21 of whom were abstinent at 30 days and 8 at 150 days. Survival analysis with a Cox regression model ($\square = 0.05$) was performed to predict the days to relapse post-treatment using the 16 circuit strengths and age, gender, years of education and a motion index as possible explanatory variables. Forward conditional stepwise selection method identified the variables providing statistically significant contribution towards predicting relapse status. Time-dependent receiver operating characteristics (ROC) curves were plotted at 30-day intervals to estimate the predictive power of the resulting relapse model over the 168-day follow-up period using the pROC software package in R (cran.r-project.org).
**Results:** NTSCU had reduced cortical thickness in bilateral insulae and increased cortical thickness in bilateral temporal poles (TP). NTSCU had reduced rsFC from both insulae to dACC and from left insula to left SFG/MFG. NTSCU had reduced rsFC from both TP to MTG/STG, PCC/precuneus and SFG. In addition, right TP had reduced rsFC to mPFC and supramarginal gyrus and left TP had reduced rsFC to cerebellum. rsFC in regions showing group differences from the right TP was correlated with right TP cortical thickness only in NTSCU, and this rsFC correlated

negatively with intensity of current cocaine use, especially from right TP to medial SFG. rsFC in the regions differing from the right insula seed (primarily dACC) correlated negatively with years of cocaine use. When applied to the TxCD cohort, a model including right TP - mPFC rsFC strength and years of education best predicted relapse status (more years of education and greater rsFC in this circuit was associated with longer time to relapse). Using time-dependent ROC curves with time-varying sensitivity and specificity, this model was 69% accurate in predicting relapse status at 30 days, rising over the follow up period to 89% accurate at 150 days.

**Conclusions:** Using a multi-modal imaging approach with an independent treatment outcome prediction cohort, we identify a rsFC circuit between right TP and mPFC which, when combined with years of education, predicted treatment outcome with a high level of accuracy. TP, thought to be involved in social emotional functioning, showed increased thickness in NTSCU but reductions in rsFC to numerous nodes of the default mode network, also important for emotional functioning including empathy. Intriguingly, right TP thickness correlated positively with rsFC only in NTSCU; however the rsFC of regions differentially connected to right TP in NTSCU was negatively correlated with current use and right TP - mPFC was highly predictive of treatment outcome. These results indicate that social emotional circuits are negatively impacted, perhaps even hijacked, by cocaine use and that proper functioning of these circuits is important for recovery. In addition, salience network (insula - dACC), important for identifying relevant internal and external stimuli, was impaired in NTSCU, especially with longer duration of use, although these circuits did not predict treatment outcome.

**Keywords:** cocaine addiction, treatment outcome prediction, morphological and resting state brain activity

**Disclosures:** Nothing to disclose.

### M238. Persistent Inflammatory Pain Alters Motivated Behavior via Dysregulation of the Opioid System in the Mesolimbic Pathway

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**Background:** During inflammatory pain, the mesolimbic pathway undergoes long term changes affecting mood and rewarding properties of reinforcers. The opioid system is at least partially involved in these long-term modifications, as adaptations in this system alter dopaminergic transmission, and could explain the alterations in the reinforcing properties of natural rewards and drugs of abuse. Our results provide evidence that inflammatory pain dysregulates both mu- and kappa-opioid systems in the mesolimbic circuit. Our findings indicate that inflammatory pain alters the rewarding properties of opioids and the overall motivational state, thus altering the pattern of opioid self-administration.

**Methods:** In the present study, we examined the regulation of mu- and kappa- opioid systems 48 hours after the induction of inflammatory pain using the complete Freund's adjuvant (CFA) rat model of inflammation. In addition, we conducted behavioral studies to examine the role of these opioid systems in the effects of pain on motivated behavior. We used a combination of electrophysiology and biochemistry to assess pain-induced changes in mu-opioid and kappa-opioid receptors (MOPR, KOPR). In addition, we assessed the role of KOPR in the motivation to obtain sucrose or morphine, using a progressive ratio (PR) schedule of reinforcement. All experimental protocols in animal studies were approved by the Institutional Animal Care and Use Committee at Columbia University.

**Results:** Inflammatory pain affects reward-induced dopaminergic transmission in the mesolimbic pathway. Because opioid systems are deeply involved in both pain processes and the regulation of dopamine release in the VTA-NAc pathway, we first analyzed the effect of inflammatory pain on mu- and kappa-opioid receptor function and expression 48 hours following CFA injection in rats. Using patch-clamp recording and microdialysis we showed that pain itself desensitizes MOPRs in the VTA. In midbrain slices containing the VTA, DAMGO superfusion decreases amplitude and frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) in neurons from control rats, but not from CFA-treated rats. Expanding on these findings, we used microdialysis to demonstrate that DAMGO-induced release of dopamine in the NAc is diminished by the presence of inflammatory pain. These pain-induced alterations in mu-opioid function in the VTA were accompanied by an increase in expression and function of KOPRs in the NAc. Using microinjections of both NorBNI and U50488 (a long acting antagonist and a short acting agonist of KOPR respectively), we partially uncovered the role of KOPR system in pain-induced decrease in motivation for both sucrose and morphine as follows; Using a PR schedule of reinforcement we clearly show impaired motivation for sucrose one hour after U50488 injection in the NAc-shell cold spot (Castro&Berridge, JNeuro 2014). Furthermore, in this paradigm, blockade of KOPR before CFA injection reverses the observed alterations in animal's motivation for reward seeking. These results suggest a prominent role of KOPRs in the deleterious effects of inflammation-induced impairment of motivation. Altogether, our data provide evidence that inflammatory pain dysregulates mu- and kappa-opioid function leading to altered dopamine transmission which in turn deeply affects the rewarding properties and motivational value of natural and drug reinforcers.

**Conclusions:** Pain patients with a prior history of drug use are sensitized to opioid exposure such that they are more prone to opioid abuse, dose escalation and/or relapse. However, the effect of pain on opioid intake patterns in animal models of opioid abuse has not been investigated. The results presented here reveal that the presence of inflammatory pain impacts the reinforcing effects of morphine and alters sucrose-seeking behavior. Moreover, KOPR activation during persistent inflammatory pain seems to be greatly involved in motivational deficits observed in rats. Taken together, our data suggest that the presence of pain impacts the effects of opioids and natural

reinforcers in the mesolimbic reward pathway by 1) decreasing mu-opioid regulatory function and 2) enhancing kappa-opioid repressive effects on dopamine release in the NAc. We are currently investigating the involvement of opioid systems in the regulation of rewarding properties of positive stimuli during painful conditions, as well as the role of adaptations in these systems in the development of addiction.
**Keywords:** opioids, motivation, pain, reward pathways, dopamine
**Disclosures:** Nothing to disclose.

### M239. Modafinil Reduces Smoked Cocaine Self-Administration in Humans: Effects Vary as a Function of Cocaine "Priming" and Cocaine Cost

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**Background:** No medications have been FDA-approved for the treatment of cocaine use disorder (CUD) despite extensive preclinical and clinical study. The failure to develop an effective cocaine pharmacotherapy may partly reflect the use of one medication for distinct clinical scenarios. For example, some patients in clinical trials for CUD pharmacotherapy enter the study already abstinent from cocaine while others are continuing to use cocaine while in treatment. It may be unrealistic to expect a single medication to both initiate abstinence, i.e., interrupt ongoing cocaine use and prevent relapse, i.e., reduce the impact of potential triggers for relapse, such as exposure to cues associated with cocaine. In human laboratory models testing the effects of potential pharmacotherapies in nontreatment-seeking cocaine users, modafinil has been one of the only medications to significantly reduce cocaine self-administration. Yet in clinical trials, modafinil has shown mixed results, with some studies reporting reduced cocaine use and others showing no effect. The objective of this study was to test the effects of modafinil on cocaine self-administration under experimental conditions that model the wide-ranging clinical scenarios by varying (a) whether participants had recent cocaine exposure, (b) were exposed to cues associated with cocaine, and (c) the cost of cocaine, in order to more precisely define how modafinil influences the decision to use cocaine.
**Methods:** Nontreatment-seeking current cocaine smokers who were not dependent on any other drug of abuse (other than tobacco) and with no history of or current alcohol dependence were enrolled in a 52-day, placebo-controlled, double-blind, within-subject study comprising both inpatient and outpatient phases. Participants were maintained on placebo capsules (0 mg/day) in one inpatient phase and modafinil (300 mg/day) capsules in another inpatient phase in counter-balanced order. A minimum of 8 medication-free days separated the two phases to allow for medication clearance. Participants, maintained on placebo or modafinil for at least 10 days, chose to self-administer smoked cocaine (25 mg) under 9 conditions: when they had been exposed to (a) both the cues associated with cocaine and were 'primed' with a non-contingent, single administration of cocaine (12-25 mg), (b) only the cues associated with cocaine use, and (c) neither cues nor cocaine. In order to determine if the effect of priming and cues differed as a function of cocaine cost, each of these conditions was tested when the cost of each cocaine administration was low ($5), moderate ($10), and high ($15); participants used actual study earnings to purchase cocaine for self-administration. There were 7 opportunities to purchase cocaine within a session so the maximum cost for self-administration was $105/session.
**Results:** Participants [3F (Black), 15M (14 Black, 1 Asian)], 44.3 ± 5.2 years of age who reported to spend $401 ± 225 per week on cocaine completed the study. Under placebo medication conditions: (1) the presence of cocaine cues alone did not significantly influence cocaine choice, but the presence of cocaine cues plus a cocaine 'prime' significantly increased cocaine choice relative to the no cue or prime condition, and (2) the cost of cocaine significantly influenced the amount of cocaine self-administered, with the number of doses self-administered within a session decreasing as a function of cost. Modafinil's effects on cocaine self-administration varied as a function of cocaine cost and the cue/prime condition. When cocaine cost $10 or $15 per dose and participants had no 'priming' dose of cocaine, modafinil robustly decreased cocaine self-administration. When participants were 'primed' with cocaine, modafinil did not reduce cocaine choice regardless of the cocaine cost. Further, when cocaine was inexpensive ($5/dose), modafinil had no effect on cocaine self-administration relative to placebo under any of the cue/prime conditions.
**Conclusions:** These findings suggest that modafinil's effects on cocaine-taking significantly vary as a function of recent cocaine exposure and cocaine cost. Modafinil was highly effective at reducing cocaine use if participants had no cocaine 'on board.' Once cocaine use was underway, modafinil was without effect. Similarly, modafinil had no influence on choice when participants had access to inexpensive cocaine ($5), but when the cost of cocaine was greater modafinil decreased cocaine choice relative to placebo. Overall, our findings may help explain the mixed clinical findings with this medication. Modafinil appears to be most effective for relapse prevention (reducing the likelihood that abstinent smokers will relapse to cocaine use), rather than initiating abstinence (reducing cocaine use in individuals who have not achieved abstinence), particularly under conditions in which cocaine is costly.
**Keywords:** cocaine addiction, pharmacotherapy, human laboratory
**Disclosures:** Nothing to disclose.

