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W2. Interoceptive Awareness in Meditators During Cardiorespiratory Deviations in Body Arousal

Sahib Khalsa*, David Rudrauf, Richard Davidson, Daniel Tranel

UCLA Semel Institute for Neuroscience and Human Behavior, Los Angeles, California

Background: Attention directed towards internal body sensations is commonly practiced in many meditation traditions, and is a core skill cultivated when learning mindfulness. This practice is commonly proposed to increase 'awareness' of internal body states, despite a lack of supporting scientific evidence. In a previous study we found that extensive meditation experience was not associated with differences in the ability to perceive heartbeat sensations at rest, despite the fact that meditators perceived their performance to be superior than nonmeditators. Since most individuals are unable to reliably detect their resting heartbeat, it is possible that we failed to assess the intended function. Furthermore, since meditators most commonly direct attention towards breathing sensations, it is possible cardiac perception is a poor index of the type of interoception cultivated by meditation practice. In the current study we extended our investigation to assess awareness of heartbeat and breathing sensations across a wide range of arousal levels, using bolus isoproterenol infusions. We hypothesized that meditators would display greater interoceptive awareness than nonmeditators. We predicted this would be indexed by increased discrimination (ie, detection) of isoproterenol induced body changes at lower doses of isoproterenol than nonmeditators. We also predicted meditators would be more accurate than nonmeditators at tracking the ongoing experience of interoceptive sensations at higher doses.

Methods: Using a randomized, double-blinded and placebo controlled design, we assessed interoceptive awareness in 15 meditators and 15 nonmeditators who were individually matched on age-, gender- and body mass index. Recruitment emphasized Vipassana meditators as this tradition strongly emphasizes cultivating attention to internal body sensations, and specifically teaches that it results in increased awareness of the body. Meditators were eligible if they reported a daily or near daily meditation practice during the previous two years, and if they had also attended one or more weeklong meditation retreats within the previous year. Nonmeditators were eligible if they had never received formal or self-taught meditation or yoga training. All were screened and excluded for psychiatric, neurological, cardiac or respiratory disease. Participants rated the experience of internal body sensations during multiple bolus infusions of isoproterenol (0.1, 0.25, 0.5, 0.75, 1.0 and 2.0 micrograms) and saline. During infusions, participants indicated their ongoing experience of the intensity of heartbeat and breathing sensations by rotating a dial. Discrimination was measured by a positive dial deflection above baseline during an infusion trial. Accuracy was measured via maximum cross correlation between each subject's dial ratings and their corresponding heart rate response. After each infusion participants also traced the body locations where they had felt heartbeat sensations, on a manikin template.

Results: Bolus isoproterenol infusions elicited equivalent increases in heart rate in both groups (Repeated measures ANOVA; $F(1,5) = 50.1$, $p < 0.0001$). There were no significant group ($F(1,28) = 1.27$, $p = 0.27$) or group x dose interactions ($F(1,5) = 0.04$, $p = 0.998$). There were also no group differences in adrenergic sensitivity as measured by CD25 (dose required to elevate heart rate by 25 beats per minute; $t(28) = -0.35$, $p = 0.73$). Examination of individual dial ratings revealed that both groups correctly detected increases in heartbeat and breathing sensations at increasing doses of isoproterenol compared with saline, with 100% of participants endorsing increased sensations at the highest dose (2.0 mcg). There were no significant differences in the proportions of meditators *vs* nonmeditators detecting increased sensations, across all doses (χ^2, $p > 0.05$). At the highest dose, peak sensation ratings were highly correlated with peak heart rate changes in nonmeditators ($r = 0.864$, $p = 0.0001$) but not meditators ($r = 0.281$, $p = 0.310$). Examination of accuracy revealed that all participants generated greater maximum cross correlations at increasing doses of isoproterenol (Repeated measures ANOVA; $F(1,5) = 39.92$, $p < 0.0001$). However, there were no significant group ($F(1,28) = 0.00$, $p = 0.99$) or group x dose interactions ($F(1,5) = 2.23$, $p = 0.06$). Overlap maps of heartbeat sensation location revealed that nonmeditators perceived heartbeat sensations in the anterior chest, particularly in the lower left region. Meditators also perceived heartbeat sensations in the chest but with greater variability in body localization, across the midline chest, neck, abdomen, head, back, arms and legs.

Conclusions: Contrary to predictions, meditators did not demonstrate increased awareness of heartbeat or breathing sensations across a range of levels of arousal. The absence of an effect cannot be ascribed to an inability of most subjects to perceive interoceptive sensations. Furthermore, this finding covers assessment of both heartbeat and respiratory sensations, the latter of which is more commonly the focus of attention during meditation. Although predominantly negative, these findings address significant gaps in our knowledge and suggest a revision of some basic assumptions about the sensory effects of meditation may be warranted.

Keywords: interoception, meditation, interoceptive awareness, mindfulness, heartbeat, respiration, isoproterenol, attention, perception.

Disclosures: S. Khalsa, Nothing to Disclose; D. Rudrauf, Nothing to Disclose; R. Davidson, Nothing to Disclose; D. Tranel, Nothing to Disclose.

W3. Neural Mechanisms of Extinction Learning for Monetary Reward in Health and Cocaine Addiction

Anna Konova*, Muhammad Parvaz, Nelly Alia-Klein, Rita Goldstein

Mount Sinai School of Medicine, New York, New York

Background: Addiction is characterized by continued drug-seeking despite reduced pleasure derived from the drug and even in the face of catastrophic personal, social, and legal consequences, suggesting that addicted individuals may have diminished ability to form and/or maintain new associations for stimuli that previously predicted rewards. Therefore, here we used classical appetitive conditioning to examine the psychophysiological and neural correlates of extinction learning in healthy individuals and individuals addicted to cocaine.
Methods: 22 individuals with cocaine use disorders (CUD) and 13 healthy controls completed two days of testing. On day 1, subjects learned to associate a cue, the conditioned stimulus (CS), with monetary reward ($4, CS+) or no reward ($0, CS−) using partial reinforcement. Extinction training was conducted immediately following acquisition and involved repeated presentation of the CS without the paired monetary reward. Retention of extinction learning was assessed a day later (day 2). Skin conductance responses (SCR) and functional MRI were acquired throughout.
Results: Differential SCR, indexing conditioned response to the CS+ *vs* CS−, did not differ between the groups or the learning phases (reward acquisition, day 1 extinction, or day 2 extinction; $F<2.34$, $P>0.11$). Whole-brain analyses revealed that, across subjects, extinction learning was associated with a progressive increase in activation of the right inferior frontal gyrus to the CS+ *vs* CS− (acquisition < day 1 extinction < day 2 extinction). In addition, while in controls the opposite pattern was observed in the bilateral thalamus/globus pallidus and ventromedial prefrontal cortex (VMPFC; CS+ > CS−, acquisition > day 1 extinction > day 2 extinction), in CUD it was not, and this represented a significant group × learning phase interaction. CUD also had greater activation in the right hippocampus/amygdala compared with controls across the learning phases. Follow-up whole-brain regression analyses indicated that suppression of activation in the rostral anterior cingulate and bilateral thalamus/striatum during extinction learning was associated with reduced differential SCR (CS+ > CS−) across subjects and with lower frequency of cocaine use in CUD (for all analyses, $P<0.05$ cluster-corrected).
Conclusions: Consistent with studies demonstrating that the thalamus, striatum, and hippocampus/amygdala are involved in the expression of learned associations and arousal states, the lateral prefrontal cortex in emotion regulation, and the VMPFC/anterior cingulate is critical for the success of extinction of aversive conditioning (eg, involving electric shock), our results provide preliminary evidence for the involvement of these regions in the extinction of appetitive conditioning involving monetary gain, highlighting common neural correlates of aversive and appetitive conditioning in humans and respective abnormalities in individuals with CUD.

Keywords: addiction, extinction learning, money, reward, fMRI.
Disclosures: A. Konova, Nothing to Disclose; M. Parvaz, Nothing to Disclose; N. Alia-Klein, Nothing to Disclose; R. Goldstein, Nothing to Disclose.

W4. Automated Analysis of Disorganized Communication Predicts Transition to Psychosis Among Clinical High Risk Patients

Gillinder Bedi, Facundo Carillo, Guillermo Cecchi, Diego Fernandez Slezak, Mariano Sigman, Jordan E DeVylder, Felix M Muchomba, Cheryl M Corcoran*

Columbia University Medical Center, New York, New York

Background: Subthreshold thought disorder has been identified as predictive of psychosis onset among patients at clinical high risk (CHR) for psychosis (Bearden *et al*, 2011). Assessment of thought disorder is achieved through clinical ratings of speech production. Analyzing speech with automated methods may present a direct, objective measure to complement existing methods, potentially offering a unique 'window into the mind'. We evaluated the trajectory of disorganized communication leading to psychosis using clinical rating scales in a large cohort of clinical high risk (CHR) patients. We also assessed whether a novel, automated method of speech analysis could differentiate those who went on to transition to psychosis from those who did not over a 2.5 year period.
Methods: 100 patients at CHR for psychosis were ascertained and followed quarterly for up to 2.5 years, or until time of dropout or transition to psychosis. Disorganized communication was assessed for predictive value for psychosis both at baseline and as a latent trajectory over time. A subcohort of 35 CHR patients had transcribed interviews, which were analyzed for semantic and syntactic coherence using a novel automated speech analysis approach. We employed machine learning with leave-one out cross validation to assess whether the semantic and syntactic indices identified predicted conversion to psychosis over the period of follow up. To further validate the method, we applied the classification algorithms developed to two separate cohorts of schizophrenia patients and healthy controls.
Results: Psychosis transition in the overall sample was 26%. Both baseline disorganized communication and a trajectory of high persistent disorganized communication were predictive of psychosis, with similar sensitivity and specificity ~0.6. Automated speech-based analyses of speech from a single time point predicted transition to psychosis with a sensitivity of 0.8 and specificity of 0.93. The algorithms developed also accurately discriminated between schizophrenia patients and healthy controls in two separate cohorts.
Conclusions: Persistent disorganized communication predicts psychosis onset; importantly this feature of psychosis risk can be accurately identified using automated speech analyses. These findings are consistent with neural and electrophysiological studies of psychosis risk, and have important implications for treatment strategies and prognosis assessment in CHR individuals.

Keywords: psychosis; risk; thought disorder; graph theory; semantic.
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W5. BDNF, Synaptic Function and Cognitive Decline in Healthy Subjects and Pre-clinical Alzheimer's Disease

Pradeep J Nathan*

UCB Pharma, Brussels, Belgium

Background: Clinical trials in Alzheimer's disease (AD) are extremely challenging. Its slow progression and huge patient heterogeneity require lengthy studies with a large number of patients. Lack of sensitive and reliable biomarkers for its core pathophysiology makes it difficult to track disease progression and drug efficacy. Increasing evidence suggests that synaptic dysfunction is a key pathophysiological hallmark for AD. Success of a 'synaptogenic' therapy depends on whether synaptic dysfunction and repair/regeneration can be measured in the clinic. In this context, the discovery of the val66met polymorphism in the human brain-derived neurotropic factor (BDNF) gene provides a unique opportunity. Cellular, imaging and behavior studies have revealed that the BDNF-met allele is associated with a decrease in activity-dependent BDNF secretion, leading to impairments in synaptic plasticity and synaptogenesis, as well as various cognitive functions. Taking advantages of the BDNF val66met polymorphism, the present study aimed to address two critical issues in AD clinical trial design. First, assuming that a reduction in BDNF secretion in BDNF-met carriers leads to proportional impairment in synaptic function in the brain, we systematically compared a number of 'synaptic' markers in individuals carrying val/val, val/met, met/met genotypes (Study 1). Second, given that Aβ amyloid deposit is a key pathogenic factor for AD, we examined the interaction between BDNF val66met polymorphism and Aβ loading (measured by PiB PET imaging) in the brain (Study 2).
Methods: For study 1, 60 healthy subjects (20 val/val; 20 val/met and 20 met/met) were recruited. For study 2, 165 healthy older subjects (107 val/val and 58 met carriers) and 34 patients with mild cognitive impairment (MCI) (24 val/val and 10 met carriers) were recruited. Synaptic activity and cognition were quantified using Imaging (electrophysiology, functional magnetic resonance imaging) and behavioural (cognitive performance) methods. Aβ loading was quantified with PiB PET Imaging. Study 1 was conducted at the GSK Clinical Unit Cambridge (CUC) while study 2 was conducted as part of the Australian Imaging Biomarker and Lifestyle (AIBL) longitudinal study.
Results: In study 1 we identified a number of BDNF sensitive markers of synaptic activity. Compared to val homozygotes, met carries (val/met and met/met) showed evidence of 'inefficient' synaptic activity as demonstrated by impaired EEG activity (ie decreased delta/theta power and phase synchrony) during cognitive processing in an error related negativity task of executive function (all $p<0.05$), increased frontal and parietal resting slow wave EEG power in the theta and alpha frequency (all $p<0.05$), and increased hippocampal ($p<0.05$) and inferior frontal ($p=0.004$) and parietal ($p=0.009$) cortical activation during retrieval of an episodic memory task. In study 2, we identified a number of BDNF sensitive markers related to Aβ amyloid. Compared to val/val homozygotes with high Aβ amyloid (ie PIB+), healthy elderly subjects carrying BDNF-met genotype showed significant and moderate-to-large magnitude decline in episodic memory, executive function, and language, as well as greater reductions in hippocampal volume over 36 months (all $p<0.05$; cohen's d between 0.73 and 0.8). In patients with MCI and high Aβ amyloid (ie PIB+), met carriers showed significant and large decline in episodic memory and hippocampal volume (all $p<0.05$; cohen's d between 0.83 and 0.95). In healthy elderly subjects with high Aβ amyloid (ie PIB+) the odds ratio for conversation to MCI at 36 months was 3.57 for met carriers relative to val carriers. In MCI patients with high Aβ amyloid (ie PIB+) the odds ratio for conversation to AD at 36 months was 2.1 for met carriers relative to val carriers.
Conclusions: Using BDNF val66met polymorphism, we have identified several electrophysiological and imaging biomarkers that could sensitively and reliably measure synaptic changes in the human brain, using relatively small number of subjects. These endpoints may potentially be used in AD clinical trials to monitor both disease progression and drug effects on synaptic changes. We have also demonstrated that Aβ loading (ie PIB+) combined with BDNF-met genotype is associated with greater neurodegeneration and memory decline as well as a greater odds ratio for conversion to MCI or AD. The latter finding suggests that Aβ loading (ie PIB+) combined with BDNF-met genotype could be used as criteria to select patients for a shorter clinical trial with fewer patients. Overall, these findings provide support for the use of common genetic polymorphisms in biomarker development and patient stratification for clinical studies of complex brain diseases such as Alzheimer's disease.
Keywords: BDNF, Alzheimer's disease, cognition, synaptic activity, Brain Imaging.
Disclosures: P. Nathan, **Part 1:** I work for UCB Pharma, **Part 2:** N/A, **Part 3:** I am currently emplyed by UCB Pharma. Prior to that I was employed by GlaxoSmithKline, **Part 4:** I receive grants from UCB Pharma for my research. I also received funding from GlaxoSmithKlne (my previous position) while I was emplyed there until May 2013, **Part 5:** I am currently employed by UCB Pharma.

W6. Prolonged Temporal Interaction for Peripheral Visual Processing in Schizophrenia: Evidence from a Three-flash Illusion

Yue Chen*, Daniel Norton, Charles Stromeyer

McLean Hospital, Harvard Medical School, Belmont, Massachusetts

Background: Schizophrenia patients are impaired in their capacity to organization of visual information. Coherent perception and understanding of the visual world requires orderly processing of spatially and temporally distributed visual information in the central but also equally

importantly in peripheral visual fields. In schizophrenia, we previously showed visual temporal integration at the fovea is prolonged in patients. It is however unclear how the processing of peripheral visual information is distinctly affected. In this study, we investigated this temporal interaction in the periphery.
Methods: We used a three-flash illusion paradigm in which two light flashes are perceived by healthy individuals as one, two or three flashes depending on the time interval between the flashes. In each trial, two brief, spatially-coincident light pulses (each lasting 1 msec) were presented at the fovea or in periphery (34° in the right visual field). The inter-stimulus interval (ISI) of two light pulses ranged from 30 to 310 msec. The task for patients ($n = 28$) and controls ($n = 26$) was to indicate the number of flashes (one, two or three) perceived after the presentation of two light pulses.
Results: In the periphery, the peak of three-flash illusion were shifted to longer ISIs—190 msec in controls (compared to 110 msec at the fovea), and 270 msec in patients (compared to 150 msec at the fovea). Patients had significantly greater three-flash illusion than controls for the ISIs of 230 and 270 msec in the periphery and 270 msec at the fovea.
Conclusions: Compared to central visual field, the range of temporal interaction in periphery is prolonged to a greater extent in schizophrenia. This expansion of temporal range suggests a coarse temporal resolution for visual interactive processes in this mental disorder, especially when peripheral visual processing is involved.
Keywords: schizophrenic, temporal processing, periphery, fovea, vision, perceptual organization.
Disclosures: Y. Chen, Nothing to Disclose; D. Norton, Nothing to Disclose; C. Stromeyer, Nothing to Disclose.

W7. Social Isolation Stress Markedly Reduces the Response of Cortical Dopaminergic Neurons to Pleasurable Stimuli

Giovanni Biggio*, Laura Dazzi

University of Caligari, Monserrato (CA), Italy

Background: Experimental evidences suggest that exposure to both acute and chronic stress cal deeply influence the amount of food eaten, inducing both decreases and increases in food intake. Accordingly, decreased food intake and weight loss have been used as reliable markers of stress severity in rats. Social isolation (SI), a widely used animal model of depression, is able to induce anhedonia in rats, reducing the consume of sucrose. The mesocortical dopaminergic system is involved in the coping response to environmental stimuli. Accordingly, both stressful and pleasurable stimuli can induce an increase in the extracellular concentration of dopamine (DA) in the medial prefrontal cortex of rats. In light of these evidences we decided to investigate the role of a chronic stress like SI, on the sensitivity of mesocortical dopaminergic neurons to anticipation and consumption of both standard and palatable food.
Methods: For our experiments we measured extracellular dopamine concentration in freely moving rats by microdialysis. Rats were trained to consume their daily meal only two hours a day (from 11:00 A.M. to 1:00 P.M.) and after four weeks of training dopamine was measured from 9:00 A.M. to 3 P.M., thus including the 2 h before food presentation (anticipatory phase), the 2 h during food consumption (consummatory phase), and the following 2 h (satiety). Dopamine extracellular concentration was measured in group housed (GH), or socially isolated (SI) rats. Different groups of animal received a pellet of chocolate (CH) or Imipramine (IMI 20 mg/kg) dissolved in the CH pellet, once a day for 14 days one hour before food presentation.
Results: In GH rats dopamine extracellular concentration significantly decreased (+ 180% over basal values) as early as 80 min before food presentation, reached a maximum during food consumption 8 + 350%) and returned to basal values when food was taken away. On the contrary, in SI rats the increase of dopamine output induced by both food anticipation and consumption was almost completely abolished with respect control, GH animals. As most of the SI-induced changes are reverted by housing the animals in group, we measured DA output in a group of rats that, after SI for 4 weeks, have been GH for 4 more weeks. In these rats we found that, as in SI rats, neither food anticipation nor consumption induced significant changes in DA output, suggesting that, at least in this parameter, SI induced persistent changes. In order to try to restore the response of mesocortical DAergic neurons to food, we then administered the antidepressant drug IMI (20 mg/kg/day for 14 days) in CH pellet that was presented to the animals one hour before food. This way of administration was chosen to avoid to handle the animals as handling has been shown to abolish the effect of SI. Control animals received only the CH pellet. Our results showed that in Si rats neither CH alone nor CH with IMI were able to restore the food-induced increase in DA extracellular concentration. In GH rats receiving the CH pellet, the anticipatory increase in DA output was shifted before CH presentation, while food consumption still induced an increase in DA output similar to that observed in control rats. In these rats, IMI administered with CH was able to further enhance the increase in DA output observed both in the anticipatory and consummatory phase.
Conclusions: Our data confirm the crucial role of mesocortical dopaminergic neurons in the regulation of emotion and suggest that the alterations in mood state induced by Si are able to blunt the response of cortical dopaminergic neurons to pleasurable stimuli.
Keywords: social isolation stress, cortical dopaminergic neurons, pleasurable stimuli.
Disclosures: G. Biggio, Nothing to Disclose; L. Dazzi, Nothing to Disclose.