### M240. Glucagon-Like Peptide-1 Receptor Activation in the Ventral Tegmental Area or the Nucleus Accumbens Attenuates Cocaine Seeking in Rats

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**Background:** Glucagon-like peptide-1 (GLP-1) receptor signaling in the CNS is pharmacologically and physiologically

relevant for energy balance control. The GLP-1 receptor agonist exendin-4 decreases intake of palatable food when administered into the ventral tegmental area (VTA) and nucleus accumbens core. Since the VTA and the nucleus accumbens mediate the reinforcing effects of food as well as drugs of abuse, we hypothesized that GLP-1 receptor activation in these two nuclei would attenuate cocaine reinstatement, an animal model of relapse in human addicts.

**Methods:** Initially, rats were surgically implanted with indwelling jugular catheters and bilateral guide cannulas aimed at the VTA, nucleus accumbens core or nucleus accumbens shell. Following a recovery period, rats were allowed to self-administer cocaine (0.25 mg/infusion i.v.) for 21 days on a fixed-ratio 5 (FR5) schedule of reinforcement. Cocaine self-administration was then extinguished by replacing cocaine with saline. Once cocaine taking was extinguished, rats received an acute priming injection of cocaine (10 mg/kg, i.p.) to reinstate cocaine-seeking behavior. During subsequent reinstatement test sessions, the GLP-1 receptor agonist exendin-4 (0, 0.005 and 0.05 μg) was infused directly into the VTA, nucleus accumbens core or nucleus accumbens shell in separate cohorts of rats prior to a priming injection of cocaine. Rats were then placed into the operant chambers and the reinstatement test session began immediately. Potential nonspecific rate-suppressing effects of central exendin-4 administration were evaluated by assessing the influence of exendin-4 administration into the VTA, nucleus accumbens core or nucleus accumbens shell on the reinstatement of sucrose-seeking behavior in separate cohorts of rats.

**Results:** Administration of exendin-4 directly into the VTA, nucleus accumbens core or nucleus accumbens shell dose-dependently attenuated cocaine priming-induced reinstatement of drug-seeking behavior. To determine if the suppressive effects of exendin-4 in the VTA and nucleus accumbens on cocaine seeking were due to drug-induced motor impairments, we also examined the effects of intracranial exendin-4 infusions on the reinstatement of sucrose seeking. Administration of exendin-4 directly into the VTA, nucleus accumbens core and nucleus accumbens shell had no effect on sucrose reinstatement.

**Conclusions:** Taken together, these results indicate that increased activation of GLP-1 receptors in the VTA and nucleus accumbens is sufficient to reduce cocaine seeking and that these effects are not due to general motor suppressant effects of drug treatment.

**Keywords:** Self-Administration, relapse, cocaine addiction
**Disclosures:** Nothing to disclose.

### M241. N-Acetylcysteine in the Treatment of Alcohol Dependence

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**Background:** N-acetylcysteine (NAC), an antioxidant and procysteine drug, has been shown to be effective in the treatment of several addictive disorders, such as cocaine dependence, cannabis dependence, and gambling disorder. Preclinical research has shown that NAC decreases alcohol intake by stabilizing glutamate system. However, NAC has not been tested in patients with alcohol dependence. The purpose of this study was to evaluate the short-term safety and efficacy of NAC (3600 mg/day) for treating alcohol dependence. We hypothesized that NAC would be superior to placebo in reducing alcohol consumption and alcohol craving.

**Methods:** We conducted an 8-week, randomized, double-blind, placebo-controlled trial of NAC 3600 mg/day in patients with alcohol dependence. Inclusion criteria were: age 18-65 years, current alcohol dependence by DSM-IV, and heavy drinking at least 4 times in the past month. Main exclusion criteria were: current drug abuse or dependence, psychotic disorders, bipolar disorders, cognitive disorders, current suicidal or homicidal ideation, Clinical Institute Withdrawal Assessment for Alcohol (Revised) $>15$, initiation of counseling or changes in doses of psychiatric medications in the past 3 months, clinically unstable cardiac, hepatic, renal, neurologic, or pulmonary disease, and current use of naltrexone, disulfiram or acamprosate. Subjects were recruited from an outpatient clinic and were assessed weekly for safety and efficacy during the study. All subjects received manual-guided Medical Management (MM) weekly. The primary outcome variable was percentage of heavy drinking days as measured by the Time Line Follow Back (TLFB). The secondary outcome variables were alcohol craving and quality of life. Comparisons of changes in outcome measures were conducted using a linear mixed effects model. The covariates were Alcoholics Anonymous attendance and baseline alcohol craving. All p-values were 2-tailed, and p-value $< 0.05$ indicated statistical significance.

**Results:** 44 subjects were randomized and took at least one study medication. NAC group ($n = 21$) received 3600 mg/day of NAC (1800 mg twice daily) and placebo group ($n = 23$) received matched placebo. NAC was safe and well-tolerated in patient with alcohol dependence. Dropout rates were similar in the NAC group (33%) and the placebo group (30%). No serious adverse effects were reported. Both NAC and placebo groups reduced percentage of heavy drinking days (primary outcome), but the between-group difference was not statistically significant. Compared with placebo, NAC significantly reduced alcohol craving as measured by the Penn Alcohol Craving Scale (PACS) ($p < 0.05$) and enhanced quality of life as measured by the Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q) ($p < 0.05$).

**Conclusions:** This is the first study testing NAC in patients with alcohol dependence. Although NAC was not superior to placebo in reducing alcohol consumption, NAC reduced alcohol craving and improved quality of life. With regard to the safety and tolerability of NAC, 3600 mg/day of NAC demonstrated a good safety and tolerability profile in alcohol-dependent patients. Since most other NAC studies used 600-2400 mg/day, our safety data can be informative for researchers testing NAC in addictive or psychiatric disorders. In summary, these findings suggest that NAC may be beneficial for patients with alcohol dependence by reducing craving and enhancing quality of life. Although the mechanisms of these benefits are not clear, NAC may treat alcohol craving and protracted withdrawal from alcohol by stabilizing glutamate system. Since this investigation is a small pilot study, larger studies are needed to further investigate the efficacy of NAC in alcohol dependence.

**Keywords:** N-acetylcysteine, Alcohol dependence, Treatment, Clinical trial
**Disclosures:** Nothing to disclose.

### M242. The Next Generation: Passive Exposure to Personal Vaporizer Use Evokes Combustible Cigarette Desire and Urge in Smokers

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**Background:** Use of electronic cigarettes (e-cigarettes) has increased substantially in the past few years. These devices aerosolize liquid nicotine and involve inhalation and exhalation behaviors from users that resemble combustible cigarette smoking. Product types are evolving rapidly, with newer second-generation "vape pen" devices forecasted to overtake use and sales from older first-generation "cigalikes" in the next 5-7 years. However, unlike cigalikes, the next generation vape pens have less resemblance to regular cigarettes and users often report they produce a more enjoyable experience and greater nicotine "hit". We investigated whether these next generation e-cigarette devices would generalize as a conditioned cue to evoke combustible smoking urges in observers, as we have shown previously with cigalikes.
**Methods:** In this interim analysis of a controlled investigation, we tested the effect of in-vivo passive exposure to personal vaporizer ("vape pen") e-cigarette use compared to regular cigarette smoking. Participants were 43 young adult daily smokers (42% female; age mean (SD) = 28.6 ± 4.1 years, smoke 10.0 ± 4.1 cigarettes/day) and were randomized to passive exposure while conversing with a study confederate for five minutes. The study confederate used either a regular combustible cigarette (n = 22) or a personal vaporizer (n = 21) during this conversation period. The main dependent variables were pre- and post-exposure ratings on the Brief Questionnaire of Smoking Urges (B-QSU), regular and e-cigarette desire from visual analogue scales (VAS), and the Diener Positive and Negative Mood Scale.
**Results:** Results showed a main effect of time (ps ≤ .004) as observation of both the regular cigarette smoking and vape pen use significantly increased participants' VAS desire for a cigarette and BQSU smoking urge ratings. In addition, observing vape pen use, but not regular smoking, increased VAS desire for an e-cigarette (p = 0.02). Finally, vape pen observation did not affect mood, but regular smoking observation increased negative mood and decreased positive mood (ps = .04).
**Conclusions:** In sum, these analyses indicate that passive exposure to use of a next generation personal vaporizer evokes regular cigarette smoking urge and desire, as well as e-cigarette desire, without effects on general mood. Directly viewing use of these next generation devices, with their associated frequent hand to mouth movements and inhalation and exhalation behaviors, appears to share enough salient features of combustible smoking to generalize as a Pavlovian cue and elicit regular smoking urge and desire. These findings may have implications for public health given the increasing prevalence of passive exposures to vape pen use, particularly among young adult smokers who may be exposed at concerts, parties and other social events. Future research examining urge increases in relation to smoking behaviors after these exposures is warranted.
**Keywords:** Behavior, Pavlovian conditioning, Electronic cigarette (e-cigarette), Smoking urge
**Disclosures:** Nothing to disclose.

### M243. Riluzole Impairs Reinstatement to Cocaine Seeking

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**Background:** A major cellular adaptation observed following withdrawal from numerous drugs of abuse, in particular cocaine, is decreased expression and activity of the glutamate transporter GLT-1/EAAT2. GLT-1 is responsible for approximately 90% of glutamate uptake in the brain, and is critical for neuroprotection and fidelity of synaptic processing. Previous studies have reported that compounds which restore expression of GLT-1 can also reduce behavioral measures of drug seeking. We sought to test the hypothesis that a known regulator of GLT-1, riluzole, might reduce cocaine seeking. Riluzole is FDA-approved for the treatment of amyotrophic lateral sclerosis, and has been shown to upregulate GLT-1 both in vitro and in vivo.
**Methods:** To determine whether riluzole has an effect on cocaine relapse, we employed the rat self-administration/extinction/reinstatement model of cocaine abuse. Following two weeks of cocaine self-administration, rats received chronic intraperitoneal injections of vehicle or riluzole (1 or 4 mg/kg), thirty min before each of sixteen extinction sessions. Cocaine seeking was then measured during independent cue-primed and cocaine-primed reinstatement tests. Sucrose seeking was performed identically to cocaine seeking, except that reinforcement was achieved with a 45 mg sucrose pellet, instead of a 0.2 mg intravenous cocaine infusion. To determine the effects of cocaine history and riluzole administration on neuronal excitability, whole cell patch clamp recording was performed on pyramidal neurons within the prelimbic and infralimbic regions of the prefrontal cortex, following self-administration and extinction training performed identically as for behavioral reinstatement testing.
**Results:** We observed a significant, dose-dependent effect of riluzole to reduce both cue- and cocaine-primed reinstatement to cocaine. However, the effective dose of riluzole (4 mg/kg) had no effect on cue-primed reinstatement to sucrose seeking. In addition, whole cell recording data indicate a cocaine-dependent increase in prelimbic neuron excitability, which was reversed by administration of riluzole. This effect was specific to prelimbic pyramidal neurons and was not observed in infralimbic neurons.
**Conclusions:** These results indicate that chronic treatment with riluzole impairs behavioral measures of cocaine seeking without affecting pursuit of non-drug reward. Electrophysiology data also indicate that riluzole, a $Na+$ channel inhibitor, restores levels of intrinsic excitability in the prefrontal cortex, which may contribute to its effect on cocaine seeking. These results further support a growing body of literature that

implicates GLT-1 regulators as effective therapeutic candidates for psychostimulant addiction.
**Keywords:** cocaine, GLT-1, riluzole, reinstatement, Excitability
**Disclosures:** Nothing to disclose.

### M244. A Double-Blind, Active- and Placebo-Controlled Evaluation of the Neuropsychiatric Safety and Efficacy of Varenicline and Bupropion for Smoking Cessation in Subjects with (Pre-Existing) Psychiatric Disorders: An Objective Blinded Analysis

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**Background:** Neuropsychiatric Symptoms (NPS) have been reported and highly publicized with respect to both Chantix® (varenicline) and Zyban™ (bupropion). Further complicating any cause-and-effect inferences is the fact that anxiety and/or depressed mood may be symptoms of nicotine withdrawal. To investigate the neuropsychiatric profiles of varenicline and bupropion, as compared with placebo, we enrolled smokers with a prior history of a psychiatric disorder, who were motivated to stop smoking.
**Methods:** Our Southern California-based research center, along with approximately 200 other research sites in multiple countries, enrolled a total of 8,000 subjects in this Phase-IV, double-blind, Nicotine Replacement Therapy (NRT)- and placebo-controlled study. We are reporting (only) on data generated by and statistically analyzed at Pharmacology Research Institute (PRI). There was a 3-to-14 day screening phase into which 51 subjects were entered and six were excluded. Forty-five subjects who met all of the entry criteria were randomly assigned on a 1:1:1:1 ratio to varenicline, bupropion, NRT patch or placebo. The duration of the active treatment phase was 12 weeks, followed by a non-treatment follow-up phase for an additional 12 weeks.
There were weekly visits up to and including Week 6 and then bi-weekly visits between Weeks 6 and 12. On weeks with no scheduled clinic visits, telephone contact visits were performed to collect the smoking status of participants. All subjects set a target quit date (TQD) to coincide with their Week 1 visit, study Day 8. Brief (10-minute) smoking cessation counseling sessions, consistent with the Agency for Healthcare Research and Quality (AHRQ), were incorporated into each clinic visit. The primary objective was to evaluate the neuropsychiatric profiles of varenicline and bupropion as compared with placebo, including any neuropsychiatric adverse experiences (NAEs) at endpoint. The primary efficacy endpoint was 4-weeks carbon monoxide (CO)-confirmed abstinence from Weeks 9→12. The secondary efficacy endpoint was CO-confirmed continuous abstinence from Weeks 9 through 24.
Bivariate summary statistical analyses for continuous variables in successful quitters versus non-quitters were performed. The non-parametric Wilcoxon rank sum test was used to compute the p values for this comparison. The p values for comparing categorical variables in quitters and non-quitters [also ignoring time to quit] were computed using Fisher's exact test (2 x 2) or the chi-square test. Multivariate analyses using both Cox proportional hazard regression model, looking at quit rate per week, and a regression tree were used to simultaneously evaluate fifteen (15) potential predictors.
Inclusion Criteria (partial)Male and female cigarette smokers, 18-75 years of age
Smoked at least 10 cigarettes per day during the past year
Exhaled carbon monoxide (CO) $>$ 10 ppm at screening visit
Current and/or past Axis I and/or II diagnosis using DSM-IV-TR criteria based on clinical assessment and confirmed by SCID
**Results:** The "top line" results indicated that only one (1) subject had a neuropsychiatric adverse experience (NAE), panic attack. She began the study with a primary diagnosis of Generalized Anxiety Disorder (GAD) and Social Phobia, under the care of a therapist. At approximately "Week 9," she began taking Abilify® (aripiprazole) [15 mg./day], missing study medication doses and 'losing' her NRT patches. She was discontinued from the study at Week 12 [end of active treatment] at which time she had successfully stopped smoking. Smoking cessation (e.g., quit) rates were such that 15 of the 42 evaluable subjects, or 35.7%, were categorized as successful quitters utilizing the protocol-specified definition of CO values of less than ten parts per million [$<$ 10 ppm]. Notwithstanding the very small sample size(s), statistical analyses of previously observed and reported predictors of successful cessation, again demonstrated statistically significant results, including the average number of cigarettes per day ($p < .05$) and the number of lifetime quit attempts ($p < .05$). Similarly, there was also a statistical trend with respect to the age one started smoking ($p < .16$) and Body Mass Index (BMI) ($p < .07$) as having potential predictive utility with respect to successful cessation.
**Conclusions:** Our objective "still blinded-to-treatment assignment" analyses of neuropsychiatric adverse events, all involving subjects with a prior and/or current diagnosis of a psychiatric disorder, indicated that only one subject had a relatively mild NAE, which may have pertained more to her non-compliance than study drug-related causality. At her completion in the active treatment phase of the trial, she had successfully stopped smoking.
Previously reported positive baseline indicators of successful smoking cessation were replicated and re-verified vis-à-vis our analyses.
**Keywords:** Smoking Cessation, nicotine dependence, Neuropsychiatric Adverse Events
**Disclosures:** Nothing to disclose.

### M245. KCa2 Channel Inhibition in the Infralimbic Cortex is Necessary for mGluR5-Dependent Enhancement of Synaptic Plasticity and Extinction of Alcohol-Seeking Behavior

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**Background:** Postsynaptic activation of metabotropic glutamate receptors (mGluRs) enhances induction of

long-term potentiation (LTP) of synaptic transmission, and mGluR5 positive allosteric modulators (PAMs) show promise in facilitating cognitive function. Relapse to drug-seeking behaviors is a major obstacle for the successful treatment of individuals with substance use disorders and repeated exposures to drug-associated cues leads to craving that promotes consumption and facilitates relapse. Although cue-exposure therapy (inhibitory learning) should reduce the impact of cues through repeated, non-contingent cue exposure, this approach has not been effective in preventing relapse to drugs. Using cognitive enhancers to facilitate extinction learning of drug-associated cues is a promising therapeutic approach to reduce relapse rates, and results of preclinical studies show that mGluR5 PAMs facilitate extinction of drug-seeking behaviors. In the current study, we targeted a novel molecular target (i.e., small-conductance calcium-activated potassium channels (KCa2) that facilitates inhibitory learning and examined how pharmacological manipulation of KCa2 function in the infralimbic prefrontal cortex (IL-PFC) affects mGluR5-dependent synaptic plasticity and extinction learning of alcohol-associated cues.

**Methods:** Adult Wistar rats were first trained to self-administer alcohol and then underwent extinction training and a spontaneous recovery test session of alcohol-seeking behaviors. Drugs or vehicle were administered systemically or were microinjected into the IL-PFC prior to each extinction training session. Using slices from adult alcohol naïve rats, whole-cell patch-clamp electrophysiology and field recordings were used to determine the molecular mechanisms the drive mGluR5-dependent enhancement of LTP of IL-PFC neurons.

**Results:** Systemic administration of the KCa2 channel inhibitor apamin prior to each extinction training session enhanced extinction of alcohol-seeking behavior. Apamin treated rats responded significantly fewer times on the previously active lever on multiple days of extinction training and required fewer sessions to reach extinction criteria without demonstrating locomotor impairments. Rats treated with apamin prior to each extinction session also responded significantly fewer times on the previously active lever during the spontaneous recovery test performed 3 weeks following extinction. Consistent with previous findings, mGluR5 activation reduced the amplitude of KCa2 channel-mediated currents in layer V IL-PFC pyramidal neurons, and systemic treatment with an mGluR5 PAM facilitated extinction learning of alcohol-associated cues. Microinjection of the KCa2 channel positive modulator 1-EBIO into the IL-PFC prevented the ability of an mGluR5 PAM to facilitate extinction learning. Similarly, 1-EBIO co-exposure blocked the ability of an mGluR5 agonist to potentiate LTP in acute IL-PFC slices.

**Conclusions:** These data provide compelling evidence that activation of mGluR5s reduce function of KCa2 channels and enhance extinction learning of alcohol-seeking behavior. A critical finding from the present study is that the ability of mGluR5 activation to facilitate extinction learning and synaptic plasticity requires a reduction in KCa2 channel function in the IL-PFC. The results also demonstrate that blockade of KCa2 channels facilitates extinction learning of alcohol-associated cues and leads to a persistent reduction in alcohol-seeking behavior. Overall, the present findings provide strong preclinical evidence that KCa2 channels are a novel and effective target for enhancing cue exposure therapy in the treatment of alcohol use disorder.