W8. Impaired Neural Functioning Following Internally Focused Cognition in Obsessive-Compulsive Disorder

Emily R Stern*, Alexandra F Muratore, Stephan F Taylor, James L Abelson, Patrick R Hof, Wayne K Goodman

Mount Sinai Hospital, New York, New York

Background: Obsessive-compulsive disorder (OCD) is associated with excessive absorption in distressing

thoughts, images, or urges, which may be due to an inability to disengage flexibly from an internally focused (IF) cognitive state and attend to external information. Functional magnetic resonance imaging (fMRI) studies have linked IF cognition to activation of the 'default mode network' (DMN), a large-scale brain system that is hyperactive in OCD. Externally focused (EF) cognition, by contrast, is subserved by a fronto-parietal network (FPN) involved in detecting and acting on information in the environment. We previously reported widespread reductions of FPN activity during target detection (TD) when is follows a perceptually decoupled IF task compared to a perceptually guided EF task. This suggests that excessive internal absorption in OCD may impact patients' subsequent FPN functioning. To test this hypothesis, we examined the association between brain activity during TD and prior cognitive state in patients with OCD and controls.

Methods: fMRI data have been obtained from 12 healthy individuals and 9 OCD patients. Subjects imagined personal event scenarios that were positive or negative (IF condition) or performed a color-word Stroop task (EF condition) for 12–18 s before switching to a target detection (TD) task also requiring attention to external information (15 s). A block design was employed to reduce collinearity between sequential conditions, enabling the unique estimation of neural activity associated with the IF, EF, and TD blocks. Behavioral analysis compared OCD patients with controls on reaction time (RT) and accuracy during TD using independent samples *t*-tests (alpha = 0.05). Neuroimaging analysis employed two-sample *t*-tests (whole brain analysis, $p < 0.005$ voxelwise threshold with 20 contiguous voxels) to compare patients and controls.

Results: OCD patients made more errors during TD after imagining negative personal scenarios (negative IF) than controls ($t = 3.0$, $p = 0.008$), with no significant group differences in accuracy for TD following positive IF or TD following EF. There were no group differences in RT for any of the conditions. During the IF task, there were few differences between OCD patients and controls, with both groups showing robust activation of DMN as would be expected. However, on TD blocks following negative IF, OCD patients showed reduced activity compared to controls in FPN regions including dorsolateral prefrontal cortex (DLPFC)/frontal pole, dorsal anterior cingulate cortex, precuneus, cuneus, putamen, and thalamus. These group differences were not found for TD following positive IF or TD following EF. Among OCD patients, greater symptom severity as measured on the Yale-Brown Obsessive-Compulsive Scale (YBOCS) was associated with reduced activation of DLPFC during TD following negative IF.

Conclusions: These preliminary findings reveal altered behavior and brain activity in OCD patients when directing attention to external information following engagement with a negative, internally focused state. OCD may involve a deficit in the ability to activate FPN regions that are required for redirection of attention from internal states to external, perceptual information. This may help explain their failure to attend to or tendency to discount available perceptual evidence that could otherwise counteract obsessional thinking.

Keywords: obsessive-compulsive disorder neuroimaging attention imagination cognition.

Disclosures: E. Stern, Nothing to Disclose; A. Muratore, Nothing to Disclose; S. Taylor, Nothing to Disclose; J. Abelson, Nothing to Disclose; P. Hof, Nothing to Disclose; W. Goodman, Nothing to Disclose.

W9. Hyperconnectivity of Default Mode and Emotion-salience Neural Networks in Late-adolescent, Remitted Depression

Rachel Jacobs*, Laura Gabriel, Kelly Ryan, Sara Weisenbach, Amanda M Baker, Amy T Peters, Rachel Ringrose, Gloria Harrington, Jon-Kar Zubieta, K Luan Phan, Scott Langenecker, Robert Welsh

University of Illinois at Chicago, Chicago, Illinois

Background: Brain regions that are more active during rest or during self-reflective thought than during cognitively demanding tasks have been labeled the default mode network (DMN). This describes the 'default mode' of the brain when not attending to external stimuli. Analyses that capture inherent low frequency connectivity between nodes in this network suggest that it is stable and may be related to a number of diseases, such as Major Depressive Disorder (MDD). Functional connectivity MRI (fcMRI) analyses of individuals lying quietly in the scanner with eyes open have focused primarily on those *currently* within a depressive episode. These studies have reported hyperconnectivity of the DMN and emotion/salience networks (ESN). Only a very small number of studies have examined whether these abnormalities are a *trait* that can be observed outside of episode and not a *state* feature only present during the episode. We examine low frequency neural network connectivity among a sample of young adults with remitted MDD (rMDD). These individuals are hypothesized to bear less of a lifelong cumulative burden of illness, and based on existing literature we hypothesized that we would observe hyperconnectivity between regions of the DMN and the ESN among rMDD youth when compared to HCs.

Methods: fcMRI data were acquired over eight minutes in a 3.0 Tesla GE scanner using a forward-reverse spiral sequence and a 2 sec TR. Eighteen young adults (ages 18–22, mean age = 20.5, 68% Female) who had experienced between 1–3 prior episodes of MDD (using Diagnostic Interview for Genetic Studies; DIGS) and who had been remitted for at least one month were scanned and compared with 18 healthy control (HC) individuals with no personal or family history of a mood or anxiety disorders. All participants were medication free for 30 days prior to enrollment. Participants rested with eyes open while viewing a fixation crosshair to elicit coherence of the DMN at rest. Data were processed and analyzed using MATLAB and SPM8, including slice timing, realignment, coregistration, warping, and smoothing with a 5 mm FWHM. The left posterior cingulate cortex (PCC, −5, −50, 36) and left subgenual anterior cingulate cortex (sgACC, −4, 21, −8) were used as seeds to analyze connectivity within the DMN and ESN based upon prior literature and confirmed with the average anatomy of the current sample of 36. Three-dimensional correlation coefficient images were trans-

formed to z scores and z images were used to conduct two-sample *t*-tests. AlphaSim was used with 1000 Monte Carlo simulations to determine whole brain correction with a joint threshold of height and extent ($p < 0.005$, cluster extent of 440 mm^3) for group comparisons. Images are displayed on an averaged brain anatomy of current subjects.

Results: As expected, the use of the left PCC seed in low frequency fcMRI analyses indicated connectivity with the medial prefrontal cortex, posterior superior temporal gyrus, and bilateral hippocampus. rMDD participants demonstrated greater connectivity between the PCC and a number of frontal and parietal regions, including the rostral anterior cingulate cortex (rACC, 14, 46, 8, $z = 3.82$) and putamen/insula (20, −4, 0, $z = 3.67$) when compared to HCs. When evaluating the sgACC as a seed for the ESN, connectivity was primarily with nearby areas including the OFC and ventral striatum. rMDD participants demonstrated greater connectivity between the left sgACC and the bilateral medial thalamus (0, −10, 6; $z = 4.65$), bilateral dorsolateral prefrontal cortex (DLPFC; Right 42, 24, 54; $z = 4.04$; Left −48, 22, 48, $z = 3.70$), and additional medial and lateral prefrontal regions when compared with HCs.

Conclusions: his is the first study to show hyperconnectivity of the DMN with fronto-limbic regions, and of the ESN with cognitive control regions in late-adolescents with a history of depression who are currently in remission. Findings of abnormalities in DMN connectivity are similar toresults deriving from currently depressedindividuals, suggesting that disturbances in the DMN may be likely contributors to the persistence of depression across the lifespan and could be trait features (Pizzagalli, 2011). Given recent evidence that the DMN can be modulated with Serotonin Reuptake Inhibitors (SSRIs; Posner *et al*, 2013), understanding the role of this network in depressive relapse and acute illness is particularly important. Future work will evaluate the clinical correlates and other potential mediators of the pattern of hyperconnectivity of the DMN to cortical regions and the ESN to cognitive control regions. In addition, critical future research can test whether these abnormalities can be detected before the onset of depression to guide preventive efforts.

Keywords: depression, fmri, functional connectivity, remission.

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W10. Altered GABAergic Signaling in Prefrontal Cortex Contributes to Impaired Working Memory in Aged F344 Rats

Jennifer Bizon*, Cristina Banuelos, Sofia Beas, Joseph McQuail, Ryan Gilbert, Barry Setlow

University of Florida, Gainesville, Florida

Background: Prefrontal cortical (PFC) systems subserve a wide range of complex executive functions that are essential for adaptive behavior and necessary to maintain independence across the lifespan. Key among such functions is 'working memory', which may be defined as the ability to briefly store and act on a mental representation of information, even when that information is no longer associated with a persistent sensory input. Working memory is critically dependent upon the PFC, which is comprised of excitatory glutamatergic pyramidal neurons and inhibitory GABAergic interneurons. The balance between excitation and inhibition is crucial for optimal working memory, and disruption of GABAergic signaling contributes to working memory impairments in schizophrenia and related disorders. Across species, working memory deficits also emerge at advanced ages; however, surprisingly little is currently known regarding how PFC GABAergic systems are influenced by normal aging. The current study employed a rat model to determine if PFC GABAergic signaling alterations contribute to decline of working memory abilities at advanced ages, and if pharmacological manipulations of PFC GABAergic signaling can attenuate age-related working memory impairments.

Methods: Experiment 1 evaluated the relationship between expression of GABAergic signaling proteins in the medial PFC of young adult (6 mo, $n = 8$) and aged (12 mo, $n = 12$) male F344 rats in relation to performance on a PFC-dependent delayed response test of working memory. This task was performed in operant test chambers and on each trial, rats were required to remember the location of a sample lever over a delay period (0–24 s) in order to obtain a food reward. One week after completion of behavioral testing, rats were sacrificed and brains extracted. In medial PFC homogenates, western blotting was used to evaluate expression of (1) the GABA synthetic enzyme glutamic acid decarboxylase (GAD67); (2) the neuronal GABA transporter (GAT-1); (3) the vesicular GABA transporter (vGAT); and (4) both subunits of the metabotropic GABA(B) receptor (GABA(B)R1 and GABA(B)R2). Experiment 2 used two cohorts of young ($n = 8$) and aged ($n = 12$) rats and a counterbalanced, within-subjects design to test the effects of the high-affinity GABA(B)R antagonist CGP55845 on delayed response performance. In cohort 1, CGP55845 was administered systemically (0, 0.01, 0.1 mg/kg) and in cohort 2, CGP55845 was administered directly into medial PFC (0, 200, 600, 2000 nmol).

Results: Performance in the delayed response task was delay-dependent, with working memory accuracy decreasing as a function of delay duration. Aged rats performed comparably to young at short delays, but exhibited reliable deficits relative to young at longer delays. In Experiment 1, immunoblots of PFC homogenates showed that whereas vGAT was unchanged by age, there was a significant increase in GAD67 and a significant decrease in GAT-1 and in both subunits of the GABA(B)R in aged relative to young rats (ps < 0.05). Linear regression analyses showed that among aged rats, expression of vGAT, GAD67 and GAT-1 was not associated with working memory abilities (vGAT, GAD67, GAT-1: rs < 0.3, n.s.). However, GABA(B)R expression was significantly and negatively associated with working memory performance ($r = -0.60$, $p < 0.05$), such that lower GABA(B)R expression predicted better working memory in aged rats. In Experiment 2, systemic adminis-

tration of CGP55845 in aged rats dose-dependently enhanced working memory relative to vehicle administration (main effect of drug: $p<0.05$). This effect was mimicked in aged rats by direct intra-PFC administration of this drug, which caused a similar enhancement in working memory (main effect of drug: $p<0.05$). Notably, neither systemic nor intra-PFC administration of CGP55845 enhanced performance in young rats, suggesting that the enhancing effects of PFC GABA(B)R blockade in aged rats are countering an age-specific deficit, rather than a result of general effects of this drug on PFC function.
Conclusions: The data suggest that dysregulation of PFC GABAergic signaling during normal aging contributes to working memory deficits. Specifically, the increase in GAD expression and reduction in GAT-1 expression suggest there may be an increase in extracellular GABA availability and tonic inhibition in the PFC at advanced ages. This hypothesis is supported by recently published work (Bories *et al*, 2013) and preliminary electrophysiological recordings from our rat model in which greater GABA(B)R activation is observed in PFC pyramidal neurons in aged rats (Carpenter, Kelly, Bizon, & Frazier, 2013, Soc. for Neurosci Abs.). Against this context, the finding that lower GABA(B)R expression significantly predicted better working memory among aged rats suggests that downregulation of GABA(B)Rs may represent an endogenous mechanism to compensate for increased GABA availability in aging. The fact that working memory in aged rats is restored by GABA(B)R blockade is consistent with this hypothesis, and supports the potential of targeting GABA(B)Rs for ameliorating age-related impairments in executive function.
Keywords: aging, executive function, prefrontal cortex, working memory, GABA.
Disclosures: J. Bizon, Nothing to Disclose; C. Banuelos, Nothing to Disclose; S. Beas, Nothing to Disclose; J. McQuail, Nothing to Disclose; R. Gilbert, Nothing to Disclose; B. Setlow, Nothing to Disclose.

W11. Negative Symptoms in the Early Course of Schizophrenia: Their Longitudinal Stability and Relationship to Early Cognitive Processes

Joseph Ventura*, Kenneth Subotnik, Michael J Gitlin, Denise Gretchen-Doorly, Gerhard S Hellemann, Kathleen F Villa, Keith H Nuechterlein

UCLA Semel Institute for Neuroscience and Human Behavior, Los Angeles, California

Background: Understanding the longitudinal course of negative symptoms in patients with a first episode of schizophrenia is relevant to developing intervention approaches. The longitudinal relationship between negative symptoms and cognitive deficits may also impact intervention strategies. We examined the longitudinal course of negative symptoms following a first psychotic episode and the longitudinal association between negative symptom severity and cognitive deficits.
Methods: The study included 149 patients with a first episode of schizophrenia, from 3 National Institute of Mental Health-funded protocols at the Aftercare Research Program at the University of California, Los Angeles, who had a mean age of 23.7 (standard deviation [SD] = 4.4) years and mean education level of 12.9 (SD = 2.2) years. Treatment programs included a range of psychosocial interventions, including a novel vocational rehabilitation program for some patients, as well as either oral or injectable atypical antipsychotic medications. Negative symptom assessments (Brief Psychiatric Rating Scale [BPRS] and Schedule for Assessment of Negative Symptoms [SANS]) were conducted frequently by trained raters from the point of medication stability throughout the first outpatient year. Cognitive assessments (Degraded Stimulus Continuous Performance Test [CPT], Span of Apprehension Test [SPAN, 3–7 CPT]) were administered at baseline and at 1-year follow-up. Cross-lagged panel analyses examined relationships between negative symptoms and cognitive functioning at baseline and 12 months.
Results: After antipsychotic medication stabilization, negative symptoms during the first outpatient year were moderately stable (intraclass correlation coefficient [ICC] = 0.64). In addition, specific negative symptom domains were moderately stable: blunted affect (ICC = 0.61), emotional withdrawal (ICC = 0.53), and motor retardation (ICC = 0.63). Beyond this overall moderate stability, 24% of patients experienced at least 1 period of negative symptom exacerbation, defined using operational criteria as remission followed by relapse or significant exacerbation, or persisting symptoms followed by significant exacerbation. Notably, 5% of patients had at least 2 periods of negative symptom exacerbation. Furthermore, negative symptom severity at baseline significantly predicted poorer sustained attention (Degraded Stimulus CPT) at 1 year ($p<0.01$) and showed a similar tendency for early perceptual processing (SPAN) ($p<0.08$).
Conclusions: This study is among the first to systematically examine longitudinal patterns of negative stability and fluctuations of negative symptoms in a sample of patients with a first episode of schizophrenia. We found that negative symptoms during the first outpatient year were generally stable over time but did exacerbate in a subset of patients. In addition, negative symptom severity at baseline appeared to contribute to deficits in early cognitive processing at 1 year. We conclude that negative symptoms under current treatment conditions often continue during the first outpatient year, may contribute to later cognitive functioning, and are an important target for intervention to promote recovery.
Keywords: early course of schizophrenia, SANS, negative symptom stability, cognition, cross-lagged panel analysis.
Disclosures: J. Ventura, **Part 1:** Research grants from Genentech, Inc., and Janssen Scientific Affairs, LLC.; Consulting to Genentech Inc.; **Part 4:** Research grants from Genentech, Inc., Janssen Scientific Affairs, LLC.; K. Subotnik, **Part 1:** Research grants from Janssen Scientific Affairs, LLC.; Consulting to Genentech Inc., **Part 4:** Research grants from Janssen Scientific Affairs, LLC, and Genentech, Inc.; M. Gitlin, **Part 1:** Bristol Myers, **Part 3:** Bristol Myers; D. Gretchen-Doorly, Nothing to Disclose; G. Hellemann, Nothing to Disclose; K. Villa, **Part 1:** I own stock in Genentech, Inc/Roche., **Part 5:** I am an employee of Genentech, Inc.; K. Nuechterlein, **Part 1:** Research grants from Genentech, Inc., and Janssen Scientific Affairs, LLC.; Consulting to Genentech Inc.; research grants from Brain Plasticity, Inc., **Part 3:** Research grants from Genentech, Inc., Janssesn Scientific Affairs, LLC., and Brain Plasticity Inc.

W12. Error Monitoring in Autism: Correlates to Symptom Severity

Melisa Carrasco*, Gregory L Hanna, Catherine Lord, William J Gehring

University of Rochester, Rochester, New York

Background: Autism spectrum disorders (ASD) are associated with deficits in social function and communication, and the presence of repetitive behaviors (RBs). Children with ASD also display significant variation in behavioral impairment and often display co-occurring emotional and behavioral symptoms. The electrophysiological mechanisms underlying social deficits and RBs in ASD are still unclear. The purpose of this study was to evaluate two error-related ERPs (the error-related negativity, ERN and the error positivity, Pe) and their behavioral correlates in ASD youth performing the Eriksen flanker task.
Methods: The dataset consisted of 26 ASD and 26 healthy controls ages 10–17. ASD and control subjects were equivalent for age, gender, and manual preference. ASD children were diagnosed using the ADOS and the diagnosis was confirmed by clinical consensus.
Results: There were no group differences in accuracy ($t(50) = 1.050$, $p > 0.05$), reaction time during error ($t(50) = 1.283$, $p > 0.05$) or correct trials ($t(50) = 1.502$, $p > 0.05$), or post-error slowing ($t(50) = 1.377$, $p > 0.05$) between ASD children and the healthy comparison subjects. After correcting for multiple comparisons, children with ASD had a more negative ERN at Cz ($t(50) = 2.416$, $p < 0.05$), in comparison to healthy subjects. In addition, ASD children had a less positive early Pe ($t(50) = 3.151$, $p < 0.05$) and late Pe at Cz ($t(50) = 2.820$, $p < 0.05$), in comparison to healthy subjects.
Conclusions: Results provide further support for group differences in error processing between healthy and ASD youth. Future analyses will seek to evaluate whether social and repetitive behavior symptom severity, as measured by the ADOS, significantly predict ERN and Pe amplitude.
Keywords: autism, ERN, ERP, error monitoring.
Disclosures: M. Carrasco, Nothing to Disclose; G. Hanna, Nothing to Disclose; C. Lord, **Part 1:** Dr Catherine Lord has received royalties from the Western Psychological Services for the Autism Diagnostic Observation Schedule (ADOS); all royalties are donated to charity.; W. Gehring, Nothing to Disclose.