**Keywords:** synaptic plasticity, extinction learning, alcohol self-administration, KCa2 channels, mGluR5 receptors

**Disclosures:** Nothing to disclose.

### M246. Differential Effects of Recent Vs. Past Trauma and Stress on Mood, Social Support, Binge Alcohol Intake, Emotional Eating and BMI, and on Neural Responses to Acute Stress

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**Background:** Cumulative stress and trauma adversely impact brain corticostriatal limbic circuits and are significantly associated with psychiatric disorders, other than post-traumatic stress disorder (PTSD). However, the separate effects of past and recent trauma and stress on maladaptive coping, neural responses and health behaviors are not well known. We conducted two related studies to assess separate and combined effects of cumulative recent and past trauma and stress on depression, social support, alcohol use, emotional eating and body mass index (BMI) (study 1) and on neural responses to acute stress exposure in a subsample of individuals (study 2).

**Methods:** Study 1 included a large cross-sectional sample of 847 community adults' ages (18-50 years) with minimal prevalence of PTSD who were assessed on cumulative life stress, including past and recent traumas using the Cumulative Adversity/Stress Interview (CAI, Turner & Wheaton, 1995). Participants were grouped according to two factors: recent trauma /stress (high/low scores for events in past 12 months) and past trauma/stress events (high/low scores for events prior to past 12 months) based on median cutoffs in a 2 X 2 factorial design. Social support, depressive symptoms, binge alcohol use and alcohol-related problem scores, emotional eating and BMI were assessed. Study 2 included a subsample of 75 subjects who were scanned using functional magnetic resonance imaging (fMRI) to assess recent and past trauma effects on neural responses to brief script-driven guided imagery of stress and neutral relaxing states.

**Results:** Study 1 results indicated high past trauma was associated with higher depressive symptoms, low social support and higher BMI (all $p$'s $< 0.05$), while specific effects of high recent trauma (without past trauma) were seen on increased binge drinking, alcohol-related problems and higher emotional eating scores. Study 2 fMRI findings indicated greater recent trauma exposure was correlated with a hyperactive neural response in the ventromedial prefrontal cortex (VmPFC) and ventral striatum (VS) during neutral relaxed state, and a blunted VmPFC response to acute stressful imagery ($p < 0.05$, whole brain corrected:WBC). By contrast, higher past trauma was associated with significantly increased neural responses to stress in the corticolimbic-striatal regions involving the amygdala, hippocampus, insula and striatum ($p < 0.05$, WBC), critical regions for reward and emotion processing.

**Conclusions:** These findings indicate specific effects of high recent trauma on increasing maladaptive coping behaviors of excessive alcohol use and emotional eating, separate from the well-known effects of cumulative past trauma and stress on lower mood, social support and BMI. Recent and past trauma show distinct neural responses to acute stress and neutral relaxed states. Results have implications for specific effects of recent and past trauma/stress on the risk of developing addiction versus mood and weight related disorders.

**Keywords:** Traumatic stress, mood and addiction, fMRI

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## M247. Estimated Risk of Cannabis Dependence Soon after Onset among Cannabis Only Users: Possible Influence of Tobacco and Alcohol

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**Background:** Epidemiological estimates for the United States (US) suggest that roughly 1 in 9-11 cannabis users develop a cannabis dependence syndrome (Anthony et al., 1994; Lopez-Quintero et al., 2011), but this expected value does not take into account the possibility that use of other internationally regulated drugs (IRD) might be accelerating the cannabis dependence process or might create 'polydrug' diagnostic complexities. Recent estimates focused strictly on 'cannabis only' users (no other IRD use) suggest that as few as 1 in 50-60 'cannabis only' users develop a cannabis dependence syndrome (Lopez-Quintero & Anthony, 2015). In this study, we turn to quite recent US epidemiological survey data for new estimates of the risk of becoming cannabis dependent among newly incident 'cannabis only' users, with a hypothesis that the cannabis dependence process for 'cannabis only' users might be shaped by past or concurrent alcohol and/or tobacco use.

**Methods:** We estimated cannabis transition probabilities, using data from US National Surveys on Drug Use and Health, 2004-2013, which produced an aggregate nationally representative sample of 13,889 newly incident users of cannabis (NICU). We formed subgroups to distinguish NICU with no other IRD use ('New Only Cannabis Users', NOCU, n = 9,247) versus NICU who had used at least one other IRD (n = 4,642). Survey assessments of all drug use and DSM-IV cannabis dependence are via confidential computer assisted self-interviews. Estimation includes appropriate survey analysis weights and Taylor series linearization for variance estimation and derivation of 95% confidence intervals (CI).

**Results:** Among newly incident cannabis users, an estimated 1 in 22 became cannabis dependent within 12 months after onset of cannabis use (4.5%; 95% CI = 4.1, 5.0). The corresponding estimate for NICU with a history of other IRD use is 1 in 11 (9%; 95% CI = 8, 10), while the corresponding estimated transition probability for 'cannabis only' NOCU is 1 in 42 (2.4%; 95% CI = 2.0, 2.9). A much smaller transition probability is seen for NOCU who had tried neither alcohol nor tobacco ($\sim$0.5%), and a larger estimate is seen for NOCU with concurrent drinking and tobacco dependence (12.6%; 95% CI = 3.9%, 33.9%). The estimate for a small NOCU subgroup with concurrent dependence on both tobacco and alcohol is 9%, with wide and inconclusive CI (2.7%, 26.5%).

**Conclusions:** The main finding replicates recently published evidence about lower risk of cannabis dependence among 'cannabis only' users, with greater risk when other IRD have been used. We also discovered possible enhancement of cannabis dependence transition probabilities in relation to histories of alcohol and tobacco involvement in NOCU. Kandel & Kandel (2014) suggest molecular substrates for a nicotine-enhanced acceleration of cocaine use. Preclinical evidence on corresponding nicotine and/or ethanol-affected molecular substrates for cannabis is needed. Some potential implications for preventive and therapeutic interventions merit discussion.

**Keywords:** Cannabis Dependence, Tobacco Smoking, Alcohol

**Disclosures:** Nothing to disclose.

## M248. Cocaine Cue-Induced Striatal Dopamine Release in Non-Dependent Cocaine Users

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**Background:** Drug-related cues increase drug-seeking behaviors and striatal dopamine transmission. Studies in laboratory animals suggest that these dopaminergic effects are seen in the ventral striatum following relatively little drug use, before shifting to the dorsal striatum once stimulus-response habit-like behaviors have been established; an accumulation of these habits has been proposed to lead to compulsive drug abuse (Everitt & Robbins 2015). In humans, there is evidence that these same transitions occur. Stimulant drug-related cues provoke dopamine release in the ventral striatum of healthy controls following exposure to three prior doses of amphetamine (Boileau et al 2007) and in the dorsal striatum of those with cocaine use

disorders (Wong et al 2006; Volkow et al 2006; Fotros et al 2013). It is unknown how soon this shift in anatomical locus can occur. In the present study, we measured the effects of drug related cues in recreational cocaine users without a substance use disorder.
**Methods:** Cocaine users were interviewed in-depth with the structured clinical interview for DSM-IV-TR to rule out the presence of psychiatric disorders including addictions. Criteria-meeting cocaine users then underwent two PET [11C]raclopride scans on separate days (n = 6; all male; mean ± SD lifetime cocaine use: 64 ± 44 occasions). On Day 1, subjects were scanned while watching a 60-minute video of someone typing. Prior to the scan, subjects were instructed to type a short paragraph for 3-5 minutes, which served as a neutral motor control task. On Day 2, participants returned to the PET Unit with a friend that they used cocaine with. They were then videotaped while self-administering cocaine hydrochloride powder together (4mg/kg/person, intra-nasal). Sixty-minute videos were then created, one per person. On Day 3, subjects were presented with another bag of cocaine powder (4mg/kg) that they could ingest immediately after the scan. After manipulating the powder into four lines, subjects were scanned while watching the video of their friend taking cocaine. PET images were co-registered to individual MRIs, and parametric [11C]raclopride BPND data were generated at each voxel using a simplified reference tissue model. A t-map was created to assess the change in [11C]raclopride BPND between cocaine and control cues using a residual t-statistic (Aston et al 2000). Subjective drug craving was measured using visual analog scales.
**Results:** Cocaine cue exposure had two main effects. First, it increased cocaine craving, with the strongest response immediately after manipulation of the drug paraphernalia ($p = 0.04$). Second, it decreased [11C]raclopride BPND values, and this was evident in the dorsal caudate (peak t = 6.56; MNI coordinates: -17, 12, 14; cluster size = 387mm3) and putamen (peak t = 7.31; MNI coordinates: -30, -11, 9; cluster size = 994mm3).
**Conclusions:** These results suggest that exposure to cocaine related cues can increase extracellular dopamine levels in the dorsal striatum in non-dependent cocaine users. This might contribute to the development of addictive behaviors but can precede their onset.
**Keywords:** cocaine addiction, Dopamine, Dorsal striatum, habit, compulsivity
**Disclosures:** Nothing to disclose.
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## M249. Nicotine Withdrawal Alters Neural Responses to Psychosocial Stress

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**Background:** Psychosocial stress is considered to be an important mechanism underlying smoking behavior and relapse. Although the neural correlates of stress have been explored in smokers, understanding effects of acute nicotine withdrawal is important to intervene to prevent stress-induced relapse.
**Methods:** In this functional magnetic resonance imaging (fMRI) study, 39 treatment-seeking smokers were randomized to one of two conditions: abstinent 24 hours (deprived; n = 21) or smoking as usual (non-deprived; n = 18). Blood oxygen level-dependent (BOLD) signal was acquired while participants completed the Montreal Imaging Stress Task (MIST), which requires participants to solve difficult mental arithmetic problems while receiving negative performance feedback. Subjective stress was assessed in the scanner prior to and immediately after the MIST via the 11-item short form of the Profile of Mood States. To characterize group differences, a between-group (deprived vs. non-deprived) t-test of the experimental minus control contrast was conducted. Resulting Z (Gaussianised F) statistic images were cluster corrected for multiple comparisons using $Z > 3.10$. Mean percent signal change was calculated for each significant cluster and separate linear regressions were used to predict BOLD signal from subjective measures of stress (pre- to post-MIST change score).
**Results:** Subjective measures of stress increased following the MIST, compared to baseline, but these results did not vary by smoking status. Whole brain between-group analysis identified clusters in four regions: inferior frontal gyrus (IFG), paracingulate gyrus, precuneus, and right supramarginal gyrus. In all regions, the deprived group showed significantly greater activation compared to the non-deprived group. Increases in subjective stress were negatively related to BOLD signal in the IFG and paracingulate gyrus ($ps < 0.05$).
**Conclusions:** These findings provide new evidence that brain regions previously shown to be predictive of relapse, such as the precuneus and IFG, display heightened neural

responses to stress during nicotine deprivation. The current data elucidate the neural mechanisms that may be associated with stress-precipitated relapse and suggest that increased stress-related activation during nicotine withdrawal may represent a neural marker to identify those most vulnerable to relapse.
**Keywords:** stress, nicotine dependence, Withdrawal, fMRI, imaging
**Disclosures:** Nothing to disclose.

### M250. Discriminative Stimulus Effects of Drug Mixtures: Studies with Cocaine, MDPV, and Caffeine

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**Background:** Many illicit drug preparations include more than one pharmacologically active compound. For example, powdered cocaine often contains other stimulants such as caffeine or lidocaine, as well as compounds with dissimilar mechanisms of action (e.g., levamisole; an anthelmintic). The same is true for preparations of designer stimulants (synthetic cathinones, "bath salts"), which often contain two or more synthetic cathinones or a synthetic cathinone and caffeine. Although the primary reason for including cheap and legal drugs, such as caffeine or lidocaine, in illicit drug preparations is to increase volume without increasing the amount of cocaine or synthetic cathinone, it is also possible for adulterants to augment the effects of the primary drug of abuse. The current studies were designed to characterize the nature of the interactions for binary mixtures of cocaine, caffeine, and the widely abuse cathinone methylenedioxypyrovalerone (MDPV).
**Methods:** Seven adult male Sprague Dawley rats were trained to discriminate 10 mg/kg cocaine from saline using a multiple cycle, two-lever discrimination procedure. Training sessions consisted of 2-6 cycles, with injections administered 10 min before a 10-min response period in which responses on the injection-appropriate lever were reinforced under an FR10 schedule of food presentation. Test sessions were always 6 cycles long, with saline always administered prior to the first cycle, and cumulative doses of a single drug (cocaine, caffeine, MDPV, methamphetamine, or midazolam) or mixtures of two drugs (cocaine-caffeine, cocaine-MDPV, or MDPV-caffeine) administered prior to cycles 2 through 6. Dose-response curves for each drug and for each mixture were determined in triplicate. Drug mixtures were based on the concept of dose equivalence, with each pair of drugs evaluated at three ratios (3:1, 1:1, and 1:3) relative to the mean ED50 for each drug. Predicted, additive effect levels were calculated for each pair of doses for each combination of drugs using the Emax, ED50, and slope parameters derived from dose-response curves obtained for each drug alone in individual rats.
**Results:** Dose-dependent increases in cocaine-appropriate responding were observed following administration of cocaine, caffeine, MDPV and methamphetamine; midazolam failed to increases cocaine-appropriate responding. Although there were individual differences with respect to the nature of the drug interactions (i.e., supra-additive for some rats and strictly additive, or sub-additive for other rats), when the data were grouped a supra-additive interaction was only observed for the 1:1 cocaine: caffeine mixture; all other combinations exhibited strictly additive interactions with respect to their cocaine-like discriminative stimulus effects.
**Conclusions:** Combining two drugs with similar mechanisms of action (i.e., cocaine and MDPV are both dopamine transporter [DAT] inhibitors) would be expected to produce a strictly additive effect, whereas combining two drugs with different mechanisms of action (i.e., cocaine, and caffeine, an adenosine receptor antagonist) could result in effects that differ from additivity, depending upon the nature of their effects. The results of these studies support these predictions, with strictly additive interactions observed with mixtures of cocaine and MDPV over a range of fixed dose ratios, and supra-additive interactions with a mixture of cocaine and caffeine at the 1:1 dose ratio. Although it is unclear why a similar supra-additive interaction was not observed when caffeine was combined with MDPV, it is possible that the discriminative stimulus effects of this mixture were only partially overlapping with the discriminative stimulus effects of cocaine, and that a supra-additive interaction would have be observed in rats trained to discriminate MDPV. Moreover, it is unclear if similar interactions exist between these drugs for other abuse-related or toxic effects.
**Keywords:** bath salts, drug discrimination, MDPV, cocaine, caffeine
Disclosures: none.

### M251. Experimental Conditions Facilitating Escalation of Ethanol Consumption in Male and Female C57BL/6J Mice

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**Background:** We have previously found that mice that were allowed to consume 8% ethanol on a limited access schedule whereby alcohol was only available for two 24 hour periods per week, increased consumption of alcohol over a 3 week period. Under these conditions, ethanol consumption increased by about 80% in female mice, but by only 50% in male mice. To determine whether conditions could be identified under which greater escalation could be observed in both male and female mice a series of studies was conducted that varied ethanol concentration, the intervals between ethanol availability, and the number of ethanol concentrations available at one time.
**Methods:** All experiments used adult C57BL/6J, male and female mice ($N = 10$ per experimental condition). Experiment 1 examined the effect of ethanol concentration (4%, 8%, 16% and 32% v/v) on escalation of ethanol consumption in separate groups of mice. Voluntary ethanol consumption was compared in two-bottle, 24 hr access, home-cage preference tests (versus water), 2 days per week, with only water available on intervening days, over a period of 6 weeks. Experiment 2 examined the effect of access

interval on escalation of consumption of 16% ethanol, comparing groups of mice that had different schedules of alcohol availability: 1, 2, or 3 periods of 24 hr access to ethanol per week, versus continuous access. Experiment 3 examined the effects of a 4 bottle preference procedure (4%, 8% and 32% EtOH v/v; and water) on escalation of ethanol consumption under conditions of continuous (daily) access.
**Results:** Experiments 1 and 2 demonstrated that escalation in a 2 bottle test was dependent on both concentration and interval. In both experiments, however, escalation of ethanol consumption continued to be much less in male than female mice, and similar in magnitude to our previous observations. In Experiment 3, however, far greater escalation of ethanol consumption was observed in both male and female mice. Initial consumption of alcohol (calculated as the total ethanol consumption in g/kg for all 3 ethanol concentrations) was greater than in the other studies using a 2-bottle procedure. Moreover, there was a much greater increase in ethanol consumption over the period of study (e.g. escalation), than previously observed, greater than a 100% increase in both male and female mice, to more than 20 g/kg in male mice and more than 40 g/kg in female mice.
**Conclusions:** Although both interval and concentration in a limited access procedure led to increases in ethanol consumption over time (e.g. escalation of ethanol intake), a 4-bottle procedure with continuous access was found to produce far greater escalation of ethanol intake. In the 4-bottle procedure both male and female mice consumed more ethanol initially, which may have contributed to the escalation of ethanol consumption. Comparing across the experiments, escalation appeared to be at least somewhat dependent on the overall amount of ethanol consumed, so that the conditions that produced the greatest consumption initially, produced greater escalation of ethanol consumption over time. This procedure, given the large amounts of alcohol consumed using a completely voluntary procedure, will prove useful for examining the underlying mechanisms that may contribute to escalated ethanol consumption, to levels that may be relevant to alcohol dependence.
**Keywords:** ethanol, sex differences, Alcoholism
**Disclosures:** Nothing to disclose.

### M252. Risk-Preferring Rats Make Worse Decisions and Show Increased Incubation of Craving after Cocaine Self-Administration

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**Background:** Maladaptive decision-making may play an integral role in the development and maintenance of an addiction. Substance dependent individuals make riskier choices on the Iowa Gambling Task (IGT), and these deficits persist during withdrawal and are predictive of relapse. However, it is unclear from clinical studies whether this cognitive impairment is a cause or consequence of drug use.
**Methods:** We trained male Long-Evans rats on the rat Gambling Task (rGT), a rodent analogue of the IGT, to determine how choice preference influenced, and was influenced by, cocaine self-administration, withdrawal and incubation of craving. Testing and housing were in accordance with the Canadian Council of Animal Care, and all experimental protocols were approved by the Animal Care Committee of the University of British Columbia.
**Results:** Rats that exhibited a preference for the risky, disadvantageous options at baseline were uniquely and adversely affected by cocaine self-administration. The proportion of rats exhibiting such a maladaptive choice pattern was significantly increased by adding highly salient win-paired cues to the rGT that increased in complexity and variability with the size of the reward delivered. Risky choice was exacerbated in these rats when decision making was assessed during the same diurnal period as cocaine self-administration, whereas the choice pattern of optimal decision makers was unaffected. This effect was consistently observed in animals trained on either the cued or uncued rGT. The cocaine-induced decision-making deficit was maintained during 30 days of withdrawal, and correlated with greater cue-induced incubation of craving. Risk-preferring rats also made more drug-seeking responses during cocaine self-administration, but did not obtain more drug infusions.
**Conclusions:** These data demonstrate that poor decision-making prior to contact with addictive drugs is associated with a pro-addictive behavioral phenotype, characterized by further worsening of judgement and heightened drug-seeking both during cocaine self-administration and withdrawal. Such findings indicate that the elevated risky decision-making observed in substance-dependent populations is not merely circumstantial, but makes an important contribution to addiction vulnerability and severity that can now be effectively modeled in laboratory rats. The increased frequency of risk-preferring rats in the cued version of the rGT speaks directly to the ability of salient cues to invigorate maladaptive decision making, an important and understudied component of both gambling and substance use disorders.
**Keywords:** cocaine addiction, risk taking behaviors, individual differences, relapse, Withdrawal
**Disclosures:** Nothing to disclose.

### M253. Moderators of Varenicline Treatment Effects in a Double-Blind, Placebo-Controlled Trial for Alcohol Dependence

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**Background:** To explore if varenicline (Chantix®) showed more efficacy in treating certain subgroups of patients. In a recent multi-site trial, varenicline was shown to be effective in reducing drinking in alcohol dependent patients, both smokers and nonsmokers. Given the heterogeneity among alcohol dependent patients, secondary analyses were conducted to determine if certain subgroups responded more favorably than others to treatment with varenicline.
**Methods:** Data were drawn from a Phase 2 randomized, double-blind, placebo-controlled multi-site 13-week trial of varenicline in 200 alcohol dependent patients. Seventeen

moderator variables were selected for exploratory testing on the basis of theoretical and scientific interest.
**Results:** Of the 17 moderator variables assessed, four were statistically significant, including cigarettes per day reduction, treatment drinking goal, years drinking regularly, and age of patient. Two other variables—the type of adverse events experienced by patients and the severity of alcohol-related consequences—appeared to moderate the varenicline treatment effect at borderline statistical significance. Individuals who reduced the number of cigarettes per day experienced a significant effect from varenicline in reducing drinking, whereas those who did not change or who increased their number of cigarettes observed no beneficial effect. Reviewing the moderators related to severity, varenicline appears to have greater efficacy than placebo among less severely-dependent patients.
**Conclusions:** Varenicline appears to be more efficacious in certain subgroups, particularly in those who reduced their smoking and in the "less severe" patient. Additional studies are needed to confirm the results of these exploratory analyses.
**Keywords:** varenicline, CNS Clinical Trials, Alcohol dependence, moderators
**Disclosures:** Nothing to disclose.