W13. Prospective Examination of Prepulse Inhibition in OIF/OEF Marines Suggests Reduced Sensorimotor Gating Is a Pre-existing Factor in Those That Develop PTSD after Combat Deployment

Victoria Risbrough, Dean Acheson*, Dewleen G Baker, Caroline Nievergelt, Kate Yurgil, Mark A Geyer

University of California San Diego, La Jolla, California

Background: Development of combat-related posttraumatic stress disorder (PTSD) is one of the major health concerns arising following the wars in Iraq and Afghanistan (Smith *et al*, 2008; Polusny *et al*, 2011). To develop more effective treatment and prevention efforts, a greater understanding of theneurobiological processes involved in the etiology and course of PTSD is needed (Baker *et al*, 2012). To understand PTSD etiology, it is critical to differentiate components of PTSD-related phenotypes that are pre-existing factors from those that emerge specifically after trauma. This distinction can only be addressed by prospective studies. Here we tested the hypothesis that sensorimotor gating is a pre-existing factor in development of PTSD. Prepulse Inhibition (PPI) is a cross-species operational measure of sensorimotor gating and putative measureof pre-attentional information processing (Geyer and Braff, 1987). Presentation of a neutral acoustic 'prepulse' 30–300 ms before a more intense, startling stimulus reduces startle magnitude, possibly via direction of attentional resources toward the prepulse creating a 'gate' for the subsequent startle stimulus (Swerdlow *et al*, 1999). PPI has a well defined neural circuit and is modulated by both subcortical and cortical circuits such as the prefrontal cortex. PPI has been found to be deficient in a number of psychiatric disorders (Swerdlow *et al*, 2006; Castellanos *et al*, 1996; Perry *et al*, 2001; Ahmari *et al*, 2012; Ludewig *et al*, 2002), however its role in PTSD is currently unclear (Kohl *et al*, 2013).
Methods: These data are collected as part of the Marine Resiliency Study (MRS), a prospective study of psychological and biological markers in a sample of Marines deployed to either Iraq or Afghanistan from 2008–2011. Marines completed the PPI test as well as a clinician administered PTSD symptom scale (CAPS) prior to deployment, 3 months post-deployment, and 6 months post-deployment. PPI was assessed as previously described (Acheson *et al*, 2012). In brief the session used 114 dB acoustic startle pulses, amd 86-dB prepulses (16 dB above the 70 dB background noise) that preceded the startle pulse by 30, 60 or 120 msec (ie interstimulus intervals).
Results: Of the 1229 Marines that did not have PTSD before deployment and had complete EMG and CAPS data at the 6-month time point, 46 (4%) developed PTSD after deployment (CAPS score >65). A linear mixed effects model found that Marines who tested positive for PTSD 6-months after return from combat deployment displayed significantly lower PPI performance across all pre- and post-deployment assessment periods relative to Marines who did not test positive at 6 months. There were no main effects of time point. PPI reductions in the PTSD group were greatest at 30 and 60 ms interstimulus intervals. No significant differences in either general startle magnitude or startle habituation emerged at any assessment period, although there was a trend for the PTSD group to show higher baseline startle at the pre-deployment time point. Effect of group remained when covarying for cohort, traumatic brain injury, and hearing loss. Further, post-deployment PPI performance did not correlate with Marine self-report of combat experience, suggesting that PPI performance is a stable trait unaffected by trauma experience itself.
Conclusions: This study represents the first longitudinal test of PPI performance in relation to risk for later development of PTSD following combat experience. These results suggest that deficient PPI performance may represent a pre-existing risk factor for development of PTSD in response to traumatic experience. Ongoing studies are now in progress to determine if environmental or genetic perturbations mediate the role of PPI as a risk factor for PTSD.

Keywords: sensorimotor gating, PTSD, prospective study, risk factor, combat trauma.
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W14. Relationship Between Peritraumatic Distress and Attentional Avoidance of Trauma-relevant Threat in the Prediction of Posttraumatic Stress Disorder: Preliminary Results from a Prospective Study

Charmaine Thomas, Christopher Sears, Etienne Very, Juliette Salles, TH Eric Bui*

Massachusetts General Hospital, Boston, Massachusetts

Background: Although early interventions have been shown to be useful in preventing posttraumatic stress disorder (PTSD), reliably identifying those at risk for PTSD following a potentially traumatic event remains elusive. Recent evidence suggests that two potential indicators may be useful in this regard. First, peritraumatic distress, a measure of immediate emotional and physical response to trauma exposure (Brunet *et al*, 2001), has been consistently found to prospectively predict PTSD symptoms in a number of populations (eg, Bui *et al*, 2010). Second, while it has been found that individuals with chronic PTSD exhibit a threat-related attentional bias where they allocate more visual attention to threat-related information (eg Bar-Haim, *et al*, 2007), recent preliminary evidence suggests that an attentional bias away from threat (threat avoidance) during or immediately after trauma exposure may be a risk factor for later PTSD symptoms (Wald *et al*, 2011). To date, however, little is known about the relationship between peritraumatic distress and early attentional threat avoidance in the prediction of PTSD. The present study aims to examine the relationship between these two factors in the immediate hours following trauma exposure, and their combined ability to predict future PTSD symptoms.
Methods: Within six hours of admission to a hospital emergency department, patients admitted for either a physical assault or motor vehicle accident were approached and provided consent to participate (current sample enrolled $n = 29$, mean (SD) age = 36.6 (14.6), 34.5% ($n = 10$) women). Participants were assessed for prior trauma exposure and PTSD symptoms using the 17-item self-report PTSD Checklist (PCL; range 17–85; Blanchard *et al*, 1996), and peritraumatic distress using the 13-item self-report Peritraumatic Distress Inventory (PDI; range 0–52; Brunet *et al*, 2001). At baseline, participants were also assessed for threat-related attentional bias using a laptop computer administered dot-probe task (DPT; Bar-Haim *et al*, 2007). The DPT consisted of 10 practice trials and 90 experimental trials (60 trauma-relevant/neutral image pairs, and 30 neutral/neutral control image pairs, presented in randomized order). Attentional bias scores were calculated by subtracting participants' response time to probes replacing neutral images to response time to probes replacing trauma-relevant images, so that decreased scores indicated increased threat avoidance. Participants were reassessed for PTSD symptoms 1 and 3 months later. All procedures were approved by the Ethics Committee of Toulouse University Hospital and the Conjoint Faculties Research Ethics Board at the University of Calgary.
Results: Preliminary analyses conducted among the first 29 participants revealed that at baseline, mean (SD), PCL, PDI, and PDEQ scores were 30.7 (13.3), 18.0 (8.6) and 25.0 (9.5) respectively, and mean (SD) DPT score was 17.0 (39.1). At 1-month, 25 participants completed the PCL (mean (SD) score = 35.0 (11.6)), with 7 scoring above the cut-off for probable PTSD (>44). Preliminary correlational analyses revealed that 1-month PTSD symptoms were significantly associated with PTSD symptoms prior to emergency department admission ($r = 0.62$, $p < 0.01$), with peritraumatic distress ($r = 0.64$, $p < 0.01$), but not with threat avoidance ($r = 0.08$). The correlation coefficient between peritraumatic distress and threat avoidance was moderate ($r = 0.30$), although not quite significant ($p = 0.14$) due to limited power. A linear regression analysis predicting 1-month PTSD symptoms with these peritraumatic distress and threat avoidance, was significant ($F(2, 22) = 10.48$, $p < 0.001$, $R^2 = 0.49$) with peritraumatic distress predicting (beta = 0.73, $p = 0.001$), and attentional threat attendance (not avoidance) marginally predicting (beta = 0.30, $p = 0.07$) 1-month PTSD symptoms.
Conclusions: First, the predictive ability of peritraumatic distress measured in an emergency department adds to the literature attesting to its clinical utility in a wide range of settings and populations (eg Bui *et al*, 2010). Second, we failed to replicate Wald *et al*'s results (2011) of attentional threat avoidance during life threatening danger, and its association with later PTSD. Instead, our findings suggest that, when controlling for peritraumatic distress, those who go on developing PTSD symptoms at one month may express higher threat-related attentional hypervigilance, as opposed to avoidance, in the immediate hours following trauma exposure. These findings are however in line with a large body of research indicating that chronic PTSD is associated with threat-related attentional bias (eg Bar-Haim, *et al*, 2007). Thus, it is possible that the relationship between attentional bias towards threat and 1-month PTSD symptoms may reflect an association with preexisting chronic PTSD symptom. Completion of the current study with the full sample size will enable an examination of the moderating effects of pre-existing PTSD, and will further expand current knowledge about the relationship between prior trauma, emotional, and attentional responses to traumatic stress in the immediate hours following exposure and the development of PTSD.
Keywords: attentional biais, trauma, peritraumatic reactions, PTSD.
Disclosures: C. Thomas, Nothing to Disclose; C. Sears, Nothing to Disclose; E. Very, Nothing to Disclose; J. Salles, Nothing to Disclose; T. Bui, Nothing to Disclose.

W15. Reduced Impairment, Yet Increased Reliability of Cognitive Control Measurements in Remitted MDD

Scott Langenecker*, Rachel Jacobs, Natania Crane, Kelly Ryan, Sara Weisenbach, Olusola Ajilore, Michelle Kassel, Laura B Gabriel, Jon-Kar Zubieta

University of Chicago, Chicago, Illinois

Background: Executive dysfunction is one of the most consistently reported effects of active-state, major depressive disorder (MDD). Despite many experiments in the active illness state for MDD, few studies have investigated whether the Cognitive Control (CC) system is disrupted in the remitted state of depression. Furthermore, studies often fail to account for the severity of illness, or the number and chronicity of episodes. One strategy to avoid these past challenges is to study individuals early in the course of illness, in the remitted state, at a point where CC system maturity in development would not be expected to differ between healthy control and MDD groups. To more specifically address the question of whether CC deficits are a trait risk factor for MDD, we studied remitted participants with former MDD using a Parametric Go/No-go Test (PGNG).
Methods: Participants were recruited at 2 sites, either the University of Michigan ($n=32$) or the University of Illinois at Chicago ($n=45$). rMDD ($n=41$) did not differ in age, education, verbal IQ, or sex from the HC group ($n=36$, $ps>0.37$). The remitted MDD group had minimal depressive symptoms on the HDRS (mean = 2.4, SD = 2.9) and had been remitted for at least 1 month prior to evaluation. The PGNG was completed as part of a neuropsychological test battery. Repeat assessment at 4 weeks was used to evaluate for stability of effects. The task was also completed during functional MRI. The primary dependent variables for the CC were the PGNG variables; Go Accuracy, No-Go Accuracy, and Go Response Time. In active MDD, Go Accuracy and Go Response Time are typically impaired, with effect sizes in the medium to large range.
Results: There were no significant differences between rMDD and HC groups in Go Accuracy with small effect size ($d=0.31$, $p=0.26$). Reliability in measurements between baseline and 4 weeks was weak ($r=0.50$, $p=0.0001$). Intraclass correlation coefficients (ICC) were modest (ICC = 0.72, $p<0.0001$). For No-Go Accuracy, reliabilities were improved over previous estimates (ICC = 0.66, $p<0.0001$), yet still modest. Differences between groups were borderline at baseline ($d=0.49$, $p=0.065$) and attenuated at 4 weeks ($d=0.16$, $p=0.50$). For Go Response Time, there were no differences between groups ($d=-0.07$, $p=0.74$), yet ICC was excellent (ICC = 0.85, $p<0.0001$).
Conclusions: CC measures demonstrated increased stability and reliability in a more homogenous sample of young adults with and without remitted MDD. Those with remitted MDD do not appear to demonstrate significant abnormalities in CC function in the early, remitted state. Follow-up measures may indicate that this system is predictive for recurrence of illness and risk for chronic illness as has been suggested by longitudinal studies. fMRI may be more sensitive to baseline trait differences between HC and rMDD and these analyses are underway.

Keywords: depression, euthymia, cognitive control, reliability, trait.
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W16. Neural Substrates of Spatial Working Memory Deficits in Patients with Neurofibromatosis 1 (NF1)

Amira Ibrahim, Caroline A Montojo, Tena Rosser, Nicole Enrique, Katherine H Karlsgodt, Alcino Silva, Carrie Bearden*

UCLA Semel Institute for Neuroscience and Human Behavior, Los Angeles, California

Background: Neurofibromatosis Type 1 (NF1) is a genetic disorder resulting from a mutation in the neurofibromin gene, which is part of the Ras/mitogen-activated protein kinase (MAPK) pathway. As a single-gene disorder, NF1 provides a unique genetic tool to identify and dissect molecular and cellular mechanisms underlying cognitive dysfunction. Behavioral research suggests that NF1 is associated with marked impairment in visuo-spatial working memory, as well as other frontally-mediated cognitive functions. In healthy individuals, there is consistent evidence that neural activity within the intraparietal sulcus (IPS) is sensitive to manipulations of load levels within working memory (WM). Here we investigated the neural underpinnings of visuo-spatial deficits in NF1 patients utilizing a spatial WM capacity task.
Methods: Study participants consisted of 24 patients meeting clinical diagnostic criteria for NF1 and 25 healthy controls matched on age and gender. BOLD fMRI data were acquired while each participant performed the WM task. In this task participants were presented with target displays consisting of one, three, five, or seven dots; after a variable delay period, a single probe dot was displayed and participants then judged whether it appeared in the same position as one of the target dots. fMRI data were analyzed using a voxel-wise general linear model run in FSL.
Results: The primary contrast of interest for WM load, high *vs* low load (Control mean, Load5-Load1), revealed the predicted recruitment of IPS for healthy controls, in agreement with previous neuroimaging and electrophysiological research. NF1 patients exhibited a more diffuse pattern of activation, with activity in IPS, as well as anterior cingulate cortex, occipital cortex, and basal ganglia. A between-group contrast for WM load (NF1 > Controls, Load5-Load1) revealed that NF1 patients had significantly greater activation than controls in multiple cortical regions not typically associated with spatial WM load, including bilateral inferior and middle frontal gyrus, bilateral middle temporal gyrus, bilateral supramarginal gyrus, and cingulate gyrus. There were no regions showing greater neural activity in controls relative to NF1 patients as a function of increased WM load.
Conclusions: Together, these findings indicate that NF1 patients exhibit a more diffuse pattern of neural activity

during spatial WM performance compared to controls, suggesting neural inefficiency, or possibly the engagement of compensatory mechanisms. Notably, these results suggest a possible neural substrate for previously reported behavioral findings of visuo-spatial deficits in NF1. We have previously observed defects in the regulation of Ras activity, resulting in increased GABA-mediated inhibition in prefrontal/striatal networks and working memory impairment, in an Nf1 mouse model. These mechanisms may underlie neurophysiologic abnormalities during working memory performance in NF1 patients, a possibility which requires further study in both human and animal models.

Keywords: neurofibromin Rasopathy working memory capacity intraparietal sulcus.

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W17. Positive and Negative Symptom Correlates of Second-generation Antipsychotic Adherence in Recent-onset Schizophrenia

Kenneth Subotnik*, Joseph Ventura, Denise Gretchen-Doorly, Gerhard S Hellemann, Elisha R Agee, Laurie R Casaus, John S Luo, Kathleen F Villa, Keith H Nuechterlein

UCLA Semel Institute for Neuroscience and Human Behavior, Los Angeles, California

Background: To determine to what extent initial severity of positive or negative symptoms in patients with recent-onset schizophrenia is related to medication nonadherence during the first outpatient year.

Methods: The study involved patients with first-episode schizophrenia from 2 National Institute of Mental Health-funded longitudinal protocols ('Sample 3' and 'Sample 4') conducted at the Aftercare Research Program at the University of California, Los Angeles, who received treatment with second-generation antipsychotic medication, regular psychiatrist visits, individual case management, and group psychosocial interventions and/or cognitive training interventions. Antipsychotic medication adherence, rated on a 1–5 scale (1 = best, 5 = worst), was based on pill counts every 2 weeks, plasma concentrations measured every 4 weeks, patient report, clinician assessment, and the Medication Event Monitoring System (MEMS-6 [Sample 4 only]). Symptoms were evaluated using the Scale for the Assessment of Negative Symptoms (SANS) and the Scale for the Assessment of Positive Symptoms (SAPS) completed every 3 months. Correlations between medication adherence and symptoms were examined over each 3-month interval during 12 months of follow-through treatment. For these analyses, only the 64 patients receiving oral risperidone and with complete 12-month follow-through data were examined.

Results: As expected, adherence with antipsychotic medication was generally associated with lower levels of Reality Distortion (mean of SAPS Delusions and Hallucinations; Pearson correlation coefficients (r values) ranged from 0.22 (nonsignificant [ns]) to 0.36, $p<0.02$). Adherence was also associated with lower levels of Affective Flattening within each 3-month interval (r values ranged from 0.08 (ns) to 0.31, $p<0.02$). Antipsychotic medication adherence was associated with lower Avolition-Apathy during the initial 3-month period ($r=0.27$, $p<0.03$), and with Alogia in the 3–6 month interval ($r=0.31$, $p<0.02$), but was not significantly correlated with Anhedonia during any time interval. Medication adherence during the initial 3 months was associated with lower levels of Avolition-Apathy (r values ranged from 0.16 (ns) to 0.31, $p<0.02$) and of Alogia (r values ranged from 0.32, $p<0.01$, to 0.38, $p<0.002$) during subsequent time points. However, adherence was not associated with decreases in Avolition-Apathy or Alogia over time. It is possible that the relationship between antipsychotic medication adherence and negative symptoms reflects their common relationship with 1 or more additional variables that are known to be associated with both, such as insight, cognitive impairment, or possibly psychotic symptoms. Indeed, in the current dataset, most of the significant correlations between antipsychotic medication adherence and negative symptoms were nonsignificant after controlling for Reality Distortion. Conversely, the correlations between adherence and Reality Distortion were strengthened after controlling for Affective Flattening (r values ranged from 0.21 (ns) to 0.51, $p<0.001$).

Conclusions: In 64 patients with first-episode schizophrenia, antipsychotic medication adherence was significantly correlated with lower levels of positive and negative symptoms. However, the associations between medication adherence and lower levels of negative symptoms appeared to be accounted for by the relationship of both variables to positive psychotic symptoms. The findings suggest that the impact of second-generation antipsychotic medication on suppression of negative symptoms might be mediated via a reduction in positive symptoms. Future analyses will directly model this proposed mediation, and will examine the influence of poor insight and cognitive impairment as additional common third variable influences.

Keywords: schizophrenia; antipsychotic agents; patient compliance; longitudinal studies.

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W18. Early Changes in Neural Responses to Emotional Information Predict Clinical Response to SSRI Treatment in Depression

Beata Godlewska, Ray Norbury, Philip Cowen, Catherine J Harmer*

University of Oxford, Oxford, United Kingdom

Background: Antidepressant treatment has been shown to modulate both behavioural and neural markers of negative affective bias in healthy volunteers and acutely depressed patients. These effects can be measured early in treatment before therapeutic changes in mood and subjective state are seen. It has been hypothesised, however, that these early changes in emotional processing are an important mechanism which mediates clinical improvement over time by allowing a more positive response to on-going environmental stimuli, stressors and social interactions. A key prediction of this account is that early changes in emotional bias should be predictive of later clinical changes in symptoms in depression.
Methods: 32 unmedicated patients meeting DSMIV criteria for major depression were prescribed the SSRI escitalopram at a dose of 10 mg over 6 weeks. The neural response to emotional facial expressions (fear, sad and happy) was assessed both before and after 7 days of treatment. Depression severity was measured before treatment, 7 days and after 6 weeks using the Beck Depression Inventory (BDI). Small volume correction was applied during fMRI analysis to assess if early change in key areas identified through previous studies predicted clinical change after 6 weeks.
Results: BDI scores fell an average of 17.5 points after the 6 weeks treatment. This response was predicted by decreased amygdala response to both fearful and sad facial expressions of emotion after 7 days of escitalopram treatment. This decreased amygdala response was uniquely related to 6 week clinical response and was not abolished by including either baseline or one week change in BDI scores as a co-variate. The prediction was stronger for the left compared to the right amygdala, though a similar pattern was seen across both hemispheres. There were no other statistically significant predictors of clinical response at the whole brain level.
Conclusions: SSRIs have previously been reported to decrease amygdala response early in treatment in depressed patients and compared to a placebo control (Godlewska *et al*, 2012). The current study shows that these early changes are predictive of later clinical response as supported by the cognitive neuropsychological model of antidepressant drug action. These data therefore add support to the idea that early changes in emotional processing have clinical significance which is expressed with a time delay, following environmental exposure and a period of learning in the context of a reduced negative bias.
Keywords: depression, amygdala, antidepressants, serotonin, fmri.
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W19. Differential Alterations of Internally-generated Behavioral Responses and Dose-dependent Treatment Effects in Antipsychotic Naïve First Episode Schizophrenia and Psychotic Mood Disorder Patients

Sarah Keedy*, James Reilly, Jeffrey R Bishop, Margret Harris, Peter J Weiden, John A Sweeney

University of Chicago, Chicago, Illinois

Background: Laboratory studies in schizophrenia have shown that antipsychotic treatment disrupts motor acts guided by internalized representations even when extrapyramidal symptoms are not clinically evident, as seen for anticipatory responses during a predictive saccade task, a type of procedural learning task. This change from normal pretreatment responses is believed to implicate an effect of D2 blockade on frontostriatal systems, where we have reported significant change after treatment in fMRI studies using saccade tasks. Here we seek to replicate the behavioral effects, and examine their diagnostic specificity, dose relatedness, and neural function correlates.
Methods: Antipsychotic naive first episode schizophrenia ($n = 29$) and bipolar patients with psychosis ($n = 18$) were assessed before and after ~ 5 weeks of similar, low-dose second generation antipsychotic treatment. Matched healthy controls ($n = 59$) were also assessed at a similar interval. Participants performed the predictive saccade task, an oculomotor test of procedural learning. The task requires tracking a visual target alternating at a constant time interval between two locations, eliciting anticipatory saccades, which are generated in accordance with internalized representations of the target. Internally generated anticipatory responses (< 90 ms latency) and externally-guided responses were analyzed separately. A subsample of the schizophrenia patients also participated in fMRI studies of predictive saccades before and after treatment.
Results: Prior to treatment, only bipolar patients displayed impaired task performance. After antipsychotic treatment, schizophrenia patients displayed reduced performance for anticipatory saccades as well, while bipolar patients displayed no change, continuing to show a deficit. Higher antipsychotic doses were associated with worse performance posttreatment for only schizophrenia patients. In the scanned subsample of schizophrenia patients, pre-to-post-treatment activation changes included reduced right dorsolateral prefrontal cortex activation and increased left intraparietal sulcus and occipital cortex activation. Reduced performance in the scanner pre-to-post treatment was associated with lower dorsolateral prefrontal cortex activation and increased caudate activation.
Conclusions: In this independent sample of first episode, antipsychotic naive schizophrenia patients, we replicated the finding of intact performance on a procedural learning task prior to treatment as well as impaired performance after acute treatment with second generation antipsychotic medication. The treatment effects were not seen in bipolar disorder; instead, deficits appeared to be more trait-like in

bipolar patients. Higher doses of antipsychotic medication were associated with more impaired performance only for schizophrenia patients, even in the narrow range of low doses typical of the initial weeks of treatment. The selective nature of treatment-associated disruption of internally-guided responses, as opposed to externally-guided responses, suggests an adverse effect on frontostriatal function supporting cognitive operations such as planning and spatial working memory in schizophrenia. Neuroimaging analyses indicate that key correlates of posttreatment alterations in internally generated responses in schizophrenia are reduced activity in dorsolateral prefrontal cortex and increased activity in caudate.