### M254. Sustained Opioid Antagonism Increases Stratal Sensitivity to Baby Schema in Opioid Dependent Patients

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**Background:** Chronic opioid abuse is associated with reduced sensitivity to natural rewards and pro-social deficits that include dysfunctional parenting. The neurophysiological mechanisms underlying these deficits and their response to treatment with opioid modulators are unknown. Naltrexone is an effective treatment of opioid addiction by competitive antagonism primarily at the mu-opioid receptors. The injectable extended-release naltrexone formulation (XRNTX, Vivitrol ®; Alkermes, Inc., Waltham, MA), gradually releases 380 mg of naltrexone from dissolvable polymer microspheres, producing therapeutic blood levels that block opioid receptors for approximately one month. The advantages of XRNTX over the oral preparation, are the greatly improved compliance profile and reduced expectancy of the drug effects, i.e. opioid craving. Baby schema (Kindchenschema) is a set of juvenile physical features defined by Konrad Lorenz (1943, 1980), which is perceived as "cute" and triggers motivation for caregiving. The baby schema effect was operationalized in healthy controls as the behavioral and brain fMRI response to the varying level of baby schema in a previously validated set of infant portraits (Glocker et al, PNAS 2009). Recent studies suggest that increasing levels of "baby schema" are associated with greater activation of the brain "reward" network and proposed this effect as an index of social cognition. In the present study, we used the brain and behavioral response to baby schema as a probe of the effects of XRNTX) on social cognition in opioid dependent patients. We hypothesized that in detoxified individuals with opioid use disorder, sustained opioid antagonism produced by XRNTX will enhance the brain fMRI and behavioral response to baby schema.
**Methods:** Forty-seven (24 F) recently detoxified opioid dependent patients (42 Caucasian, 3 African American, 2 Asian, age 28.9 $\pm$7.5, mean $\pm$SD) underwent functional magnetic resonance imaging (fMRI) while viewing infant portraits parametrically manipulated for baby schema content and rating them for cuteness, at baseline and during treatment (i.e. 12 $\pm$7 days after the first XRNTX injection). Self-reported craving for heroin and other opioids was evaluated prior to each fMRI session. Plasma concentrations of naltrexone and 6-beta-naltrexol (an active metabolite) were ascertained at the second MRI session (12 $\pm$ 7 days after injection) using liquid chromatography and tandem mass spectrometry.
**Results:** The behavioral baby schema effect, indexed by "cuteness" ratings, was present ($F(2, 36) = 151.39$, $p < 0.001$) and unaffected by XRNTX ($F(1, 37) = 0.425$, $p = 0.519$). The brain response to baby schema was absent at baseline (right: ($F(2, 36) = 0.871$, $p = 0.422$; left: $F(2, 36) = 0.592$, $p = 0.555$), and present in the bilateral ventral striatum after two weeks of XRNTX treatment (right: $F(2, 36) = 4.896$, $p = 0.010$; left: $F(2, 36) = 4.148$, $p = 0.020$). The decline in self-reported craving for opioids was positively correlated with the brain fMRI response to baby schema in the bilateral ventral striatum (right: $r = 0.427$, $p = 0.007$; left: $r = 0.385$, $p = 0.017$).
**Conclusions:** Extended release naltrexone treatment may bring the functioning of neural systems that support social cognition in detoxified opioid dependent patients closer to patterns previously reported in healthy controls. These preliminary findings set the stage for future controlled studies of the effects of opioid modulators on the brain and behavioral correlates of social cognition. The clinical significance of such studies is underscored by the fact that opioids are among the most commonly used and abused psychopharmacological agents worldwide.
**Keywords:** social cognition, Reward, Naltrexone, Human Neuroimaging, Heroin
**Disclosures:** Nothing to disclose.

### M255. Paradoxical Effects of Glucocorticoid Receptor Antagonism in the Basolateral Amygdala on Drug Context-Induced Relapse to Cocaine Seeking

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**Background:** Drug context-induced relapse to cocaine-seeking is dependent on the integrity of context-cocaine associative memories and the recruitment of these memories and other processes that trigger goal-directed behavior. Here, we investigated the role of basolateral amygdala (BLA) glucocorticoid receptors (GR) in A) contextual cocaine memory reconsolidation, a process responsible for long-term memory maintenance, and B) drug context-induced reinstatement of cocaine-seeking behavior.
**Methods:** Rats were trained to lever press for cocaine infusions in a distinct context followed by extinction training in a

different context. In Exp. 1, rats received bilateral intra-BLA microinfusions of the GR antagonist, RU038486 (3, 10 ng/hemisphere), or vehicle following exposure to the previously cocaine-paired context, a procedure that elicits cocaine memory reactivation and reconsolidation. Anatomical controls received RU038486 into the caudate-putamen (CPu). No reactivation controls were exposed to an unpaired context prior to intra-BLA microinfusion. Non-reinforced lever presses were assessed 72 h later in the cocaine-paired context. Quantitative Western blotting was used to assess the effects of RU038486 on NMDA GluN2a subunit, NMDA GluN2b subunit, ERK1, ERK2, and CREB phosphorylation and on GR levels in the BLA. In Exp. 2, rats received the same pharmacological manipulations in the BLA or CPu immediately prior to testing for drug context-induced cocaine-seeking behavior. Furthermore, the possible acute and delayed effects of RU038486 on locomotor activity were measured in a novel context.
**Results:** Remarkably, RU038486 produced a robust increase in cocaine-seeking behavior 72 h after intra-BLA (ANOVA treatment main effect only, $P<0.05$), but not intra-CPu, administration. This effect did not depend on cocaine memory reactivation; therefore, it did not indicate enhancement in cocaine memory reconsolidation. Interestingly, the RU038486-induced increase in cocaine-seeking behavior was associated with a decrease in BLA NMDA GluN2a and GluN2b subunit activation, which in turn positively correlated with GR levels and ERK1/2 activation, respectively. Although it failed to significantly alter drug context-induced cocaine-seeking behavior, re-exposure to the cocaine-paired context 72 h prior to the test session resulted in a subsequent increase in ERK2 activation and GR levels as well as a decrease in CREB activation in the BLA. Contrary to its protracted effects, RU038486 administered into the BLA immediately prior to testing dose-dependently attenuated drug context-induced cocaine-seeking behavior (ANOVA treatment simple main effect, $P<0.05$). Intra-BLA RU038486 treatment failed to alter locomotor activity either immediately or 72 h after administration, suggesting that the RU038486-induced acute and protracted changes in cocaine-seeking behavior did not reflect time-dependent alterations in motor activity.
**Conclusions:** Together these findings suggest that BLA GR stimulation is necessary for drug context-induced motivated behavior. However, BLA GR antagonism results in a protracted increase in cue reactivity, possibly by precipitating compensatory adaptations.
**Keywords:** cocaine seeking, basolateral amygdala, glucocorticoid receptor
**Disclosures:** Nothing to disclose.

### M256. Impact of Chronic Ethanol Self-Administration on Kappa Opioid Receptor Regulation of Dopamine Signaling in Nonhuman Primates

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**Background:** Although alcoholism is one of the most prevalent disorders in the United States, with over 18 million individuals meeting the criteria for an alcohol use disorder, the neurobiological bases of this condition remain obscure. Recently, it has been demonstrated that kappa-opioid receptor (KOR) signaling in the striatum plays a critical role in the increased reinforcing efficacy of ethanol following ethanol vapor exposure in rodent models. However, changes in KOR signaling following voluntary ethanol drinking remain to be elucidated. Additionally, because numerous KOR agonists/antagonists are available clinically, understanding how KOR sensitivity relates to drinking behaviors, especially in nonhuman primate models of drinking, may open a novel avenue for therapeutic interventions. Here we examined the effects of chronic voluntary ethanol self-administration in macaques on dopamine neurotransmission and the ability of KORs to regulate dopamine release in the nucleus accumbens core.
**Methods:** Three cohorts of nonhuman primates were given free access to 4% ethanol (w/v) for 22 hr/day. These cohorts were composed of either male cynomolgus, female rhesus or male rhesus macaques, and were given access to ethanol for 6, 12, or 18 months, respectively. Ex vivo fast-scan cyclic voltammetry was then conducted in brain slices containing the nucleus accumbens core to determine ethanol-induced alterations in dopamine terminal function including dopamine release and uptake kinetics as well as the ability of U50,488 (KOR agonist) to inhibit dopamine release.
**Results:** Chronic ethanol drinking increased dopamine uptake rates, which could have implications for reductions in basal dopamine tone in vivo following ethanol drinking. Further, across sex, strain and exposure length ethanol consumption augmented the ability of KORs to inhibit dopamine release, demonstrating that ethanol-induced increases in KOR sensitivity are widespread and independent of other factors. Finally, in male subjects KOR sensitivity was positively correlated with lifetime ethanol intake, suggesting that changes in KOR regulation of dopamine release may be a determinant of aberrant ethanol drinking behaviors.
**Conclusions:** Although it has been proposed that nonhuman primate models of ethanol abuse represent the most promising avenue for elucidating the neurobiology of alcoholism and for identifying molecular targets for pharmacotherapeutic compounds, investigations have been largely limited to behavioral, endocrine or brain imaging analyses due to practical constraints. Here we show, for the first time, that voluntary ethanol self-administration has a unique effect on KOR sensitivity and regulation of dopamine release directly at the dopamine terminal that was positively correlated with drinking behavior. In conjunction with human and rodent work, these data suggest that ethanol-induced dysregulation of the dynorphin/KOR system may drive the motivation to administer ethanol, and thus drive the development of addiction. The dynorphin/KOR system plays a large role in regulating affective states in humans, and KOR agonists produce conditioned place aversion in rodents. Thus, it is possible that supersensitivity of this system may produce negative affective states, leading to increased consumption and motivation to administer ethanol in an attempt to ameliorate these effects with ethanol. Further supporting this hypothesis, dopamine uptake rates were also increased, which could promote a state of low dopamine tone, which

has also been associated with anhedonia. Additionally, the relationship between KOR sensitivity and drinking provides a potential mechanism for the comorbidity of early life stress and alcoholism, as stress has been linked to increases in dynorphin/KOR system activity in both animal and human investigations. Together, these data provide novel insight into ethanol-induced dysregulation of dopamine neurotransmission and suggest that dopaminergic dysfunction may be mediating the increase in voluntary drinking during the early stages of ethanol abuse/dependence. Importantly, KOR antagonists may provide a novel avenue to reduce drinking behaviors in alcoholics.
**Keywords:** Alcoholism, kappa opioid receptor, Nonhuman Primates, voltammetry, alcohol self-administration
**Disclosures:** Nothing to disclose.

### M257. Opioid Withdrawal during Adolescence Alters Brain Glucose Metabolism Sex-Specifically

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**Background:** Americans consume approximately 80% of the global opioid supply as well as two thirds of the world's illegal drugs. The 2010 National Survey of Drug Use and Health estimated that 2.4 million Americans used opioids and prescription drugs non-medically for the first time that year. Further, young adults 18-25 years of age were more likely to use illicit drugs (19.6%) than individuals 12-17 years of age (3.0%) and adults. The survey also indicated that females are high-risk for substance abuse.
The NIH required that clinical trials adequately represented women (1994). However, only recently did the NIH require that investigators include sex in their experimental designs. Starting in January 2016, all NIH funded preclinical animal studies must include this variable. Due to a marked rise in opioid use and abuse, as well as the call for sex-specific data, we investigated the effects of opioid withdrawal on brain glucose metabolism in male and female adolescent rats. While behavioral research has shown differences in morphine tolerance and withdrawal between sexes, to our knowledge no studies have measured these differences using positron emission tomography (PET).
**Methods:** Drug-naïve adolescent male ($n = 8$) and female ($n = 8$) animals were maintained on a normal 12/12-light/dark cycle. Animals received baseline [18F]Fluoro-2-deoxy-2-D-glucose ([18F]FDG) microPET scans using a Siemen's Inveon. All emission images were corrected for attenuation. Morphine was administered (10 mg/kg/day) subcutaneously for 5 days as this treatment interval and dose were previously shown to produce morphine tolerance and a conditioned response. [18F]FDG microPET scans were acquired on the third day of withdrawal. For analysis, microPET images were reconstructed using maximum a posteriori (MAP). Brain images were placed into Paxinos and Watson stereotaxic space using PMOD (version 3.2). Subsequent post-processing steps including realignment to an atlas, normalization to a mean template, and smoothing were completed using SPM 5 (Statistical Parametric Mapping). Statistical analysis utilized a two-sample t-test basic model design with ANCOVA.
**Results:** Animals gained weight normally throughout morphine treatment. There were no significant differences in brain metabolism between males and females under baseline conditions. However, images obtained from these animals during opioid withdrawal demonstrated marked metabolic increases and decreases, both cortically and subcortically. Specifically, increases in striatal and thalamic nuclei as well as both prelimbic and frontal cortices were observed. In addition, marked decreases in metabolic activity occurred in the septum, ventral striatum and ventral hippocampus. An analysis of male vs. female scans revealed several differences between the sexes. Of note, males showed a significant increase in the anterior cingulate cortices and the dorsal hippocampus (CA3) compared to females. There were no significant metabolic decreases observed between the sexes.
**Conclusions:** We are unaware of any data examining brain glucose metabolism during opioid withdrawal. In fact, to date, no studies have measured the effects of opioid withdrawal on brain glucose metabolism in drug-naïve adolescent animals. Therefore, studies detailed here were specifically designed to address these issues.
Under resting conditions, we observed no metabolic differences between the adolescent male and female brain. That is, regional brain metabolism was similar, both cortically and subcortically, throughout the rostral/caudal extent of the cerebrum and cerebellum. However, gender differences were observed during opioid withdrawal.
Specifically, images obtained during opioid withdrawal demonstrated that males and females experienced marked metabolic increases and decreases both cortically and subcortically. These metabolic increases were observed in brain regions associated with reward (striatum), and sensory processing (thalamus), while metabolic decreases were observed in regions associated with learning and memory (ventral hippocampus). Finally, metabolic decreases were also observed anteriorly in the septum, a brain region associated with behaviors including responses to environmental stimuli, sexual activity, feeding and drinking, and rage.
When we examined sex differences, only metabolic increases were observed. These increases occurred exclusively in males and included the anterior cingulate cortices and the dorsal hippocampus (CA3).
Females demonstrate higher levels of opioid abuse-related physical issues than males, although the preponderance of data suggests that males have a greater dependence liability. Regardless of these differences, pharmacologic treatment for opioid withdrawal is gender independent. That is, opioid abusers, are treated with opioid replacement and/or maintenance therapy consisting of methadone, a synthetic μ-opioid agonist, or buprenorphine, a partial opioid receptor agonist-antagonist.
Our sex-specific findings suggest that treatment outcome might be improved if therapeutic strategies are targeted at metabolic differences that appear to exist between the male and female brain. To that end, our larger and currently ongoing study is specifically designed to examine sex differences in the response to methadone or buprenorphine treatment during opioid withdrawal in the adolescent brain.
**Keywords:** opioid, withdrawal, imaging, addiction, adolescence
**Disclosures:** Nothing to disclose.

### M258. Clonidine Increases the Likelihood that Abstinence can Withstand Leisure Time in Buprenorphine-Maintained Outpatients

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**Background:** In an RCT examining daily clonidine administration for prevention of stress-induced lapses to opioid use during buprenorphine maintenance, clonidine increased time to lapse and longest duration of abstinence and decoupled stress from craving. We have now examined Ecological Momentary Assessment (EMA) data to investigate clonidine's effects on daily-life activities.
**Methods:** Outpatients (N = 118) in buprenorphine maintenance were randomized to receive clonidine (up to 0.3 mg/day) or placebo. We categorized treatment outcome into two success levels (high vs. low) with a split half on longest duration of opioid abstinence. We then determined the likelihoods of different types of daily activity (assessed at random moments 4x/day by EMA) as a function of clonidine vs. placebo, high vs. low success, and their interaction, using SAS PROC GLIMMIX models.
**Results:** In the clonidine group, treatment success was associated with higher frequency of activities associated with leisure, whereas, in the placebo group, those associations were not present or were even reversed (TV, odds ratios: 1.1 vs. 0.6; music, ORs: 3.6 v 1.3; socializing, ORs: 1.4 v 0.7; reading, ORs: 2.7 v 0.6; thinking, ORs: 2.7 v 0.5; waiting, ORs: 1.2 v 0.6). For the placebo group, treatment success was associated with higher frequency of activities associated with responsibilities, such as working (ORs: 0.9 v 2.1) and child or elder care (ORs: 1.7 v 12.8). (All p values for Clonidine Group x Success Category $<.0001$.)
**Conclusions:** These results suggest clonidine's decoupling of stress from craving helped participants engage in free-time activities with less risk of craving than they might otherwise have had. The seemingly protective effect of time spent at work for our standard-care group is consistent with our previous EMA findings, which showed that our participants are less stressed at work than at home or elsewhere.
This research was supported by the Intramural Research Program, National Institute on Drug Abuse, NIH.
**Keywords:** ecological momentary assessment, opioids, clonidine, buprenorphine maintenance
**Disclosures:** Nothing to disclose.

### M259. Genetic Variation of the GABA(B) Receptor in Individuals with Alcohol Use Disorder and Association with Smoking Measures

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**Background:** GABAergic signaling plays a key role in several neurobiological processes and dysfunction of this system has been implicated in the pathophysiology of addictions. Preclinical studies suggest that the GABA(B) receptor is involved in acquisition and maintenance of alcohol drinking as well as smoking behavior. In clinical studies, the GABA(B) receptor agonist baclofen has been shown to be a promising pharmacotherapy for alcoholic individuals, smokers and alcoholic smokers. However, only a few genetic studies have investigated the role of this receptor in addictive behaviors. Therefore, we aimed to conduct a human genetic association study to investigate the potential role of genetic variation of the GABA(B) receptor in alcoholism and its impact on smoking measures in alcohol dependent (AD) individuals.
**Methods:** Participants were recruited under two NIAAA screening protocols conducted at the NIH Clinical Center in Bethesda, MD, USA from 2005 to 2015. DNA samples were genotyped for GABA(B) receptor subunit 1 (GABBR1) and 2 (GABBR2) genes using the Illumina OmniExpress BeadChip array (Illumina, San Diego, CA, USA). We selected all single nucleotide polymorphism (SNP) variants with minor allele frequency (MAF) $\geq$ 0.10 that have been genotyped for on the Illumina array in a 5kb window beyond the specified gene region. A total number of 135 SNPs (GABBR1 = 8, GABBR2 = 127) were selected and analyzed using PLINK v1.07. We first performed a case-control analysis in which cases (N = 812) comprised individuals with lifetime diagnosis of AD according to DSM-IV criteria, while controls (N = 442) had no past or current AD. Then, we investigated the association of these SNPs with drinking and smoking measures in current AD individuals using linear regressions. Finally, the effect of gene $\times$ drinking interaction on smoking outcomes was analyzed considering average drinks per drinking days and alcohol dependence scale (ADS) score as moderators. Age, gender, and ancestry-informative markers (AIMs) score were considered as covariates in all analyses.
**Results:** After correction for multiple testing, there were no significant differences between the two groups in the case-control analysis of the whole sample, European ancestry, and African ancestry. Similarly, No significant association was found with drinking measures based on Timeline Follow-Back (TLFB) and ADS scores. On the other hand, the minor alleles of six SNPs on GABBR2 (rs2779552, rs2779558, rs2779562, rs2779572, rs7857375, rs944761) were significantly associated with lower Fagerstrom Test for Nicotine Dependence (FTND) scores ($p<0.05$); two pairs of these SNPs are in strong linkage disequilibrium (LD): rs2779562 and rs2779572 (r2 = 0.935), rs7857375 and rs944761 (r2 = 1.000). The associations between these six SNPs and FTND scores were all significantly moderated by average drinks per drinking days [False Discovery Rate (FDR) adjusted p-value = 0.040, 0.016, 0.007, 0.007, 0.043, 0.045; respectively]. Conversely, no significant interaction effect was found for ADS scores. Two other SNPs on GABBR2 (rs1930130, rs1930421) were also shown to be significantly associated with age at first cigarette in current AD individuals ($p<0.05$).
**Conclusions:** The main findings of this study indicate a significant association of GABBR2 gene variants with smoking measures in AD individuals. Drinking level (measured as average drinks per drinking days) appears to moderate this association. The significant SNPs found in

this study are all located in a small region (~27k bp) of the GABBR2 gene suggesting a particular susceptibility region for smoking on this gene. In line with previous studies, no significant association was found for GABBR1 gene. To our knowledge, this is the first study investigating the potential impact of GABA(B) receptor genes on smoking outcomes specifically in a population with AD. As such, this study provides new evidence on the importance of the GABBR2 gene in alcohol and nicotine co-use. However, further studies are warranted to confirm these preliminary findings.
**Keywords:** GABAB, Alcohol dependence, Smoking, genetics
**Disclosures:** Nothing to disclose.