Keywords: schizophrenia, bipolar disorder, antipsychotic, fMRI, first episode.

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W20. Progressive Reduction of Visual P300 Amplitude in Patients with First Episode Schizophrenia

Robert McCarley*, Naoya Oribe, Yoji Hirano, Shigenobu Kanba, Elisabetta del Re, Larry J Seidman, Raquelle Mesholam-Gately, Martha Shenton, Jill M Goldstein, Kevin M Spencer, Margaret Niznikiewicz

Harvard Medical School/VA Boston Healthcare System, Brockton, Massachusetts

Background: To understand the underlying dynamic neurophysiological changes over the course of schizophrenia, it is important to study subjects longitudinally from the early stage of the illness. We previously reported that visual P300 was already impaired in first episode schizophrenia patients compared with healthy controls (Oribe *et al*, Schizophrenia Research, 2013). The present unpublished study demonstrates how the visual P300 findings changed at the 1 year follow-up after their initial measurement (Baseline). The P300 evoked potential is thought to represent brain activity associated with a cognitive updating. Although the auditory P300 has been frequently studied in schizophrenia, the visual P300 has been rarely examined in cross- sectional studies of schizophrenia and, to our knowledge, the visual P300 has not been previously examined in longitudinal studies of schizophrenia.

Methods: Visual P300 was recorded with the same experimental paradigm and the acquisition protocol at baseline and 1 year follow-up in first episode schizophrenia patients ($n = 18$) or control subjects ($n = 24$) silently counted infrequent target stimuli ('x') amid standard stimuli ('y') presented on the screen while the 64-channel EEG was recorded.

Results: Schizophrenia patients showed visual P300 amplitude reduction and latency delay at baseline. Furthermore, patients showed progressive visual P300 amplitude reduction in the course of the illness over the 1 year follow-up. P300 latency didn't change over time in both patient and control groups. There was a significant negative correlation between P300 peak amplitude at Pz with the BPRS positive score at Baseline but this correlation lost significance at follow-up. There was no correlation of P300 amplitude at baseline or its change over time with medication dosage (chlorpromazine equivalents).

Conclusions: Compared with controls, visual P300 amplitude was lower at the early (first episode) stage (baseline) and was further reduced at the 1 year follow-up. Patients who had more positive symptoms showed more amplitude reduction at baseline, but no significant correlation with symptoms at follow-up, perhaps because all patient group members had more P300 reduction and positive symptoms at this follow-up recording, reducing the within group differences and hence correlation. These data are significant, and we believe novel, in showing a visual modality P300 progression in the early stage of schizophrenia, unlike the unclear or absent progression in the auditory P300. These visual P300 findings support the concept of progression of schizophrenia. They suggest the usefulness of the visual P300 as a biological marker of progression in schizophrenia, both at onset and potentially even in the prodromal period.

Keywords: neurophysiology first episode schizophrenia visual P300 longitudinal progression of schizophrenia biomarkers.

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W21. Adolescents' Neural Response to Personally Relevant Social Reward: A Novel Paradigm with Relevance to Affective Symptoms and Sensation Seeking

Erika E Forbes*, Lisa Sheeber, Nicholas Allen, Jennifer Silk, Marigrace Ambrosia

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: Heightened reward seeking contributes to a variety of risky behaviors during adolescence, and alterations in neural response to reward have been implicated in both substance use and affective problems (Bava and Tapert, 2010; Forbes and Dahl, 2012). Development of neural reward circuitry is linked to reward seeking and is a likely mechanism for these behaviors (Ernst *et al*, 2006). Notably, although adolescents' risky behaviors most often occur in peer social contexts (Steinberg, 2008), few neuroimaging studies of adolescent reward function have focused on personally relevant social rewards. To examine individual differences in risky behavior during adolescence, we developed an innovative functional magnetic resonance

imaging (fMRI) task that uses ecologically valid, personally relevant social rewards as stimuli. Specifically, adolescents view affective video clips of their closest, same-sex friend, with stimuli drawn from a recent conversation in which the two friends discussed their most positive mutual experience. The goal of the task is to engage neural reward and social-processing circuitry that is relevant to peer social experiences and predictive of reward-related problems during adolescence. We particularly focused on the medial prefrontal cortex, a region critical to both reward processing and social processing (Northoff, 2011).

Methods: We assessed neural response to peer social reward in 47 healthy adolescents who were age 14–18 years (mean age 16.3 years; 64% female; 34% African American, 66% European American). Approximately 2 weeks before the scan, participants took part in a laboratory assessment that included a 10-min interaction about the most exciting or pleasant experience they had shared. Coding by a team trained to reliability identified segments of positive and neutral affect for each member of the dyad, and these segments were used to create stimuli for the fMRI task. During echoplanar imaging on a 3T Siemens TIM Trio scanner, participants performed a block design task in which they viewed video stimuli of their friends expressing positive or neutral affect from the laboratory interaction, as well as control stimuli of an unfamiliar adolescent expressing positive or neutral affect. Preprocessing and analysis of data were conducted in SPM8, and multiple comparisons were addressed by computing a minimum cluster size in AlphaSim. Participants completed self-report measures of mood symptoms (Mood and Feelings Questionnaire; Angold *et al*, 1995), sensation seeking (Sensation Seeking Scale; Zuckerman, 1971), and risky behavior (Youth Risk Behaviors Survey; CDC, 2009).

Results: The task successfully engaged regions relevant to social and reward processing, including the medial prefrontal cortex (329 voxels, [Talairach: −6 52 6], $t = 3.48$, $p_{\text{corrected}} < 0.001$) and the ventral striatum (490 voxels, [−6 2 6], $t = 3.20$, $p_{\text{corrected}} = 0.001$). Analyses with measures of reward-related problems indicated that adolescents' response in the medial prefrontal cortex was further associated with depressive symptoms (1428 voxels, $t = 3.51$), manic symptoms (183 voxels, $t = 3.24$), sensation seeking (287 voxels, $t = 2.97$), and likelihood of having had sexual intercourse (200 voxels, $t = 3.69$).

Conclusions: Using an innovative fMRI task, we were able to engage neural circuitry implicated in reward, social, and self-relevant processing, indicating that this task is valid for assessing response to peer social reward. This fMRI task also allowed us to describe neural mechanismsof individual differences in reward-related problems. Specifically, adolescents' response in a region critical to reward and social processing was correlated with their severity of affective symptoms, tendency to seek novel and intense experiences, and sexual behavior. This work is therefore relevant to investigating the development and pathophysiology of affective and substance use problems. Extensions of this work could be valuable to prevention efforts in several ways, including identifying contexts or characteristics that are promising targets for prevention; distinguishing adolescents who are likely to engage in risky behavior or develop reward-related problems; or even specifying neural regions for interventions such as neurocognitive training.

Keywords: reward; brain development; depression; mania; sensation seeking.

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W22. Startle Latency and Magnitude Predict Clinical Outcome in the Psychosis Prodrome: Findings from the North American Prodromal Longitudinal Study (NAPLS)

Kristin Cadenhead*, Jean Addington, Carrie Bearden, Tyrone Cannon, Barbara A Cornblatt, Daniel H Mathalon, Thomas McGlashan, Diana Perkins, Larry J Seidman, Ming Tsuang, Elaine Walker, Scott Woods

University of California San Diego, La Jolla, California

Background: The NAPLS Consortium is a multisite longitudinal study that investigates risk, prediction and mechanism of psychosis in individuals at clinical high risk (CHR) for psychosis. Startle reactivity and startle latency, candidate biomarkers for psychosis risk, were assessed as part of the test battery.

Methods: A total of 312 CHR subjects between the ages of 12 and 30 were assessed at baseline. All CHR subjects then received clinical follow-up for up to 2 years. CHR subjects were subsequently classified as having unchanged prodromal symptoms ($N = 95$), a progression of prodromal symptoms ($N = 105$), conversion to psychosis ($N = 36$) or clinically remitted ($N = 76$). The paradigm includes 3 blocks of startle pulse alone stimuli (115 dB white noise). Electromyographic (EMG) responses were recorded using a Biosemi system equipped with EMG electrodes that record eye blink at the obicularis oculi muscle. Baseline startle magnitude and latency were compared using clinical status at outcome as a grouping variable followed by *post hoc* *t*-tests.

Results: Greater startle latency predicted progression of symptoms (first block: $F[3, 311] = 5.0$, $p < 0.002$; second block: $F[3, 311] = 2.70$, $p < 0.05$), in that those CHR subjects who either converted to psychosis or had symptomatic progression had longer latencies relative to those who remitted or remained symptomatically unchanged (all p's < 0.05) at follow-up. Reduced startle reactivity predicted clinical outcome at a trend level ($p = 0.09$) with converted and progressing CHR subjects having significantly smaller startle magnitude compared to those whose symptoms remitted (p's < 0.05).

Conclusions: Greater startle latency and smaller magnitude of response predict progression of symptoms and conversion to psychosis in subjects at CHR for psychosis. In animal models, startle latency is increased by dopamine agonists, suggesting that these findings may indicate hyperdopaminergia in CHR subjects destined to clinically progress. If these results are replicated, the startle paradigm is a relatively simple test that has the potential for use in the clinical setting as a biomarker for psychosis risk.

Keywords: biomarkers, prodrome, schizophrenia, startle.

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W23. Developmental Trajectory of Facial Emotion Recognition in Normal Development and Across Stages of Schizophrenia

Cheryl M Corcoran*, Pamela Butler Kahn, Elisa Dias, Daniel C Javitt

Columbia University Medical Center, New York, New York

Background: Schizophrenia patients have impairments in social cognition, in both detection of prosody and sarcasm, and in facial emotion recognition and discrimination. It is not clear when these deficits occur in the course of illness and if they are already evident during prodromal stages before the onset of psychosis. The normal developmental trajectory of social cognition and underlying sensory processing also bears further study.
Methods: Participants were schizophrenia patients and age-similar controls; clinical high risk (CHR) patients and age-similar controls; and a population sample of youths ages 7–25. Assessments included demographics and measures of social and other cognition, with a focus on the Penn Emotion Recognition Task or ER40. CHR patients were followed for up to 2 years to determine 'conversion' to psychosis. Social and other cognition was assessed across groups and normal age-related trajectories were evaluated.
Results: 15% of CHR patients 'converted' to psychosis. Facial affect recognition was a significant predictor of conversion, wih an effect size >1.3 compared with controls, similar to schizophrenia. Both CHR converters and schizophrenia patients had greatest impairment in recognition of threat-based stimuli, and a similar pattern of error. In contrast, CHR non-converters had performance equivalent to controls. Facial affect recognition was unrelated to age and was stable across normal development.
Conclusions: Impairment in facial affect recognition appears to be a stable risk marker for schizophrenia, evident even before the onset of psychosis, consistent with the relative stability of facial affect recognition, and its substrate of visual processing, over the course of normal adolescent development. A better understanding of the neural circuitry underlying this deficit is needed to optimize strategies for its remediation.
Keywords: psychosis; risk; facial affect recognition; visual processing; development.
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W24. Adults Recovered from Anorexia Nervosa Show Altered Brain Response During Delayed Discounting in Fasted and Fed States

Christina Wierenga*, Amanda Bischoff-Grethe, Andrew Melrose, Laura Torres, Laura Irvine, Ursula F Bailer, Walter Kaye

University of California San Diego, San Diego, California

Background: Individuals with anorexia nervosa (AN) restrict food consumption and become emaciated. Altered eating in AN may be related to enhanced executive ability to inhibit incentive motivational drives supported by the limbic system, perhaps as a consequence of dysregulated reward processing and/or awareness of homeostatic needs. Thus, individuals with AN may have an enhanced ability to delay rewards, thereby helping them to maintain persistent food restriction.
Methods: We used blood-oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) to examine effects of satiety and hunger on neural processing related to the motivational aspect of financial incentives to determine whether women recovered from AN (RAN) have state-dependent altered limbic and executive neural function. We compared 23 recovered adult restricting-type AN to 17 demographically matched control women (CW) during an fMRI time discounting task requiring a series of choices between monetary reward options that varied by delay to delivery (McClure, 2004) during a Fast visit (after 16 h of fasting) and a Fed visit (after being fed 30% of daily caloric needs). Statistical analyses were performed on BOLD data using two separate general linear models (GLM). The first GLM included the beta regressor, which was defined as decision trials in which the early reward option was available immediately (ie, 'Today'). The second GLM contained the delta regressor, which represented all decision trials in the task. We used a region of interest (ROI) analysis to examine group by visit interactions in executive-limbic circuitry. ROIs were based upon prior findings (McClure, 2004) and included the ventral striatum, dorsal caudate, and dorsal anterior cingulate cortex (dACC) for beta trials and the superior parietal cortex, middle frontal gyrus, ventrolateral prefrontal cortex (VLPFC), and insula for delta trials. At the group level, we conducted a group (RAN, CW) x visit (Fast, Fed) linear mixed effects analysis in R. Huber robust regressions were also conducted in R to examine the relationships between anxiety, as measured by the Speilberger State-Trait Anxiety Inventory, and the beta and delta regressor responses under Fast or Fed visits within the respective ROIs. Small volume correction was determined with Monte-Carlo simulations,

giving an a posteriori ROI-wise of $p<0.05$ for all comparisons. To assess behavioral discounting rate, we fit the participants' choice data to the β-δ discounting model (see Equation 1, McClure *et al*, 2007) to calculate each subject's k value, which represents the rate of discounting. Higher k values correspond with greater discounting, and therefore greater impulsivity (ie, steeper delay discounting curve or increased preference for the early option).

Results: No difference in discounting rate (k values) in a group x visit ANOVA was found. For trials in which the reward option was available immediately (eg, beta regressor), a significant group x visit interaction was found within the bilateral ventral striatum, dorsal anterior caudate, and dACC. CW showed greater BOLD response on the Fast visit compared to the Fed visit during trials that included immediate outcomes. In contrast, RAN had the opposite response in these regions: they exhibited greater brain response in the Fed condition relative to the Fast condition. For the delta regressor, a significant group x visit interaction was found in the left middle frontal gyrus, the bilateral insula, the right VLPFC, and bilateral superior parietal cortex. Within the bilateral insula and right VLPFC, CW responded more strongly on the Fast visit relative to the Fed visit. Within the left middle frontal gyrus, the significant interaction was associated both with the CW responding more strongly to decision trials on the Fast visit relative to the Fed visit, and for the RAN to respond more strongly than the CW on the Fed visit. For RAN in the Fed state, anxiety was negatively correlated with BOLD response in the bilateral ventral striatum, dorsal caudate and dACC for beta trials and the bilateral insula for delta trials. In contrast, for RAN in the Fast state, anxiety was positively correlated with BOLD response in the right ventral striatum, bilateral dorsal caudate, and left dACC for beta trials and the right insula for delta trials.

Conclusions: Most people are uncomfortable when hungry and experience pleasure when eating, whereas those with AN tend to be anxious when eating, and feel better when starving. Results showed that RAN and CW had opposite brain responses during delayed discounting after being fed or fasting despite similar behavioral performance. RAN had diminished responses in executive-limbic regions to immediate reward (beta) and across all decision trials (delta) when fasting and increased activation of these circuits after eating, a response opposite to CW. A seemingly paradoxical association between BOLD response and anxiety was found for RAN; increased anxiety corresponded to decreased BOLD signal in the Fed state but to increased BOLD response in the Fast state. This study further supports the possibility that AN adults have altered reward and executive circuitry that is tied to metabolic state and may contribute to anxious and inhibited behaviors. Understanding the neurobiology of AN is critical for developing more effective treatments for this disorder.

Keywords: anorexia nervosa, delayed discounting, fMRI, reward, eating disorders.

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W25. Implicit Cognition Towards Self-injury Among Teenage Suicide Attempters *vs* Teens Engaged in Non-suicidal Self-injury

Daniel P Dickstein*, Megan Puzia, Grace Cushman, Kerri Kim, Karen Seymour, Alexandra Weissman, Matthew Nock, Anthony Spirito

Bradley Hospital PediMIND Program, East Providence, Rhode Island

Background: Suicide is the third leading cause of death among 10–24 year olds, and the prevalence of suicide increases six-fold rise from childhood to late teenage years. However, completed suicide attempts (SA) among adolescents are only the tip of the iceberg. According to the 2011 Youth Risk Behavior Surveillance survey, 15.8% of high school students seriously contemplated suicide during the past year, while 12.8% made a suicide plan, 7.8% attempted suicide, and 2.4% sought medical attention for their SA.

Non-suicidal self injury (NSSI)—defined as the deliberate destruction of one's body in the absence of intent to die (eg, cutting, burning, or hitting oneself)—is another serious and growing problem among teens with prevalence estimates ranging from 15–45% among community and clinical samples.

Theories abound about how NSSI may, or may not, be related to SA. However, few studies have compared teens engaged in either SA or NSSI to improve our ability to diagnose, treat, and ultimately prevent both conditions. In particular, virtually none have examined attitudes towards suicidality or NSSI among groups of teenagers engaged in either behavior—but not both—using computerized behavioral tasks to tap implicit attitudes about suicidality and self-injury. To address this gap, we evaluated implicit attitudes towards NSSI and suicide using the computerized Self-Injury Implicit Association Task (SI-IAT) in three mutually exclusive groups of teenagers: (1) SAs who had never engaged in NSSI; (2) those engaged in NSSI who had never made an SA; (3) typically-developing controls (TDCs) who had no current or lifetime psychopathology. The SI-IAT was selected because this computerized task may tap implicit attitudes for which people lack insight or may be motivated to conceal, such as those towards NSSI and suicide.