### M260. Poor Inhibitory Control Predicts Sensitivity to Amphetamine Reward

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**Background:** Poor inhibitory control is a known prospective risk factor for drug abuse, perhaps due in part to heightened drug reward sensitivity in impulsive individuals. Preclinical and clinical studies show that impulsive action (i.e., poor response inhibition) and stimulant drug reinforcement are both linked to D2-receptor availability in overlapping frontostriatal pathways, suggesting a neural link between inhibitory control and stimulant drug reward. Here, we examined the degree to which behavioral and neural measures of response inhibition predicted the rewarding effects of a single dose of amphetamine in healthy adults. We hypothesized that poor inhibitory control and reduced frontostriatal function (activation and connectivity) during response inhibition would be related to greater sensitivity to amphetamine reward.
**Methods:** Participants completed the stop signal task to assess inhibitory control. They then attended four sampling sessions in which they received color-coded capsules containing either d-amphetamine (20mg) or placebo, in alternating order, and completed subjective self-report measures of drug response. Participants then attended a choice session in which they were allowed to choose which color capsule they preferred to take. A subset of participants also performed the stop signal task while undergoing fMRI.
**Results:** To date, 22 participants have completed the study. Preliminary results show that longer stop signal reaction time (indicating poorer inhibitory control) is associated with greater response to amphetamine compared to placebo for measures of euphoria ($r = 0.56$, $p = 0.006$), stimulation ($r = 0.45$, $p = 0.037$), like drug ($r = 0.46$, $p = 0.030$), and feel drug ($r = 0.48$, $p = 0.023$). Additional planned analyses will reveal whether poor inhibitory control is also related to choice for amphetamine over placebo, and whether frontostriatal function (activation and connectivity) during response inhibition predicts subjective and choice measures of amphetamine reward. We hypothesize that reduced activation and/or connectivity of lateral prefrontal and striatal regions during response inhibition will be associated with greater subjective and behavioral measures of amphetamine preference.
**Conclusions:** These preliminary findings support the hypothesized association between poor inhibitory control and subjective response to the rewarding effects of amphetamine. The fMRI data will provide information regarding whether reduced top-down prefrontal control of striatal regions during response inhibition is related to greater amphetamine reward. Understanding the behavioral and neurobiological mechanisms linking poor inhibitory control with drug reward could suggest potential therapeutic targets for reducing risk for drug abuse in impulsive individuals.
**Keywords:** Inhibitory control, Amphetamine, Reward
**Disclosures:** Nothing to disclose.

### M261. Evaluation of Clinically Efficacious Opioid Analgesics for Biased Agonism and Correlations to Respiratory Suppression and Antinociception

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**Background:** The clinical use of opiates for pain management is severely limited due to serious side effects, including respiratory suppression and overdose. There are considerable efforts to identify novel opiates that are effective in pain relief but have decreased side effect profiles. One such effort is based on the findings that βarrestin2-KO mice have enhanced antinociceptive responses, but decreased respiratory suppression when treated with morphine. Therefore compounds are being developed to activate the mu opioid receptor without inducing the recruitment of βarrestin2. In this study, we have characterized in vitro bias signaling profiles of clinically relevant opioid analgesics and have then tested antinociceptive and respiratory responses for the narcotic that displayed bias in our in vitro study.
**Methods:** MOR signaling via G protein signaling pathways were assessed using a [35S]-GTPγS binding assay and an inhibition of cAMP accumulation assay in transfected cell lines. To assess βarrestin2 recruitment, a DiscoveRx PathHunter βarrestin2 translocation assay was used. In addition, βarrestin2 recruitment was verified using confocal microscopy to assess translocation of βarrestin2-GFP to MOR. Using DAMGO as the reference full agonist in all assays, bias factors were calculated by the operational model. For compounds that displayed biased, we assessed antinociceptive responses in C57Bl/6J mice via the hot plate and warm water tail immersion tests. We then determined their ability to suppress respiration using a pulse oximeter designed for rodents (MouseOx, Starr Life Sciences). Full dose response curves were carried out in all the assays.
**Results:** We find that most clinically used opiates, including morphine do not display bias towards G protein coupling, cAMP or βarrestin2, when compared to DAMGO. Fentanyl, however, is biased towards recruiting βarrestin2 over both GTPγS binding and inhibition of cAMP accumulation. Moreover, when compared to morphine in vivo, fentanyl induces greater respiratory suppression than morphine at doses that are equiefficacious in the antinociception assays.
**Conclusions:** These results demonstrate that a compound that is biased towards βarrestin2 over G protein coupling induces greater respiratory suppression than an agonist that

displayed no bias in the cell based assays used in these studies. These findings are in concert with the hypothesis that the development of MOR agonists that are biased towards G protein coupling rather than βarrestin2 may provide a way to develop analgesics with less respiratory suppression.
**Keywords:** mu-opioid receptors, beta arrestin, Functional Selectivity, g protein signaling
**Disclosures:** Nothing to disclose.

### M262. Deficits in Striatal Dopamine Release in Cannabis Dependence

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**Background:** Most drugs of abuse lead to a general blunting of dopamine release in the chronic phase of dependence, which contributes to poor outcome. To test whether cannabis dependence is associated with a similar dopaminergic deficit, we examined striatal and extrastriatal dopamine release capacity in severely cannabis dependent participants (CD), free of any comorbid conditions, including nicotine use.
**Methods:** Eleven CD and twelve healthy controls (HC) completed two positron emission tomography scans with [11C]-(+)-PHNO, before and after oral administration of d-amphetamine. CD stayed inpatient for 5-7 days prior to the scans to standardize abstinence. Percent change in [11C]-(+)-PHNO binding potential (ΔBPND) was compared between groups. Magnetic Resonance Spectroscopy (MRS) measures of glutamate in the striatum and hippocampus were obtained in the same subjects on the day prior to PET. Correlations with MRS glutamate, subclinical psychopathological and neurocognitive parameters were examined.
**Results:** CD had significantly lower ΔBPND in the striatum ($p = 0.002$, effect size (ES) = 1.48), specifically in the associative striatum ($p = 0.003$, ES = 1.39) and sensorimotor striatum ($p = 0.003$, ES = 1.41), as well as in the pallidum ($p = 0.012$, ES = 1.16). Lower dopamine release in the associative striatum correlated with inattention and negative symptoms in CD, and with poorer working memory and probabilistic category learning performance in both CD and HC. No relationships to MRS glutamate were detected.
**Conclusions:** This study provides definitive evidence that severe cannabis dependence- without the confound of any comorbidity- is associated with a deficit in striatal dopamine release. This deficit extends to other extrastriatal areas and predicts severity of subclinical psychopathology and reduced neurocognitive performance. Future studies should investigate the significance of this pattern for the behavioral effects of cannabis, especially in terms of increasing the vulnerability to psychosis.
**Keywords:** striatum, Dopamine, Cannabis Dependence, MRS, glutamate
**Disclosures:** Nothing to disclose.

### M263. Towards Developing Procedurally Facile Murine Models of Methamphetamine-Alcohol Co-Abuse

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**Background:** Methamphetamine (MA) ranks 3rd as the drug most co-abused in individuals with alcohol use disorders (AUDs) and the percentage of current MA users that report alcohol co-abuse (a.k.a. mixing) ranges from 34-99%. Despite this, there has been very little research effort devoted to studying this severe and prevalent neuropsychiatric conditioning. One of the major obstacles related to the study of MA-alcohol co-abuse relates to the lack of procedurally facile, high throughput, animal models that are critical to facilitating discoveries related to the cellular and molecular underpinnings of co-abuse required for the development of rationale therapeutic interventions.
**Methods:** As oral amphetamine administration can lead to addiction, we have developed simple models of both sequential and simultaneous oral MA-alcohol mixing using C57BL/6J (B6) mice as subjects. In a model of sequential mixing, B6 mice are first presented with 4 sipper tubes containing varying concentrations of unadulterated MA (10-80 mg/L) and allowed to consume the drug for 2 weeks. Then mice are presented with varying concentrations of unadulterated alcohol (5-40% v/v) under similar access procedures. In a model of simultaneous mixing, MA is dissolved in alcohol and mice allowed to choose between mono-drug or mixed solutions across days.
**Results:** When presented with unadulterated MA solutions (5-80 mg/L) under 2-h, limited-access, procedures in the home cage, B6 mice consume on average 1 mg/kg MA/day, with the majority of intake derived from the high-dose solution. Prior oral MA intake augments the subsequent intake of alcohol, doubling the intake of the 40% concentration. When presented with the choice between solutions containing a MA-alcohol mix (10 mg/L MA in 20% alcohol) versus MA alone, B6 mice consume nearly twice the amount of the mix solution as that exhibited for the MA solution alone.
**Conclusions:** These preliminary results support the feasibility of simple, limited-access, binge drinking procedures for the study of MA-alcohol co-abuse in B6 mice. These simple models of sequential and simultaneous MA-alcohol mixing should provide fruitful for advancing our scientific understanding of the etiology of co-abuse & the development of effective treatment strategies.
**Keywords:** co-abuse, Alcoholism, Methamphetamine, Animal Models, binge drinking
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