Methods: This study was IRB approved and conducted at Bradley Hospital. Participants ages 13–17 years old were enrolled in three mutually exclusive groups: (1) teens who had attempted suicide duirng the past 30 days who had never engaged in NSSI ($n=36$) (2) teens meeting the DSM-V criteria for NSSI including >5 episodes of NSSI during the past year who had never made an SA ($n=42$); (3) typically-developing controls (TDCs) who had no current or lifetime psychopathology ($n=40$). The SI-IAT is a performance-based task founded on the assumption that it is easier to respond behaviorally (ie, pressing a key on a keyboard) when two concepts are strongly associated than when two concepts are weakly associated. Thus, the SI-IAT compares the reaction time for classifying one set of semantic stimuli related to either suicide or NSSI (eg, 'death' 'me;' 'cutting' 'me') to the reaction time for classifying another set of semantic stimuli (eg, 'life' 'me;'

'not cutting' 'me'). This comparison is quantified as a standardized difference score; higher values reflect stronger identification with self-injury.
Results: We found a main effect of group in our MANOVA of performance differences in three categories of SI-IAT semantic stimuli (ie, cutting/no cutting; death/life; and suicide/life; Wilks' $\lambda = 0.69$, $F\ (6, 240) = 8.03$, $p < 0.001$). Decomposing these data, we found significant main effects of group for all semantic categories (cutting/no cutting: $F\ (2, 129) = 19.23$, $p < 0.001$; death/life: $F\ (2, 128) = 5.43$, $p = 0.005$; suicide/life: $F\ (2, 127) = 3.26$ $p = 0.04$). *Post-hoc* comparisons showed that NSSI participants had stronger identification with 'cutting' than did SAs or TDCs ($ps < 0.001$). Additionally, the NSSI group had stronger identification with both death and suicide than did TDCs ($p = 0.004$ and 0.05, respectively), but did not differ significantly from the SA group. The SAs did not differ from TDCs in identification with any self-injury category.
Conclusions: Our study, the first to evaluate implicit attitudes about suicide and NSSI in mutually exclusive samples of teens engaged in either NSSI or SA, had two important findings. First, as expected, teens engaged in NSSI had stronger implicit associations with self-cutting than teen SAs without history of NSSI. Second and unexpectedly, teens engaged in NSSI had the strongest associations with death and suicide. Further work is necessary to evaluate this relationship, including what resilience factors enable teens engaged in NSSI to have strong associations with death and suicide without actually attempting suicide, as well as to probe the neural underpinnings of these differences.
Keywords: suicide, non-suicidal self-injury, adolescence, cognition.
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W26. Decision Making in Avoidance-reward Conflict: Behavioral Performance in Non-human Primates and Humans

Darin Dougherty*, Amanda R Arulpragasam, Andrew K Corse, Navneet Kaur, Demetrio Sierra-Mercado, Tina Chou, Alexandra Rodman, Amanda Duffy, Eric J McDonald, Christine A Eckhardt, Emad N Eskandar, Thilo Deckersbach

Harvard Medical School, Boston, Massachusetts

Background: The ability to avoid dangerous stimuli and seek reward is conserved across species. Converging lines of evidence suggest a homology between non-human primates (NHPs) and humans in the brain circuits involved in reward and aversion. There has been extensive research evaluating reward and fear circuitry. However, there is little information known about the interaction of reward and aversion in decision making. The Avoidance-Reward Conflict (ARC) Task is the first paradigm that takes both these conditions into account. This study examined the interaction of varying levels of reward with varying levels of fear in decision-making in both NHPs and humans in this behavioral task.
Methods: 2 adult male NHPs (*macaca mulatta*) and 20 healthy controls (12 females, AGE: M = 24.05, SD = 2.21; Education years: M = 16.4, SD = 1.1) participated in the ARC task. The ARC paradigm is a two-choice discrimination task, where the subject's performance on each trial was modified by the expectation of both varying probability of aversive stimuli and varying magnitude of reward. Trial onset began with the subject fixating on a central point on a computer monitor. Next, two cues represented as circles were presented on the periphery of the monitor. One cue was the combined option cue that represented two distinct stimuli: the probability of aversion and an expected reward of varying magnitude. The probability of aversion was indicated by the color of the cue (Blue: low, 10%; Black: medium, 50%; Red: high, 90%) and consisted of an air-puff administered to the eye by an air-puff stimulus device safe for use in NHPs and humans (Eyeblink Conditioning System, San Diego Instruments, Inc.). For reward, the magnitude was determined by the thickness of the cue (Thin: small; Medium: medium; Thick: large). For NHPs, increasing amounts of liquid juice were used for small, medium, or large reward. Pictures of money were used for increasing amounts of reward in humans: small ($0.05), medium ($0.25), and large ($1.00). The other cue was always a white 'pass' circle that predicted a smaller reward (nominal volume of juice for NHPs, $0.01 for humans) as a safe alternative with no aversion. The side of the screen in which the cues were presented in each trial was randomized. Data were analyzed using a random effects analysis of variance (ANOVA). Significant ($p < 0.05$) main effects were followed by simple F-tests. All statistical analysis was carried out in Statistical Packages for the Social Sciences (SPSS v17).
Results: Our results demonstrate that NHPs and humans perform similarly on the ARC task. For NHP #1 the ANOVA indicated a main effect condition between the reward and aversive conditions ($F_{4, 36} = 90.27$, $p < 0.001$). Similarly, for NHP #2, the ANOVA indicated a main effect condition between the reward and aversive conditions ($F4, 36 = 67.61$, $p < 0.001$). For humans, the ANOVA indicated a main effect condition between the reward and aversive conditions ($F_{4, 76} = 8.80$, $p < 0.001$). Simple F-tests indicated that with a high probability of receiving an aversive stimulus, both NHPs and humans are more likely to forgo reward if it is small or medium, than when it is large (NHP#1: $F_{1, 9} = 102.92$, $p < 0.01$; NHP #2: $F_{1, 9} = 168.48$, $p < 0.001$; Humans: $F_{1, 19} = 4.560$, $p = 0.046$).
Conclusions: To our knowledge this is the first study that investigates the interaction of aversive and rewarding stimuli in decision-making. We observed that both NHPs and humans were able to distinguish between varying amounts of aversion and reward in the ARC task. Notably, NHPs and humans performed similarly on the same behavioral task suggesting the reliability of animal models in predicting human behavior.
Keywords: non-human primates, reward, aversion, decision-making, humans.
Disclosures: D. Dougherty, **Part 1:** Medtronic: Research Support and Honoraria, Cyberonics: reserach support, Eli Lilly: Research Support, Roche: Research Support and

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W27. The Effects of Tobacco Smoking Status on Theta-gamma Coupling in Patients with Schizophrenia and Non-psychiatric Controls

Mera S Barr*, Kristen M Mackowick, Reza Zomorrodi, Victoria C Wing, Caroline Wass, Zafiris J Daskalakis, Tony P George

Centre for Addiction and Mental Health, Toronto, Ontario, Canada

Background: Patients with schizophrenia smoke more cigarettes compared to the general population, and have greater difficulty quitting. Increased prevalence may be related to the underlying pathophysiology of this disorder, and possible remediation of cognitive deficits such as working memory. Working memory represents a core deficit in patients with schizophrenia that is associated with alterations oscillatory activity in the gamma frequency band (30–50 Hz) and in a mechanism called theta (4–7 Hz)—gamma coupling. Evaluating oscillations mediating working memory in the dorsolateral prefrontal cortex (DLPFC) in smokers and non-smokers in patients with schizophrenia compared to non-psychiatric controls may increase our understanding of the mechanisms contributing to co-morbid tobacco addiction in schizophrenia.
Methods: In an on-going study, we have recruited 19 patients with schizophrenia (smokers $N=9$; non-smokers $N=10$) and 13 non-psychiatric controls (smokers $N=8$; non-smokers $N=5$). All subjects completed the verbal N-back task administered at the 1- and 3-back working memory load while electroencephalography (EEG) was collected with a 64-electrode cap and DC-coupled EEG system at 1000 Hz with a 0.3–200 bandpass filter. EEG data was processed offline using MATLAB 7.04 (The Mathworks, Inc. Natick, MA, USA) and the EEGLAB toolbox custom scripts developed by Co-author RZ. Oscillatory activity was measured from the frontal electrodes (AF3/4, F1/2, F3/4, F5/6, and F7/8) encompassing the DLPFC for correct responses to targets. EEG signals were filtered into theta (4–7 Hz) and gamma (30–50 Hz) frequencies with a zero-phase shift and then Hilbert transformed to separate phase and amplitude of the signal. Gamma power was calculated and averaged across the entire epoch from −1000 to 3095 relative to stimulus onset. Theta-gamma coupling which evaluates the relationship between the phase of theta and amplitude of gamma was measured using the Modulation Index (MI). With this method, the phase of theta is binned into 18 (20° intervals) and mean gamma amplitude of each bin is calculated and normalized. MI is a measure of the divergence of the phase-amplitude distribution from the uniform distribution (MI = 0). The greater MI away from 0, the greater the coupling.
Results: Notable differences were observed in the 3-back, but not 1-back, conditions. Reduced gamma power was observed in smokers compared non-smokers with schizophrenia (Cohen's $d=-1.0$) though gamma power was still greater in these patients compared to controls who were non-smokers ($d=-0.51$). Evaluation of 3-back theta-gamma coupling showed reduced coupling in controls who were smokers compared to non-smokers ($d=1.8$), while the opposite effect was found in patients ($d=-0.76$). Patients also demonstrated increased coupling regardless of smoking status (smokers $d=-1.3$; non-smokers $d=0.85$) compared to the controls.
Conclusions: Our preliminary findings suggest that oscillations mediating working memory are modulated both by smoking status and the diagnosis of schizophrenia. Further understanding of these mechanisms may have implications for tobacco assessment and treatment in people with and without schizophrenia.
Supported in part by Canadian Institute of Health Research (CIHR) operating grant MOP#115145 to Dr George.
Keywords: schizophrenia, smoking, working memory, oscillations.
Disclosures: M. Barr, Nothing to Disclose; K. Mackowick, Nothing to Disclose; R. Zomorrodi, Nothing to Disclose; V. Wing, Nothing to Disclose; C. Wass, Nothing to Disclose; Z. Daskalakis, **Part 1:** Roche Advisory Panel, **Part 3:** Brainsway Inc Operating; T. George, **Part 1:** Data Monitoring Committee (DMC) for Novartis, **Part 3:** Pfizer Global Research.

W28. Baseline Neurocognitive Predictors of Responders to Treatment at 12 Month Follow-up for Major Depressive Disorder by Deep Brain Stimulation

Shane McInerney, Sakina Rizvi, Heather McNeely, Helen S Mayberg, Lozano Andres, Peter Giacobbe, Joseph Geraci, Sidney Kennedy*

University Health Network, Toronto, Ontario, Canada

Background: Advances in understanding the neurobiology and brain circuitry of depression are driving novel treatment approaches for patients with treatment-resistant depression (TRD). Deep brain stimulation (DBS) represents an adjustable and reversible method of focally modulating the activity of dysfunctional brain circuits with electrical stimulation. It has been reported that deep brain stimulation (DBS) of the subcallosal cingulate gyrus (SCG) may provide benet in treatment-resistant depression (TRD). In a previous study on this sample, several areas of cognition that were below average or impaired at baseline improved over 12 month follow-up. These changes were not correlated with improvements in mood. We now report the results of a

one-year follow-up of neurocognitive measures to assess baseline predictors of cognitive functioning on response to DBS treatment for depression at 12 month follow up period.
Methods: Twenty patients with TRD underwent neurocognitive assessments before and after SCG Deep Brain Stimulation. All patients met criteria for major depressive disorder (MDD) and were in a current major depressive episode (MDE) and had a minimum score of 20 on the 17-item Hamilton Rating Scale for Depression (HRSD-17). We determined the response (50% or greater reduction in the 17-item Hamilton Rating Scale for Depression [HRSD-17]) after surgery. Performance in a battery of neuropsychological assessments tapping 4 domains of frontal lobe function and general cognitive abilities were completed before implantation and at 12 months post-onset of continuous DBS in 20 TRD patients.
Results: There were no significant differences between responder and non-responders in relation to age, gender, duration of depressive episode or number of episodes. 55% ($N=11$) responded to DBS using the HRSD-17. Using non-parametric testing, Finger Tap test dominant hand ($Z=-2.1$, $P=0.035$), non-dominant hand ($Z=-2.6$, $P=0.007$) and Wisconsin Card Sorting Test total score ($Z=-2.0$, $P=0.035$) at baseline were predictive of response to DBS treatment at 12 month follow up.
Conclusions: Subcallosal cingulate gyrus DBS likely acts by modulating brain networks whose dysfunction leads to depression. This study suggests that DBS is relatively safe and provides signicant improvement in some patients with TRD.DBS treatment for depression may provide positive benefits in relation to executive and motor functioning to those suffering from major depressive disorder.
Keywords: deep brain stimulation, depression, neurocognitive testing, response to treatment.
Disclosures: S. McInerney, Nothing to Disclose; S. Rizvi, Nothing to Disclose; H. McNeely, Nothing to Disclose; H. Mayberg, Nothing to Disclose; L. Andres, Nothing to Disclose; P. Giacobbe, Nothing to Disclose; J. Geraci, Nothing to Disclose; S. Kennedy, Nothing to Disclose.

W29. Relationship Between Frontal P300 Event-related Potential and Brain Glutamine/Glutamate Ratio Measured *In Vivo*

Mei-Hua Hall*, Jordan W Smoller, Deborah L Levy, Mary Lohan, Kevin M Spencer, Dost Ongur

Harvard Medical School, Belmont, Massachusetts

Background: Deficits of the P300 event related potential (ERP) are one of the most replicated neurobiological abnormalities in schizophrenia. However, the neurochemistry associated with the P300 deficit is unclear. Because gamma-amino butyric acid (GABA) and glutamate (Glu) are the primary inhibitory and excitatory neurotransmitters in the brain and putatively play a role in information processing and the pathophysiology of schizophrenia, it is reasonable to hypothesize that brain GABA and glutamate levels may be related to features of the P300 ERP responses. Previous MRS studies have show that the Gln/Glu ratio in particular is an index of glutamate neurotransmission. We investigated the relationship between P300 ERP, Gln/Glu, and GABA concentrations measured *in vivo* with proton magnetic resonance spectroscopy (^{1}H MRS). We hypothesized that higher GABA and Gln/Glu levels would associate with larger P300 amplitude and shorter P300 latency.
Methods: Target P300 ERPs measured at the Fz and Pz sites were collected from 22 healthy participants who performed an auditory oddball task (20% 1000 Hz target tone, 80% 1500 Hz standard tone). Measures of GABA and Gln/Glu ratio were obtained on a 4 Tesla MR scanner in anterior cingulate (ACC) and posterior cingulate (POC) cortices. Linear regression and partial correlations were used for statistical analysis.
Results: Controlling for age, sex, and the time difference between EEG and MRS testing dates, a significant positive correlation was found between P300 amplitude at Fz and glutamine/glutamate ratio (but not GABA) in ACC (partial $R=0.49$; $P=0.03$). No significant relationship between P300 amplitude and latency at Pz and the glutamate or GABA levels in the parietal cortex were detected.
Conclusions: These results indicate a specific connection between glutamate neurotransmitter concentration in ACC and frontal amplitude of P300 response, providing a novel insight into the relationship between the neurochemical and neurophysiological processes underlying cognition. Baseline glutamate neurotransmission may be the underlying substrate for P300 generation, and its abnormalities may explain the abnormalities seen in P300 in schizophrenia.
Keywords: event related potential, MRS, neurophysiology, glutamte and GABA neurotransmission.
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W30. Ethnic Differences in Physiological Responses to Fear Conditioned Stimuli

Karen G Martinez*, Jose A Franco, Mohammed R Milad, Gregory J Quirk

University of Puerto Rico, San Juan, Puerto Rico

Background: There is increasing evidence that people with anxiety disorders show exaggerated fear responses during experimental fear conditioning, in which a visual cue is paired with a mild shock to elicit increases in skin conductance responses (SCR). Subjects' sex and age have been shown to affect SCR, but it is unclear if there are ethnic differences in fear responses. We therefore compared SCRs of healthy Caucasians with those of healthy Hispanics in Puerto Rico, during experimental fear learning and extinction.
Methods: SCRs during a fear learning and extinction protocol were obtained at two sites: University of Puerto Rico School of Medicine (PR) and Harvard School of Medicine (Bos.) using the same methodology. A total of 78 healthy subjects (39 Hispanics from PR and 39 Caucasians from Bos.) were matched for sex, age, and educational level. All subjects were evaluated with the SCID and presented no psychopathology. Subjects were trained in an established fear conditioning and extinction paradigm (Milad, *et al*, 2005), employing visual CS+ and CS− stimuli. On day 1, subjects received habituation, conditioning, and extinction trials. On day 2, subjects were tested for memory of

extinction (recall), and contextual renewal. ANOVAs with repeated measures were used to evaluate ethnic and sex differences in SCRs. When significant, Tukey *post-hoc* tests were performed for individual trials.

Results: The baseline skin conductance level (SCL) was significantly higher in the PR sample compared to the Bos. Sample, in both females (Bos. females = 2.17; PR females = 4.34; $t_{(1,38)} = 3.56$, $p = 0.001$) and males (Bos. males = 4.38; PR males = 7.55; $t_{(1,36)} = 2.19$, $p = 0.034$). There were no ethnic or sex differences to the unconditioned responses to the shock. Despite the difference in initial SCL values, PR and Bos. females did not significantly differ in their responses to the CS + or CS− during the any phase of fear conditioning and extinction. In contrast, PR males showed significantly higher CS + responses than Bos. males in multiple phases: habituation (main effect of ethnicity [$F_{(1,37)} = 9.787$, $p = 0.034$]), early extinction (effect of ethnicity [$F_{(1,37)} = 8.138$, $p = 0.007$] and trial [$F_{(4,37)} = 9.116$, $p < 0.001$]), late extinction phase (effect of ethnicity: [$F_{(1,37)} = 5.545$, $p = 0.024$]), recall of extinction (main effect of ethnicity [$F_{(1,37)} = 7.126$, $p = 0.011$] and trials [$F_{(4,37)} = 3.259$, $p = 0.014$]), and renewal of conditioning (main effect of ethnicity [$F_{(1,37)} = 11.224$, $p = 0.002$] and trials [$F_{(4,37)} = 17.332$, $p < 0.001$]). The two ethnicities did not show significant differences in differential learning (CS + minus CS−), for either males or females. Compared to Bos. males, PR males did not show increased anxiety (State/Trait anxiety inventory), nor did they choose significantly different levels of shock intensity.

Conclusions: In comparison to Caucasian males, Hispanic males showed increased fear responses in most phases of conditioning and extinction. Because no differences were observed between females, this difference in males is unlikely due to any procedural differences between sites. Subtracting out the differences between males observed during the habituation phase, which precedes conditioning, eliminated significant differences in all subsequent phases, suggesting a heightened response to novelty rather than heightened fear learning. Our findings are consistent with the increased risk for post-traumatic stress disorder (PTSD) in Hispanic combat veterans (Lewis-Fernandez, *et al*, 2008) and suggest that Hispanic males, at least those living in Puerto Rico, show augmented physiological reactivity.

Keywords: fear learning, anxiety, ethnic differences, sex differences, biological markers.

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W31. Preventing the Return of Fear Using a Retrieval + Extinction Reconsolidation Update Mechanism: The Integration of Fear-potentiated Startle and US-expectancy Ratings

Seth D Norrholm*, Victor T Warren, Kemp Anderson, Cliffe Kwon, Lauren Bosshardt, Tanja Jovanovic, Bekh Bradley, Kerry J Ressler

Atlanta VA Medical Center, Atlanta, Georgia

Background: Disruption of the reconsolidation of conditioned fear memories has been suggested as a non-pharmacological means of preventing the return of learned fear in human populations. A reconsolidation update paradigm was developed in which a reconsolidation window is opened by a single isolated retrieval trial of a previously reinforced CS + which is then followed by extinction training within that window (Monfils *et al*, 2009; Schiller *et al*, 2010). However, follow-up studies in humans using different methods fear conditioning indices (eg, fear-potentiated startle, skin conductance, US-expectancy) have failed to replicate the retrieval + extinction effects (eg, Soeter and Kindt, 2013).

Methods: In the present study, we further investigated the retrieval + extinction reconsolidation update paradigm by directly comparing the acquisition, extinction, and return of fear-potentiated startle. Startle was measured through electromyographic activity of the *orbicularis oculi* muscle. The conditioned stimuli (CSs) were geometric shapes presented on a computer screen and the unconditioned stimulus (US) was a 250-msec, 140 p.s.i. airblast directed at the larynx as in our previous work (eg, Norrholm *et al*, 2013). Fear conditioning sessions occurred in the absence or presence of US-expectancy measures (using a trial-by-trial response keypad) with and without retrieval of a previously acquired CS-US association. The experiment occurred over three consecutive days. On Day 1, participants were fear conditioned to two distinct CS + 's (CS + a, CS + b). Twenty-four hours later, one group (Retrieval) was administered one of the previously reinforced CS + 's (CS + a) as a single, isolated retrieval trial before extinction training. A second group (No Retrieval) did not receive a retrieval trial and was extinguished as usual. Extinction training (to both CS + 's) was followed 24 h later by an Extinction Test for fear recovery, Re-extinction, and finally a test of fear Reinstatement.

Results: The results show that the inclusion of US-expectancy measures strengthens the CS-US association to provide enhanced fear conditioning (Keypad x Trial Type interaction; $F(2, 106) = 7.41$, $p < 0.001$) and sustained expression of fear memories over the experimental sessions (Main effect of Keypad; $F(1, 50) = 11.44$, $p = 0.001$). In addition, in the groups that used on-line US-expectancy measures, the retrieval + extinction procedure reduced reinstatement of fear-potentiated startle (Main effect of Reinstatement; $F(1, 8) = 6.56$, $p = 0.03$) as compared to the group that did not receive a retrieval trial ($p > 0.1$). The retrieval + extinction attenuation of reinstatement generalized to both previously reinforced CS + 's regardless of whether the CS + was used in the retrieval trial.

Conclusions: These data support the further investigation of reconsolidation update mechanisms as a potential non-pharmacological intervention for reducing relapse in anxiety disorders such as posttraumatic stress disorder (PTSD). The observed generalization of reinstatement blockade by retrieval + extinction has compelling clinical implications in that traumatic experiences can often generalize to multiple cues, yet reactivation of a single cue may significantly reduce re-experiencing symptoms.

Keywords: fear extinction, reconsolidation, reinstatement, startle.

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W32. Neural Correlates of Relational and Item-specific Encoding and Retrieval (RiSE) in Schizophrenia

J Daniel Ragland*, Charan Ranganath, Deanna M Barch, James M Gold, Michael P Harms, Evan Layher, Angus W MacDonald, Joshua Phillips, Andrew Poppe, Steve M Silverstein, Dennis Thompson, Cameron S Carter

University of California Davis, Sacramento, California

Background: The RiSE was developed as part of a multi-site Cognitive Neuroscience Task Reliability and Clinical Applications Consortium (CNTRACS) to provide a time-efficient task (20 min) to dissociate specific encoding and retrieval processes and enhance discovery of pro-cognitive interventions in clinical trials research. A previous study (Ragland *et al*, 2012) established that the RiSE is valid and reliable, well tolerated, related to functional capacity, and has good psychometric characteristics and alternative forms to facilitate treatment studies. It also revealed a three-way interaction in which memory performance in patients with schizophrenia was most preserved under conditions promoting item-specific encoding and familiarity-based recognition and was most impaired under relational encoding and recollection-based retrieval conditions. The goal of the current study was to use fMRI to identify neural correlates of these relative memory strengths and weaknesses.
Methods: As part of a multi-site study, the RiSE was administered during event-related BOLD fMRI to a demographically matched sample of 50 patients with schizophrenia, and 55 healthy controls. Participants were visually presented with a series of 54 pairs of common visual objects, and asked to alternate between two encoding conditions: during item-specific encoding, participants indicated whether either object in the pair was living; during relational encoding, participants indicated whether one of the objects in the pair could fit inside of the other object in the pair. This was followed by two recognition memory tasks: during item recognition, all 108 previously studied objects were presented one at a time together with an equal number of new objects and participants indicated whether the object was previously studied; during associative recognition, participants viewed 27 original and 27 new object pairings (of previously seen objects), and were required to determine whether or not the object pairing was new. fMRI data were pre-processed using standard procedures with the FMRI Expert Analysis Tool (FEAT) in the FMRIB Software Library (FSL version 4.1). Statistical analysis was performed using the general linear model in FEAT, with research site added as a co-variate to account for any site differences. Contrast images were calculated to identify neural correlates of retrieval success (item recognition hits minus misses) for each group and each encoding condition. Resulting statistical maps were cluster corrected for multiple comparisons at $p<0.05$, with a height threshold of $p<0.01$ ($Z=2.3$).
Results: Recognition accuracy (d') was impaired in patients across encoding and retrieval conditions, with a trend-level interaction between group and encoding condition for the item recognition task [$F(1, 103)=3.09$, $p=0.08$]. This trend was consistent with the previously observed disproportionate patient impairment in relational *vs* item-specific memory. fMRI results were consistent with these behavioral findings. Controls had greater activation than patients in memory specific medial temporal lobe regions (right hippocampus and parahippocampal gyrus) when successfully retrieving (ie, hits > misses contrast) objects that had undergone relational encoding. In addition, when participants made errors following relational encoding (ie, misses > hits contrast), controls showed greater activation than patients in a set of bilateral prefrontal regions (inferior and middle frontal gyrus, anterior cingulate gyrus) often associated with error detection. Conversely, in the item encoding condition, there were no group differences observed in medial temporal or prefrontal activation during either successful or unsuccessful item recognition.
Conclusions: Previous work on the RiSE revealed that it is a reliable, time-efficient and easy to administer task that can dissociate specific memory encoding and retrieval processes, and reveal patterns of relative memory strengths in item-specific memory and disproportionate weaknesses in relational memory in patients with schizophrenia. Initial fMRI findings indicate that it can also be easily administered to patients during neuroimaging. fMRI results support neural construct validity in revealing predictable mnemonic and error detection results in medial temporal and prefrontal brain regions. Moreover, fMRI results paralleled behavioral findings in revealing group differences during item recognition primarily in the relational and not in the item-specific memory condition. These combined findings suggest that the RiSE is a promising behavioral and imaging biomarker of declarative memory that may facilitate discovery of cognitive enhancing interventions in clinical trials research.
Keywords: declarative memory, episodic memory, functional neuroimaging, imaging biomarker.
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W33. Differential Effects of Aging and High Fat Diet on Cognitive Function in Mice

James Kesby*, Svetlana Semenova, Jane J Kim, Jerrold M Olefsky, Cristian L Achim, Virawudh Soontornniyomkij, Benchawa Soontornniyomkij, Dilip V Jeste

University of California San Diego, La Jolla, California

Background: Aging is associated with a decline in multiple aspects of cognitive function. Evidence suggests that high fat diet (HFD) consumption and presence of metabolic dysfunction lead to exacerbated brain aging and promote the development of cognitive deficits. The present work investigated whether exposure to HFD accelerates age-related cognitive deficits in adult *vs* aged mice.
Methods: Adult (6-month old; $n=32$) and aged (16-month old; $n=32$) C57BL/6J male mice were exposed to regular diet or HFD (60% fat) for 3 months prior to behavioral testing and maintained on the HFD during testing for an additional 2 months. Mice were tested in a battery of

cognitive tests including the novel object and novel location recognition tasks, and the Barnes maze. In addition, locomotor activity was assessed.

Results: HFD resulted in a significant body weight gain with adult mice gaining significantly more weight (72% increase from baseline body weight) than aged mice (35% increase from baseline body weight). Weight gain was attributed to food calories sourced from fat and cholesterol, but not total calorie intake. Both age and exposure to HFD decreased locomotor activity. In the novel object recognition task, all mice showed similar discrimination index, indicating no effect of age or exposure to HFD on novel object recognition memory. In the novel location recognition task, independent of exposure to HFD, adult mice showed significant increased discrimination index for the novel location (mean ± SEM; 0.26 ± 0.04 test trial *vs* -0.04 ± 0.04 familiarization trial, $p < 0.001$); whereas, aged mice did not positively discriminate the novel location (mean ± SEM; 0.11 ± 0.07 test trial *vs* 0.01 ± 0.04 familiarization trial, *n.s.*), indicating age-related deficits in spatial memory. During the 5-day Barnes maze task acquisition (4 trials per day), mice exposed to HFD exhibited a slower rate of improvement (ie, decreasing errors across days of training) compared to mice exposed to regular diet suggesting HFD-induced deficits in spatial learning.

Conclusions: The results indicate that both age and exposure to HFD produced deficits in cognitive function mediated by the hippocampus but the observed deficits were task-dependent. Specifically, exposure to HFD impaired novel location recognition memory; whereas aging was associated with impaired learning and memory in the Barnes maze. Novel object recognition memory mediated by perirhinal cortex was unaffected by age or exposure to HFD. These findings highlight the compelling link between age, diet-induced obesity, and cognitive impairments. Ongoing studies have been designed to determine the neuropathologic and metabolic mechanisms underlying the effects of aging and obesity on cognitive function.

Keywords: aging obesity cognition learning mice.

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W34. Age-at-Onset and Cognitive Control-related Brain Circuitry in First Episode Schizophrenia

Tara Niendam*, Emilio Ferrer, Tyler A Lesh, Stefania Ashby, Marjorie Solomon, J Daniel Ragland, Jong Yoon, Michael J Minzenberg, Cameron S Carter

University of California Davis, Sacramento, California

Background: Examining the impact of age at onset on brain function in schizophrenia may elucidate pathophysiological mechanisms contributing to individual differences in clinical course and outcome. Using fMRI and an established measure of cognitive control (AXCPT), we examined relationships between age at onset and prefrontal network functioning across ages 12–24 years in individuals with first episode schizophrenia (SZ) and healthy controls (HC). At baseline, across the span of age at onset, we predicted that first episode SZ participants would show poor performance (relative to controls) in conjunction with reduced DLPFC activation under conditions of high cognitive control. We also hypothesized that earlier age at onset would be associated with poorer cognitive control performance than later age at onset.

Methods: HC ($n = 82$) and First Episode SZ ($n = 73$) participants from the UC Davis Early Psychosis Programs were identified using the Structured Clinical Interview for DSM-IV (SCID-I/P). Participants completed the AX-CPT during fMRI on a 1.5 Tesla scanner at baseline. D'-context was used as the primary measure of participants' ability to utilize context to guide response selection on the AX-CPT. Behavioral differences between groups were examined with ANOVA using SPSS 21. Functional MRI data were processed using SPM8 and focused on between-groups contrasts of the cue phase of high control (B cue) *vs* low control (A cue) trials, representing proactive control processes. Whole-brain voxel-wise and *a priori* DLPFC region of interest analyses were performed. Llinear contrasts across age bins identified activity associated with increasing age at onset.

Results: Consistent with previous findings with the AXCPT, First Episode SZ show impaired performance (d'context, $p < 0.05$) and lower activation in the DLPFC and parietal regions ($p < 0.05$ FWE) when compared to HCs under conditions of high cognitive control. When age at onset is considered, First Episode SZ continued to show a consistent pattern of impaired cognitive control performance across the age range. Both SZ and HCs showed better cognitive control performance as age increased. A linear age contrast across both groups showed increased DLPFC activity with increasing age ($p < 0.005$ uncorrected). Within groups, HCs show a pattern of increased DLPFC activity with increasing age, but SZs do not.

Conclusions: Individuals with SZ show impaired cognitive control associated with reduced DLPFC activation when compared to HCs, regardless of the age of illness onset. SZ cognitive control performance is higher with later age at onset, suggesting that continued cognitive development prior to illness onset somewhat mitigates cognitive control dysfunction. However, it is not clear when the impairment in cognitive control and associated changes in DLPFC function appears in illness course. Longitudinal imaging and clinical are ongoing and data will be presented to determine if SZ cases show improvement, deterioration or stability in cognition over time that is associated with clinical or functional outcome as well as how this varies with age of onset.

Keywords: schizophrenia, psychosis, cognitive control, prefrontal, development.

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W35. Ecologically-valid Assessment of Attention in Schizophrenia in a Virtual Environment

George Foussias*, Ishraq Siddiqui, Krysta McDonald, Eliyas Jeffay, Konstantine K Zakzanis, Gary Remington

Centre for Addiction and Mental Health, Toronto, Ontario, Canada

Background: Among the constellation of cognitive impairments that characterize schizophrenia, attentional deficits have been identified as a reliable and enduring feature of the illness. In addition, attentional deficits have shown relationships to functional outcomes in schizophrenia, and have been identified as one important domain for the focus of neurobiological and treatment research. However, assessments of attention to date have relied on paradigms using abstract targets and tasks that have limited direct relevance to everyday life. In recognition of this, we sought to evaluate a novel strategy for evaluating attention deficits in schizophrenia in an ecologically valid manner. To achieve this, we used a virtual reality (VR) environment that resembles a typical factory setting, whereby participants function as quality inspectors that must identify defective or incorrect objects on conveyor belts to either side of them. Within this environment, different task parameters enable the examination of selective attention (SA), divided attention (DA), and alternating attention (AA).
Methods: Stable outpatients with schizophrenia (SZ) and healthy controls (HC), between the ages of 18 and 55, with no other Axis I disorders or current substance abuse or dependence were recruited for this study. All participants underwent clinical assessments for positive and negative symptom severity using the Scales for the Assessment of Positive Symptoms (SAPS) and Negative Symptoms (SANS), motivational deficits with the Apathy Evaluation Scale (AES), and depression with the Calgary Depression Scale for Schizophrenia (CDSS). Cognitive function was assessed using the Brief Assessment of Cognition in Schizophrenia (BACS), as well as the Trail Making Test (TMT) A & B. Community functioning was evaluated using the Quality of Life Scale (QLS). Neurologic side effects from medications, and video game experience were also evaluated. Subsequently, participants were administered the VR conveyor belt tasks.
Results: We recruited a total of 68 participants (37 SZ and 31 HC) for this study. Examination of performance on the VR attention tasks revealed that SZ participants exhibited significant deficits in DA and AA tasks compared to HC participants ($z=-4.28$, $p<0.001$, and $z=-4.82$, $p<0.001$, respectively), although with no difference in the SA task. Moreover, DA and AA task performance was significantly correlated with global cognitive performance as assessed by the BACS (rho $=0.583$, $p<0.001$; and rho $=0.635$, $p<0.001$, respectively), as well as with TMT performance (TMT A: rho $=-0.521$, $p<0.001$, and rho $=-0.482$, $p<0.001$, respectively; TMT B: rho $=-0.486$, $p<0.001$, and rho $=-0.523$, $p<0.001$, respectively). Further, DA and AA task performance was found to be significantly correlated with community functioning as measured by the QLS (rho $=0.386$, $p=0.001$, and rho $=0.505$, $p<0.001$, respectively). Finally, relationships between DA and AA task performance and community functioning remained significant after controlling for severity of motivational deficits and video game experience.
Conclusions: In an effort to improve the ecological validity of tests of attention in schizophrenia, we evaluated a novel strategy for evaluating attention in a virtual environment using a series of conveyor belt tasks. We found that performance in the VR attention tasks exhibited a relationship with standard tests of cognitive ability and specifically attention. However, variance in performance on these tasks was not entirely captured by standard paper-and-pencil tasks, suggesting that these VR tasks measure attentional performance beyond what is capable in standard tests. Further, the relationship between VR task performance and community functioning offers some support for the predictive validity of this approach. Finally, individuals with schizophrenia exhibited impairments in divided and alternating attention compared to healthy control participants, although with no difference in selective attention. Overall, these findings offer preliminary support for the use of this ecologically valid assessment strategy for attentional deficits in schizophrenia, which may enable the neurobiological evaluation of attentional processes under conditions resembling daily life.
Keywords: schizophrenia cognitive impairment attention deficits virtual reality ecological validity.
Disclosures: G. Foussias, Nothing to Disclose; I. Siddiqui, Nothing to Disclose; K. McDonald, Nothing to Disclose; E. Jeffay, Nothing to Disclose; K. Zakzanis, Nothing to Disclose; G. Remington, Nothing to Disclose.

W36. Effects of Amphetamine on Encoding and Retrieval of Memory for Emotional Stimuli

Jessica Weafer*, David Gallo, Harriet de Wit

University of Chicago, Chicago, Illinois

Background: Stimulant drugs improve memory in animals when administered at either encoding or retrieval. However, little is known about effects of stimulants on memory in humans. In this study we investigated the effects of d-amphetamine (AMP) on memory for emotional material in healthy young adults under the following conditions: when the drug was administered at encoding, at retrieval, or at encoding and retrieval. We hypothesized that AMP would enhance memory for emotional material in all conditions.
Methods: Participants attended an encoding session in which they viewed standardized positive, neutral, and negative pictures from the International Affective Picture System (IAPS; Lang *et al*, 1999). Exactly 48 h later they attended a retrieval session testing their memory of these stimuli. Participants were randomly assigned to one of four conditions ($N=20$ each): Condition AP (20 mg AMP at encoding and placebo (PL) at retrieval); Condition PA (PL at encoding and AMP at retrieval); Condition AA (AMP at encoding and retrieval); or Condition PP (PL at encoding and retrieval). Conditions AP, PA, and AA were individually compared to Condition PP. Conditions AP and PA were compared to Conditions AA and PP to test if memory accuracy was state-dependent.
Results: AMP did not affect memory for positive, negative, or neutral stimuli when administered at encoding

(AP compared to PP; $ps > 0.25$); retrieval (PA compared to PP; $ps > 0.13$); or at both encoding and retrieval (AA compared to PP; $ps > 0.53$). Memory accuracy was not state-dependent (AP and PA compared to AA and PP, $ps > 0.20$).
Conclusions: Contrary to hypotheses, AMP did not improve memory for emotional material when administered at encoding, retrieval, or both; nor was memory accuracy state-dependent. These findings do not support the use of AMP to improve memory. This lack of effect on memory may inform casual users, who take non-prescribed stimulants as study aids in academic settings.
Keywords: amphetamine, memory, encoding, retrieval, state-dependent.
Disclosures: J. Weafer, Nothing to Disclose; D. Gallo, Nothing to Disclose; H. de Wit, Nothing to Disclose.

W37. Training the Brain to Abstain from Alcohol: Towards the Neural Basis of Approach/Avoidance Training Effects in Hazardous Drinkers

Charles Taylor*, Akanksha Shukla, Karalani Cross, Nader Amir, Murray B Stein, Martin Paulus

University of California San Diego, La Jolla, California

Background: A central defining feature of alcohol dependence is the inability to control alcohol use despite awareness of harmful consequences. Research suggests that addictive behaviors are maintained in part by an imbalance between (1) relatively strong automatic approach-oriented processes for drug-relevant stimuli that are governed by brain systems involved in reward processing (eg, striatum) and (2) diminished regulatory processes to control drug urges that are governed by brain systems involved in monitoring and resolution of conflict and cognitive control (eg, anterior cingulate cortex, ACC). Recently, researchers have developed a novel, computerized procedure (Approach-Avoidance Training, AAT) that trains individuals to selectively avoid stimuli that are associated with alcohol use, thereby overriding habitual approach tendencies for alcohol-relevant cues. Initial evidence suggests that this training can improve an individual's ability to abstain from alcohol use compared to treatment-as-usual and may result in fewer individuals developing significant long-term problem use (eg, Wiers *et al*, 2011). However, the neurobiological mechanisms that account for such effects have yet to be delineated. As a first step toward addressing this issue, we combined a well-established computerized approach/avoidance training procedure with functional brain imaging to investigate how modifying implicit approach responses for alcohol-related cues affects brain mechanisms that influence vulnerability to relapse.
Methods: To date, $n = 11$ community volunteers endorsing hazardous alcohol use behaviors [Alcohol Use Disorders Identification Test (AUDIT) $\geqslant 8$] have completed the study procedures. Participants took part in a single functional magnetic resonance imaging (fMRI) session during which they completed a well-established alcohol cue reactivity task before and after an approach/avoidance training (AAT) procedure. The alcohol cue reactivity task presented participants with pictures of alcohol *vs* non-alcohol beverages while changes in the blood-oxygen-level dependent (BOLD) contrast were obtained. Previous studies demonstrate that individuals with alcohol-related problems tend to display exaggerated brain responses to alcohol stimuli in reward-related (eg, striatum), limbic (eg, amygdala and insula), and prefrontal regions (eg, ACC). During the computerized AAT, participants responded to pictures of alcohol and non-alcohol beverages by pulling a joystick toward themselves (approach) or pushing it away from themselves (avoid). Participants were randomly assigned to complete an AAT designed to facilitate implicit avoidance of alcohol stimuli by pushing alcohol stimuli away from themselves on the majority of trials (avoid-alcohol AAT; $n = 6$) or a sham AAT ($n = 5$) in which there was no contingency between the arm movement and alcohol stimuli (ie, 50% push; 50% pull). The fMRI analysis focused on examining changes from before to after training in the contrast (% signal difference) between processing of alcohol *vs* non-alcohol pictures. Given the small preliminary sample, we report effect size statistics illustrating between-group differences in brain activation to alcohol stimuli following training in several *a priori* regions of interest following prior research.
Results: Participants in the avoid-alcohol AAT group displayed larger reductions in activation from pre- to post-training in the putamen, insula, amygdala, and ACC during processing of alcohol stimuli relative to participants in the sham training group. Between-group covariance (baseline) adjusted effect sizes at post-training revealed medium to large effects of avoid-alcohol training on brain activation during alcohol cue processing (putamen, $d = 1.54$; insula: $d = 2.31$; amygdala: $d = 0.44$; ACC: $d = 2.01$). Planned analyses in the final sample will examine whether changes in neural activation on the alcohol cue reactivity task from pre- to post-training mediate between-group differences in subjective alcohol craving at post-assessment.
Conclusions: Our preliminary evidence suggests that approach/avoidance training alters activity in brain regions that are important for modulating the processing of alcohol-relevant stimuli and that have been implicated in increasing vulnerability to relapse. Specifically, decreased activation in reward, limbic, and prefrontal neurocircuitry following training may reflect reduced salience of alcohol cues as well as enhanced efficiency of neural processing resources needed to monitor and regulate the urge to use alcohol. Limitations of the present study include the use of a non-treatment-seeking sample, small sample size, and lack of follow-up assessments. The current findings support the notion that integrating computerized cognitive training procedures with fMRI may provide valuable information about the plasticity of neural systems that regulate the processing of alcohol-relevant stimuli. This study represents an initial step to identify new ways to 'train the brain' that can be used to improve existing interventions for substance use disorders and other psychiatric conditions characterized by approach/avoidance system deficits.
Keywords: translational; approach avoidance training; alcohol; cognitive bias modification; functional neuroimaging.
Disclosures: C. Taylor, Nothing to Disclose; A. Shukla, Nothing to Disclose; K. Cross, Nothing to Disclose; N. Amir, **Part 1:** Dr Nader Amir has a financial interest in Cognitive

Retraining Technologies, LLC, which has licensed elements of the technology described in this study for commercial purposes.; M. Stein, Nothing to Disclose; M. Paulus, Nothing to Disclose.

W38. Steady-state Gamma-band Responses in Children with Autism Spectrum Disorders During an Auditory Oddball Task

Kristina L McFadden*, Sarah Steinmetz, Susan Hepburn, Jason Tregellas, Donald Rojas

University of Colorado Denver, Aurora, Colorado

Background: Synchronous neuronal activity in the gamma range (30–80 Hz) has been shown to be abnormal in individuals with autism spectrum disorders (ASD) and could be a biological marker for the disorder. While previous studies have found gamma-band abnormalities in response to passive stimuli in ASD, gamma-band activity in ASD has not been studied during tasks that require sustained attention. The current study assessed gamma-band activity in children with ASD compared to control children during a deviant detection task (auditory oddball) to determine how attention impacts gamma-band abnormalities in ASD.
Methods: MEG recordings were obtained for a group of children with ASD ($N=10$) and a group of healthy control children ($N=17$) during completion of an auditory oddball task. During this task, participants were asked to continuously attend to the task and to respond via button press to rare 'oddball' tones (1500 Hz sine waves, 25% of stimuli) and not to respond to the 'frequent' tones (1000 Hz sine waves, 75% of stimuli). All tones were amplitude-modulated at 40 Hz to elicit an auditory steady-state response in the gamma-band range. Participants completed a total of 600 trials, with 150 'oddball' and 450 'frequent' tones. Evoked steady-state gamma-band power was measured in the left and right auditory cortex, inferior prefrontal cortex (PFC), and medial PFC, using source-space projection and time-frequency analysis.
Results: Compared to the control group, the ASD group demonstrated significantly increased steady-state evoked gamma-band responses (200–500 ms) to both rare and frequent stimuli in medial PFC (right and left hemispheres), inferior PFC (left), and auditory cortex (right and left), $p<0.05$. The ASD group also showed increased evoked responses compared to controls to frequent stimuli in right inferior PFC, $p<0.05$. However, while the control group consistently demonstrated increased evoked steady-state gamma-band responses to rare compared to frequent stimuli ($p<0.05$), responses in the ASD group did not significantly differ between stimulus type.
Conclusions: As expected from an auditory oddball task, an increased steady-state evoked gamma-band response was observed during rare compared to frequent stimuli in the control group. The ASD group demonstrated increased responses to both stimuli compared to controls, and did not show the anticipated difference between frequent and rare stimuli. This suggests that the ASD group may have been less responsive to deviant detection than control subjects. It is possible that the increased response to frequent stimuli observed in ASD indicates that this group was already in a heightened state of neuronal response, reducing their ability to engage additional neuronal resources to attend to deviant stimuli. This could suggest that attention difficulties observed in ASD are indicative of disrupted inhibitory mechanisms in the brain (eg, impaired inhibitory neurotransmission).
Keywords: autism spectrum disorders; magnetoencephalography; steady-state response; gamma-band; auditory oddball.
Disclosures: K. McFadden, Nothing to Disclose; S. Steinmetz, Nothing to Disclose; S. Hepburn, Nothing to Disclose; J. Tregellas, Nothing to Disclose; D. Rojas, Nothing to Disclose.

W39. Variation in Serotonin Transporter Gene Predicts Neural Activation in a Response Inhibition Task

Ranjani Prabhakaran*, Roberta Rasetti, Ena Xiao, Bhaskar S Kolachana, Daniel R Weinberger, Venkata S Mattay, Karen F Berman

NIMH, Bethesda, Maryland

Background: Variation in the serotonin transporter gene, *5HTT* (*SLC6A4*, *SERT*), involving a variable number of repeats in the serotonin transporter-linked polymorphic region, has been associated with individual variation in personality traits, cognitive performance, and neural activation. Specifically, the *5HTT* short (*s*) allele has been associated with higher levels of anxiety and harm avoidance, increased amygdala reactivity to negative emotional stimuli, and increased risk for depression compared to the long (*l*) allele. Interestingly, recent work indicates that *s* allele carriers demonstrate better performance on tasks involving decision-making and attentional control compared to those homozygous for the (*l*) allele, suggesting potential beneficial effects of the *s* variant for some domains of cognition. However, the extent to which genetic variation in *5HTT* impacts neural activation during cognitive control tasks is largely unknown. In this study, we investigated the effects of variation in the *5HTT* gene on the neural mechanisms underlying response inhibition, a key aspect of cognitive control.
Methods: Healthy Caucasian volunteers ($N=70$; mean age = 30.5; 31 females) underwent fMRI scanning during a modified flanker task, including congruent, incongruent, neutral, and no-go conditions. During congruent, incongruent, and neutral trials, participants were asked to indicate the direction (right or left) of a centrally presented arrow via a button-press. In the no-go condition, pairs of X's were presented on either side of the central arrow, and participants were asked to withhold their motor response. Participants completed a total of 145 trials with the following number of each trial-type: 41 congruent 41 trials, 40 incongruent trials, 31 neutral trials, and 33 no-go trials. The contrast of correct no-go and congruent trials was used as an index of participants' response inhibition performance. Participants were genotyped for the *5HTT* (*SLC6A4*, *SERT*) polymorphism. Our sample included the following number of participants in each *5HTT* genotype group: 23 *l/l* homozygotes (14 females), 31 *s/l* heterozygotes (14 females)

and 16 *s/s* homozygotes (3 females). A full factorial analysis was conducted on first-level results to assess whether *5HTT* genotype groupsshowed differences in neural activation for the contrast of correct no-go and congruent trials. Pairwise analyses comparing genotype cohorts were conducted within a mask of those voxels showing the main effect of task for this contrast at $p<0.05$, FDR corrected. *l/l* homozygotes were compared to *s* carriers (*s/l* heterozygotes and *s/s* homozygotes combined), and *l/l* homozygotes were compared to *s/s* homozygotes. Age, sex, IQ, and no-go accuracy were included as nuisance covariates.

Results: Accuracy was high for no-go trials across all genotype groups (mean = 94.8%, *SD* = 5.2%), and no differences were observed between genotypes for accuracy on no-go trials (*l/l* homozygotes: mean = 95.9%, *s/l* heterozygotes: mean = 93.5%, *s/s* homozygotes: mean = 95.8%; $p=0.15$). However, differences in neural activation between genotypes were observed. Greater response inhibition-related neural activation was found for *l/l* homozygotes compared to *s* carriers in the insula bilaterally, the right inferior frontal gyrus, and left supramarginal gyrus at $p<0.05$, FDR corrected. A similar pattern of neural activation was observed when *l/l* and *s/s* homozygotes were compared: greater response inhibition-related neural activation for *l/l* homozygotes compared to *s/s* homozygotes was observed in the insula bilaterally, the right inferior frontal gyrus and left supramarginal gyrus at $p<0.05$, FDR corrected.

Conclusions: These data provide novel evidence that variation in the *5HTT* gene is associated with differential recruitment of neural regions involved in response inhibition. Our results suggest that individuals homozygous for the *l* allele have greater activation of neural regions involved in response inhibition in order to achieve the same level of behavioral performance as individuals carrying at least one *s* allele. These results provide support for an important contribution of *5HTT* genetic variation to individual differences in response inhibition-related neural activation.

Keywords: serotonin transporter gene, response inhibition, fMRI, flanker task, individual differences.

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W40. Fear Learning Deficits in Women with PTSD: Estrogen is Associated with Extinction of Fear-potentiated Startle but Not Dark-enhanced Startle

Ebony Glover*, Vas Michopoulos, Kristina B Mercer, Seth D Norrholm, Bekh Bradley, Kerry J Ressler, Tanja Jovanovic

Emory University School of Medicine, Atlanta, Georgia

Background: Although the prevalence of posttraumatic stress disorder (PTSD) is twice as high in women as it is in men, the role of estrogen in the risk for PTSD is not well understood. Using the fear-potentiated startle paradigm to measure conditioned fear, we have previously shown that PTSD is associated with fear extinction deficits. Using the dark-enhanced startle paradigm to measure nonspecific anxiety, we previously found a greater degree of dark-enhanced startle in women than men. To further understand the role of estrogen in conditioned and unconditioned fear regulation, the current study examined the influence of estrogen levels on both fear extinction learning and dark-enhanced startle in women with and without PTSD.

Methods: We first measured dark-enhanced startle and then fear-potentiated startle during fear conditioning and extinction in women. The study sample ($N=81$) was recruited from an urban, highly traumatized civilian population at Grady Memorial Hospital in Atlanta, Georgia. We assayed serum estrogen levels in naturally cycling women and used a median split to divide the sample into high and low estradiol (E2) groups. Seventeen of 41 women (41.5%) in the low E2 group and 15 of 40 women (37.5%) met criteria for PTSD in the high E2 group.

Results: The results showed that all groups had equivalent levels of fear conditioning and there was a significant increase in startle magnitude during the dark phase compared with the light phase. However, we found significant interaction effects between high *vs* low E2 groups and PTSD diagnosis [$F(1,71)=4.55$, $p<0.05$] on extinction. Among women with low estrogen levels, fear-potentiated startle was higher during extinction in the PTSD group compared with traumatized control women [$F(1,38)=5.04$, $p<0.05$]. This effect was absent in the High E2 group. There were no significant estrogen effects associated with dark-enhanced startle between groups.

Conclusions: Modulation of the acoustic startle response occurs through different neural pathways in the limbic system: the amygdala mediates fear responses to specific cues, and the bed nucleus of the stria terminalis (BNST) mediates responses to nonspecific anxiogenic cues. Our results suggest that lower estrogen levels may contribute to risk for anxiety disorders through dysregulated conditioned fear responses but not via unconditioned anxiety-related responses. Further research on the role of estrogen in fear regulation may provide insight into novel treatment strategies for PTSD.

Keywords: PTSD, fear potentiated-startle, estrogen, extinction, anxiety.

Disclosures: E. Glover, Nothing to Disclose; V. Michopoulos, Nothing to Disclose; K. Mercer, Nothing to Disclose; S. Norrholm, Nothing to Disclose; B. Bradley, Nothing to Disclose; K. Ressler, Nothing to Disclose; T. Jovanovic, Nothing to Disclose.

W41. Pattern Separation Bias Deficit in Patients with Schizophrenia

Theo Van Erp*, Adrian Preda, Steven Potkin, Lawrence Faziola, Lisa Thom, Dana Nguyen, Andrea Weideman, Charles Kessler, Craig Stark

University of California, Irvine, California

Background: Patients with schizophrenia show robust episodic memory deficits, but the nature of these deficits and their precise neural underpinnings remain to be determined. A recent hippocampus-based model of psychosis (Tamminga *et al*, 2010) posits that schizophrenia patients show a deficit in dentate gyrus (DG)-dependent pattern separation, required for the formation of non-overlapping memory representations, and an increase in

CA3/CA1-dependent pattern completion, required for associate retrieval based on partial information; two processes critically important for the formation and retrieval of declarative episodic memories. Based on this model, we hypothesized that patients with schizophrenia show pattern separation deficits when compared to healthy controls.
Methods: We administered the Behavioral Pattern Separation (BPS) paradigm developed by Stark and colleagues (2008) in 17 patients with schizophrenia and 15 controls during high-resolution imaging of the hippocampus. The BPS is a continuous recognition task during which participants are shown novel, repeated, or similar (lure) objects and are asked to indicate whether the current object shown is 'new', 'old', or 'similar' to any of the objects shown during the task. The behavioral data produced by the task include the separation bias metric (SBM), computed as [p('Similar'[Lure]—p('Similar'[Foil]), which quantifies the extent to which items are successfully separated while correcting for response bias between groups. An initial group comparison of the SBM was performed using a *t*-test.
Results: The patients with schizophrenia (mean age = 39, SD = 12, 14 males) did not differ in mean age ($p = 0.36$) and sex distribution ($p = 0.38$) compared with the healthy volunteers (mean age = 35, SD = 11, 9 males). Patients with schizophrenia (mean = 0.24, SD = 0.20) had a significantly lower separation bias metric (SBM) than healthy volunteers (mean = 0.39, SD = 0.19; $p < 0.02$).
Conclusions: Patients with schizophrenia show a deficit in the Behavior Pattern Separation (BPS) task separation bias metric (SBM) when compared with healthy volunteers. This deficit may be at the core of patients' episodic memory deficits and provides initial cognitive neuroscience-based behavioral support for dentate gyrus dysfunction in patients with schizophrenia.
Keywords: schizophrenia, psychosis, memory, pattern separation, dentate gyrus.
Disclosures: T. Van Erp, Nothing to Disclose; A. Preda, Nothing to Disclose; S. Potkin, Nothing to Disclose; L. Faziola, Nothing to Disclose; L. Thom, Nothing to Disclose; D. Nguyen, Nothing to Disclose; A. Weideman, Nothing to Disclose; C. Kessler, Nothing to Disclose; C. Stark, Nothing to Disclose.

W42. Change in Functional Activation During Cognitive Control Across Childhood and Adolescence as Related to Risk Taking Behavior

Katherine H Karlsgodt*, Bart D Peters, Toshikazu Ikuta, Pamela DeRosse, Kimberly Cameron, Angelica Bato, Philip R Szeszko, Anil Malhotra

Zucker Hillside Hospital, Glen Oaks, New York

Background: Adolescence is a time of growing independence in which the individual begins to be held responsible for increasingly important life decisions. The potential for increased risky decision making in adolescence therefore has special importance. Decision making is supported, in part, by the frontal-parietal networks that support the executive functions, including cognitive control. This network has been demonstrated to continue maturation into late adolescence and into early adulthood, and it is of great interest how this development is reflected in functional activation changes. Here we have first investigated age-related differences in cognitive control in a large sample of healthy children and adolescents during functional magnetic resonance imaging (fMRI). Secondly, we assessed differences in activation of the cognitive control network in individuals exhibiting higher and lower risk taking behavior based on the hypothesis that those individuals with higher risk taking may have deficits in cognitive control.
Methods: We assessed 61 healthy individuals aged 8–18 years using the Multi-Source Interference Test (MSIT) during functional magnetic resonance imaging (fMRI). In the MSIT a set of three numbers ranging from 1–3 are shown, two are identical and one is different. The participant must use three fingers to indicate the numerical value (not the spatial location) of the number that differs. Interference is present in trials in which the value of the target does not match the spatial matching of its position. In control trials there is only one number, which is paired with two O's. In these trials, the number value always matches the position. Previous research has demonstrated that the MSIT typically activates an executive network including frontal cortex, parietal cortex, and anterior cingulate. Risk taking behavior was assessed using a subset of questions from the rule-breaking scale of the Youth Self Report, the child version of the Child Behavior Checklist.
Results: Across the whole group, in an analysis controlled for age and sex, the MSIT robustly engaged the fronto-parietal executive network, and activated superior parietal cortex, lateral frontal cortex and the anterior cingulate. To investigate age effects we performed a voxel-wise regression of age predicting functional activation, controlled for sex. We found an inverse correlation between age and inferior frontal, medial pre-frontal, and parietal activation, such that younger subjects showed higher activation than older subjects. This pattern may potentially indicate that the executive network is less efficient in younger subjects, while the default mode network is more suppressed during task performance as age increases. Finally, we demonstrated that while controlling for age and sex, those individuals with greater risk taking behavior showed higher activation in the cognitive control network, consistent with a less mature pattern of network activity.
Conclusions: The current findings indicate that the MSIT is a useful tool for probing executive network development. An analysis assessing differences across age indicate that younger subjects perform more poorly than older subjects, yet show higher network activation, resulting in an overall less efficient pattern of functional activation. Furthermore, individuals with higher scores on the risk taking scale showed less efficient activation across the cognitive control network. This may indicate that one contribution to increased risk taking behavior is immature function of the cognitive control network. This has implications for our understanding of cognitive control and risk taking in both healthy and disordered populations.
Keywords: development, cognitive control, fMRI, risk taking, adolescence.
Disclosures: K. Karlsgodt, Nothing to Disclose; B. Peters, Nothing to Disclose; T. Ikuta, Nothing to Disclose; P. DeRosse, Nothing to Disclose; K. Cameron, Nothing to Disclose; A. Bato, Nothing to Disclose; P. Szeszko, Nothing to Disclose; A. Malhotra, Nothing to Disclose.

W43. Learning in the Absence of Intact Cognitive Control: The Neural Substrates of Transitive Inference in Adolescents with Autism Spectrum Disorders

Marjorie Solomon*, Tyler A Lesh, Tara Niendam, Jonathan Beck, John Matter, Nathan Whitmore, Cameron S Carter, J Daniel Ragland

University of California Davis, Sacramento, California

Background: Individuals with autism spectrum disorders (ASD) display a unique pattern of learning strengths and challenges. They show intact (or even enhanced) lower-level learning of stimulus response associations, of single items of information, of facts, of habits, and of information learned implicitly. However, they display deficits in *generalizing* (or transferring) what they have learned during training to new similar situations. Generalization problems have a profound impact on the academic, social, and adaptive functioning of persons with ASD, and have not been well studied. The goal of the current research is to illuminate the neural mechanisms of learning differences in persons with ASD using fMRI, and to translate findings into clinically relevant insights related to classroom learning.
Methods: To better understand the neural mechanisms underlying generalization deficits characteristic of ASD, we used rapid event-related functional magnetic resonance imaging (fMRI), and a newly designed transitive inference (TI) paradigm for pediatric populations, with a 5-stimulus pair hierarchy, a game format, frequent feedback, and the opportunity to earn prizes. TI involves learning a series of ordered stimulus pairs (AB, BC, CD, DE, EF where A > B > C > D > E > F), and then transferring or generalizing this learning about order to novel pairs (AC, AD, AE, BD, BE, CE). Previously, we examined TI in adults with ASD and TYP using a 4-pair hierarchy (Solomon, Frank, Smith, Ly, & Carter, 2011), and found that individuals with ASD used a strategy involving rote memorization, which we hypothesized was more hippocampally-mediated, whereas TYP employed a more flexible reinforcement learning strategy using information about the value of the hierarchy end-items, which we hypothesized relied more on reinforcement learning involving interactions between the striatum and the PFC. Due to the use of a rote memory strategy, the ASD group performed comparably to TYP on the harder BD pair, but worse on the easier AE pair. We extended the investigation of TI to adolescents with ASD and TYP using neuroimaging. We also administered measures of academic achievement to participants. We examined their strategy use, and correlations of performance with age and classroom learning problems. Hypotheses: (1) Replication of our prior finding in adults with ASD that adolescents with ASD use a rote memory strategy; (2) TI performance that is associated with recruitment and functional connectivity of the hippocampus, striatum, prefrontal cortex (PFC), and the parietal cortex in TYP, and with recruitment of the hippocampus, striatum, and visual cortical brain regions in ASD; and (3) Poor TI performance, and less recruitment of the PFC and functional connectivity with the parietal cortex that is related to learning problems in individuals with ASD.
Results: Individuals with ASD learned the task to comparable rates as TYP by the end of training. In this format involving frequent feedback and reinforcement, they were not slower to learn. At test, the ASD group outperformed the TYP group on the most difficult previously trained internal CD and DE pairs, suggesting they showed superior rote memory. Whole brain and ROI analyses showed the ASD group exhibited greater striatal and medial temporal lobe activation during early learning that was related to task performance. At test, there was less activation in prefrontal regions in the ASD group on inference pairs, and reduced fronto-parietal functional connectivity. Indices of cognitive control deficits were related to reading comprehension and math problem solving scores on measures of academic achievement.
Conclusions: Results suggest that individuals with ASD accomplish transitive inference learning differently than TYP. When making inferences, those with ASD recruit prefrontal regions less, and show reduced functional connectivity between frontal and parietal regions. Instead, adolescents with ASD recruit striatal, medial temporal, and visual cortical regions to a greater extent, suggesting the use of these brain regions may be compensatory. Overall, those with ASD have cognitive control related learning deficits. Consequently they rely on a rote learning-based strategy as opposed to a more flexible one that can incorporate rapid updating of reward contingencies, and integrate this information in the service of goal directed behavior. This impacts their academic performance. Our interpretation is supported by the systems-level computational modeling work of Frank *et al* (2004, 2005, 2006).
Keywords: autism spectrum disorders, adolescents, learning, hippocampus, cognitive control.
Disclosures: M. Solomon, Nothing to Disclose; T. Lesh, Nothing to Disclose; T. Niendam, Nothing to Disclose; J. Beck, Nothing to Disclose; J. Matter, Nothing to Disclose; N. Whitmore, Nothing to Disclose; C. Carter, Nothing to Disclose; J. Ragland, Nothing to Disclose.

W44. Attenuated Behavioral and Brain Responses to Trust Violations Among Assaulted Adolescent Girls

Josh Cisler*, Jennifer Lenow, Scott Steele, Clinton D Kilts

University of Arkansas for Medical Sciences, Little Rock, Arkansas

Background: Physical and sexual assault during adolescence is a potent risk factor for mental health problems, particularly posttraumatic stress disorder (PTSD), depression, and substance use disorders. Moreover, early life assault exposure is also associated with heightened risk for revictimization later in life and deficits in interpersonal functioning, especially among females. While much research in the past two decades has focused on understanding the emotional mechanisms that might mediate the relationship between early life trauma exposure and subsequent risk for negative mental health outcomes, there has been scarce research devoted to understanding the social-cognitive mechanisms associated with early life trauma. In order to shed light on this understudied domain, we conducted an exploratory study comparing assaulted and non-assaulted adolescent girls in behavioral and brain responses during a social trust learning task.

Methods: Adolescent girls (14 assaulted, 16 non-assaulted) aged 12–16 engaged in a novel social learning task during fMRI scanning that manipulated the probability with which computer-simulated people delivered positive or negative social outcomes to the participant. The participant's task was to pay attention to the behavior of the computer-simulated people and decide, on a trial-by-trial basis, which is the the most trustworthy person. Contrasts of interests for behavioral and brain analyses focused on comparing responses during unexpected and expected social outcomes. Assaulted adolescent girls did not differ from control girls in age, ethnicity, or IQ.
Results: Behaviorally, we found that health control adolescent girls slowed their decision-making (ie, longer reaction times) following unexpected social outcomes (ie, a trust violation; when a presumably trustworthy person delivered a negative outcome). By contrast, assaulted adolescents demonstrated significantly *less* behavioral slowing in response to unexpected negative social outcomes relative to control girls. In regards to brain activation, healthy control girls demonstrated robust activations of a prediction error network in response to trust violations, including activation in the bilateral anterior insula and dorsal and perigenual anterior cingulate cortex (ACC). By contrast, assaulted adolescent girls demonstrated significantly less activation in these same regions. Furthermore, we found that the severity of participants' exposure to assaultive events and PTSD symptom severity scaled negatively with recruitment of these regions, but also that greater time since the last assault was actually associated with improved brain activation in these regions, which highlights plasticity of this brain circuit in response to an improving social environment.
Conclusions: These preliminary results suggest that assault victims may generate diminished prediction error signals upon unexpected negative social outcomes. The results also indicate that these deficits are worse among adolescent girls with more severe trauma history and worse PTSD symptoms, but that the deficits appear to improve in the absence of on-going assault exposures. These findings have implications for understanding impaired social behavior, risk for revictimization, and clinical symptoms among assault victims.
Keywords: early life trauma, fMRI, social-cognitive-affective neuroscience, PTSD.
Disclosures: J. Cisler, Nothing to Disclose; J. Lenow, Nothing to Disclose; S. Steele, Nothing to Disclose; C. Kilts, Nothing to Disclose.

W45. Independent and Additive Impact of Co-occurring Anxiety Disorders on Elevated Trait Impulsivity in Bipolar Alcoholics: Implications for Alcoholism Severity and Bipolar Course of Illness

Bryan K Tolliver*, James J Prisciandaro, Delisa G Brown, Helena Brenner

Medical University of South Carolina, Charleston, South Carolina

Background: Alcohol dependence co-occurs with a lifetime prevalence of roughly 50% in people with bipolar disorder and is often associated with a more severe course of illness. Impulsivity, a core feature of both bipolar disorder and alcoholism, has been shown in previous research to correlate with lifetime suicide attempts, which occur in bipolar alcoholics at a rate that is roughly double the rate in bipolar patients without comorbid alcoholism. Anxiety disorders are also extremely common in those with bipolar disorder, especially in patients with comorbid substance use disorders. Like alcoholism, comorbid anxiety disorders in people with bipolar disorder are associated with early age of bipolar onset, a more severe course of affective illness, more frequent mixed episodes, and higher rates of lifetime suicide attempts. Given that anxiety and substance use disorders often co-occur together in bipolar disorder and share similar associations with bipolar course of illness, it is reasonable to ask whether this diagnostic clustering is nonrandom and thus potentially related to other aspects of bipolar clinical phenomenology. The current study evaluated the relationship between co-occurring anxiety disorders and trait impulsivity in a sample of adults with bipolar disorder, alcohol dependence, or both diagnoses. Additional exploratory analyses assessed whether impulsivity and anxiety disorders may correlate with bipolar and alcoholism clinical characteristics.
Methods: Adults aged 18–65 were enrolled in four DSM-IV diagnostic groups as determined by the SCID-IV: (1) bipolar I or II disorder and lifetime alcohol dependence (BP + AD), (2) bipolar I or II disorder without current or lifetime alcohol dependence (BP), (3) alcohol dependence without lifetime bipolar disorder (AD), and (4) healthy controls without lifetime alcohol dependence or bipolar disorder (HC). Subjects in each group who met lifetime criteria for generalized anxiety disorder (GAD), social phobia, panic disorder, obsessive-compulsive disorder (OCD) and/or posttraumatic stress disorder (PTSD) as determined by the Mini International Neuropsychiatric Interview were recorded as having the diagnoses without regard to severity or past *vs* present status. Trait impulsivity was evaluated using the Barratt Impulsiveness Scale (BIS-11). Alcoholism severity was evaluated using the Alcohol Dependence Scale. Impulsivity data were analyzed using general linear models employing univariate linear regression and/or factorial ANOVA as appropriate. Categorical prevalence data were analyzed by logistic regression or Chi-square approaches as indicated.
Results: In total 108 subjects were enrolled ($n = 57$ BP + AD, $n = 14$ BP, $n = 18$ AD, $n = 19$ HC). All AD subjects, and 72% ($n = 41$) of BP + AD subjects met criteria for current alcoholism. Prevalence of anxiety disorders was higher in subjects with bipolar disorder ($p < 0.01$); a trend toward higher prevalence in subjects with alcoholism did not reach significance ($p < 0.07$). Among bipolar patients, prevalence of any anxiety disorder was 58% and did not differ in BP + AD *vs* BP subjects. Among alcoholics, only panic disorder was significantly more prevalent in BP + AD (41.8%) than in AD (13.6%) subjects ($p < 0.05$). In the full sample, impulsivity per the BIS-11 Total score was significantly and independently higher in subjects with bipolar disorder ($p < 0.01$) and with any anxiety disorder ($p < 0.005$), whereas the effect of alcohol dependence on BIS-11 scores did not reach significance ($p < 0.06$). No interactions among the three factors were evident. Exploratory analysis of bipolar

alcoholics alone indicated that BIS-11 Total and Attention subscale scores were significantly higher in those with at least one comorbid anxiety disorder (Total $p<0.05$, Attention $p<0.01$). Analyzed by anxiety disorder, impulsivity was higher in BP + AD subjects with GAD, social phobia, and PTSD but not in those with panic disorder or OCD. Of note, in bipolar alcoholics the presence of >2 comorbid anxiety disorders was associated with higher impulsivity, with age of bipolar onset, and with number of hospitalizations for depression, but was not significantly associated with age of onset or severity of alcoholism. BIS-11 score, but not loading of comorbid anxiety disorders, was correlated with lifetime suicide attempts in bipolar alcoholics.
Conclusions: The current results confirm and extend previous research demonstrating a high prevalence of comorbid anxiety disorders and substance use disorders in individuals with bipolar disorder. Further exploration of the relationship of trait impulsivity to each comorbid condition is warranted as improved understanding may suggest novel therapeutic targets for this important clinical population.
Keywords: addiction, comorbidity, depression, mania, suicide.
Disclosures: B. Tolliver, Nothing to Disclose; J. Prisciandaro, Nothing to Disclose; D. Brown, Nothing to Disclose; H. Brenner, Nothing to Disclose.

W46. Fronto-parietal Dysfunction and Cognitive Control Deficits in First-episode Psychosis Patients with Schizophrenia and Bipolar Disorder

Tyler A Lesh*, Benjamin Geib, Tara Niendam, Michael J Minzenberg, Jong Yoon, J Daniel Ragland, Marjorie Solomon, Cameron S Carter

University of California Davis, Sacramento, California

Background: Cognitive control deficits are consistently observed in patients with chronic and first-episode schizophrenia, and appear to be distinct from those seen in patients with unipolar depression or stable bipolar disorder without psychosis. However, recent meta-analyses reveal that individuals with bipolar disorder with psychotic features show more severe deficits, primarily on neuropsychological tests of executive functioning. Given accumulating evidence of genetic overlap in these disorders, as well as substantial shared phenotypic features, we sought to explore prefrontal-parietal circuit dysfunction and disrupted proactive cognitive control processes in patients with first-episode schizophrenia and bipolar disorder with psychotic features. We hypothesized that patients with bipolar disorder with psychotic features would show deficits in cognitive control as measured by the AX-CPT at an intermediate level compared to schizophrenia patients and healthy controls. We anticipated a similar pattern of results in brain activity during fMRI, with schizophrenia patients showing severely disrupted engagement of dorsolateral prefrontal cortex (DLPFC) and parietal cortex, and bipolar individuals showing a significant but less severe deficit in fronto-parietal activity, when compared to controls.
Methods: First-episode schizophrenia ($n=67$), bipolar disorder with psychotic features ($n=20$), and healthy control ($n=64$) participants were identified from referrals to the UC Davis Early Detection and Preventative Treatment (EDAPT) clinic using the Structured Clinical Interview for DSM-IV. Participants completed the AX-CPT, an established measure of proactive cognitive control, while undergoing fMRI. D'-context was used as the primary measure of participants' ability to utilize context to guide response selection on the AX-CPT. Functional MRI data were processed using SPM8 and focused on contrasting the cue phase of high control (B cue) and low control (A cue) trials, representing proactive control processes. Whole-brain voxel-wise and *a priori* DLPFC region of interest analyses were performed. A voxel-wise threshold of $p<0.01$ and FWE cluster threshold of $p<0.05$ was utilized for all comparisons.
Results: ANOVA revealed significant group differences on d'-context. Between group comparisons showed significantly lower performance in both bipolar and schizophrenia patients compared to control subjects. Bipolar subjects also showed significantly higher performance compared to patients with schizophrenia. During fMRI, all groups showed the expected pattern of lateral PFC and parietal engagement. Schizophrenia subjects showed significantly reduced activity in bilateral DLPFC and right parietal cortex compared to controls. Similarly, bipolar subjects showed reduced activity in right DLPFC and parietal cortex, as well as right supplementary motor area, compared to controls. Schizophrenia patients also demonstrated higher activity in right putamen compared to bipolar subjects, while bipolar patients did not show any regions of significantly greater activity compared to either group.
Conclusions: These data suggest that first-episode bipolar patients with psychosis show cognitive control deficits analogous to, but less severe than schizophrenia patients, when compared to control subjects. Furthermore, neuroimaging data revealed similar fronto-parietal circuit abnormalities in both patient groups compared to control subjects. These findings add to an expanding literature examining the common and unique factors underlying the etiology of bipolar disorder and schizophrenia.
Keywords: schizophrenia bipolar cognition fmri dlpfc.
Disclosures: T. Lesh, Nothing to Disclose; B. Geib, Nothing to Disclose; T. Niendam, Nothing to Disclose; M. Minzenberg, **Part 1:** Owns stock in Elan, **Part 4:** Research funding from GSK; J. Yoon, Nothing to Disclose; J. Ragland, Nothing to Disclose; M. Solomon, Nothing to Disclose; C. Carter, **Part 1:** one-time consultant for Pfizer, Merck, Lilly, Servier, **Part 4:** Research funding from GSK.

W47. Mismatch Negativity Predicts Psychosis Onset and is Associated with Plasma Markers of Inflammation in Youth at Clinical High Risk for Psychosis

Daniel H Mathalon*, Diana Perkins, Kristin Cadenhead, Gregory A Light, Peter Bachman, Jason Johannesen, Aysenil Belger, Margaret Niznikiewicz, Erica Duncan, Ricardo Carrion, Jean Addington, Tyrone Cannon, Barbara A Cornblatt, Larry J Seidman, Elaine Walker, Scott Woods

University of CaliforniaSan Francisco, California

Background: The mismatch negativity (MMN), an event-related brain potential (ERP) component elicited pre-attentively by deviant auditory stimuli imbedded in a

stream of standard stimuli, has been repeatedly shown to be reduced in amplitude in schizophrenia. Prior studies from several groups have shown MMN to be reduced inpatients at clinical high risk (CHR) for psychosis, particularly in those who subsequently develop a psychotic disorder, principally schizophrenia. MMN is thought to depend on neurotransmission at the N-methyl-d-aspartate (NMDA) sub-class of glutamate receptors based on human and non-human primate studies showing NMDA receptor antagonists to disrupt MMN. Recent interpretations of the MMN have emphasized its reflection of both short-term (seconds) and longer term (minutes to hours) synaptic plasticity in the service of auditory sensory/perceptual learning, since the amplitude of the MMN to a deviant stimulus increases as a function of the number of repetitions of the preceding standard stimulus and also as a function of the emergence of more enduring memory traces for complex sounds through repeated exposures to these sounds. A plethora of basic neuroscience data indicates that neuroinflammation and inflammatory cytokines compromise mechanisms of synaptic plasticity such as long-term potentiation. Moreover, prior studies have shown schizophrenia and other psychiatric disorders to be associated with elevations in peripheral markers of inflammation. Using data collected as part of the North American Prodromal Longitudinal Study (NAPLS), we examined whether MMN was reduced in CHR patients, particularly those who went on to convert to a psychotic disorder, and further, whether blood plasma markers of inflammation were associated with reductions in MMN amplitude in CHR patients.

Methods: As part of the ongoing multi-site NAPLS study, 212 CHR youth meeting criteria for a psychosis prodrome syndrome (SIPS interview) and 152 age-matched healthy controls (HC) underwent electroencephalographic (EEG) recording during a 3-deviant (pitch, duration, pitch + duration) MMN paradigm administered while subjects performed a visual distractor task. In addition, 27 CHR individuals who subsequently converted to psychosis were compared with 75 CHR individuals who had not converted by the 24-month follow-up assessment. MMN was measured at electrodes Fz and Cz, using linked mastoids as reference. Blood plasma samples were drawn from a sub-group of subjects including 32 CHR-converters, 40 CHR non-converters, and 35 healthy controls, and were subject to analysis on a Luminex® multiplex platform. Analytes of inflammatory markers that distinguished CHR converters from non-converters were identified ($n = 30$) and aggregated (z-transformed) to form an 'inflammatory threshold index'. For each subject, this index was the calculated as the number of analytes that exceeded a z-threshold set to flag deviant analyte values.

Results: Results showed that CHR patients had reduced MMN amplitudes at baseline relative to healthy controls for duration deviant MMN ($p < 0.05$), but not for pitch or combination deviant MMNs (Group x Deviant Type interaction $p = 0.011$). Moreover, baseline MMN amplitude, irrespective of deviant type, was significantly reduced in the CHR converters relative to the CHR non-converters (Group main effect $p = 0.024$). As expected based on the method used to derive it, the inflammatory threshold index was significantly elevated in CHR converters relative to CHR non-converters ($p < 0.00001$). Of note, both the CHR converters ($p < 0.00001$) and the CHR non-converters ($p < 0.005$) showed significant elevations of the inflammatory index relative to healthy controls. Reduced pitch-deviant MMN amplitudes were significantly associated with elevated scores on the inflammatory threshold index in CHR patients ($r = 0.38$, $p = 0.002$, $n = 66$), but not in healthy controls ($r = 0.11$, $p = 0.57$, $n = 35$). Furthermore, while this MMN-inflammation relationship was strongly evident in the CHR converters ($r = 0.57$, $p = 0.007$, $n = 21$), it was not evident in the CHR non-converters ($r = 0.16$, $p = 0.34$, $n = 39$).

Conclusions: Results to date replicate previous findings showing MMN amplitude to be reduced in CHR patients, particularly those who go on to convert to a psychotic disorder. Accordingly, disruption of NMDA-dependent synaptic plasticity, as reflected by the MMN, appears to precede psychosis onset and predicts the likelihood of conversion to a psychotic illness. In addition, the identification of a number of plasma analyte markers of inflammation that are elevated in CHR converters suggests that an active inflammatory process precedes psychosis onset in CHR patients who develop psychosis. Moreover, consistent with literature showing neuroinflammation to compromise mechanisms of synaptic plasticity, baseline elevations in plasma markers of inflammation were significantly associated with MMN amplitude reductions in the subgroup of CHR patients who subsequently converted to a psychotic disorder.

Keywords: mismatch negativity inflammation psychosis plasticity.

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W48. High-resolution fMRI Reveals Reward Anticipation Signaling of the Substantia Nigra that is Modulated by Reward Magnitude in Healthy Subjects and Blunted Responses in Schizophrenia

Jong Yoon*, Anthony Grandelis, Edward D Cui, Michael J Minzenberg, Tara Niendam, J Daniel Ragland, Tyler A Lesh, Marjorie Solomon, Cameron S Carter

Stanford University, Palo Alto, California

Background: The substantia nigra (SN) is a mid-brain nucleus and one of the main sources of neural dopamine (DA). Thus, the SN is well positioned to play a central role in many of the cognitive and affective processes modulated by DA. Furthermore, the SN may also play an important role in numerous neuropsychiatric conditions thought to involve impairments in DA function, such as schizophrenia. While the VTA has been the DAergic region most commonly associated with reward processing, the connec-

tivity pattern of nigral neurons with the ventral striatum suggests that the nigra may also be involved in reward processing. We tested this hypothesis by conducting a task-evoked high-resolution fMRI study with a novel method for localizing the nigral BOLD signal and one of the most widely used reward processing paradigms. We also conducted a pilot study with a small sample of subjects with schizophrenia (SZ), to explore the possibility of nigral dysfunction during reward processing in this condition.

Methods: 18 healthy subjects and 6 individuals with SZ on atypical antipsychotics performed the Monetary Incentive Delay (MID) Task while undergoing high-resolution fMRI. fMRI consisted of a single-shot, T2*-weighted echo planar imaging (EPI) sequence (TR 2000 ms, TE 34 ms, flip angle 75 degrees, FOV 224 mm × 244 mm with 25 contiguous slices in the axial oblique plane, with a voxel size of 1.8 mm × 1.8 mm × 1.9 mm). Images underwent slice time correction and spatial realignment to correct for in-scanner movement. Images did not undergo spatial warping and all analyses were conducted in the individual's 'native space.' This was done to preserve as much spatial resolution as possible so that for each individual we could manually draw the SN based on its distinct signal qualities apparent in EPI based images. This SN mask was used to derive estimates of the BOLD signal for hypothesis testing.

Results: In healthy subjects, we observed significant modulation of nigral BOLD signal during the cue-delay interval as a function of the amount of money to be won or lost. The order of BOLD signal magnitude was: Lose \$5 = Win \$5 > Lose \$1 = Win \$1 > No reward. In the pilot study with patients, we observed a different pattern of BOLD signal responses. The nigra displayed above baseline activity only in conditions with the largest amount of money to be won or lost. Compared to healthy subjects, the magnitude of responses in these conditions was decreased in SZ.

Conclusions: The pattern of nigral responses in healthy subjects demonstrated in this study is very similar to published reports of ventral striatal responses. This result, in conjunction with the known connectivity pattern between these structures, suggests the possibility that the ventral striatal responses are the downstream effects of nigral signaling. The preliminary results from patients suggest blunting of reward related signaling of the nigra, particularly in the low monetary reward/loss conditions. However, the results also suggest some preservation of nigral reward function during high reward conditions in SZ.

Keywords: fMRI, substantia nigra, reward processing, schizophrenia, DAergic.

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W49. Acute and Non-acute Effects of Cannabis on Neurocognitive Functioning

April D Thames*, Natalie Arbid

University of California, Los Angeles, California

Background: Cannabis use has steadily increased in recent years, with approximately 17.4 million individuals reporting cannabis use within the past month of a National Survey on Drug Use and Health.[1] While many studies generally support the adverse acute effects of cannabis on neurocognition, the non-acute effects are less clear. This may be partially due to methodological differences across studies that include differences in participant characteristics, operational definition of 'abstinence,' and failure to consider the role of other substances such as alcohol that are commonly reported among cannabis users. The purpose of the current study was to examine the acute and non-acute effects of cannabis use in a sample of college students. Our study hypotheses were as follows: (H1) Cannabis users will score lower on global neurocognitive performance and in domains of attention/working memory, speed of information processing, learning and memory, and executive functioning compared to non-users, (H2) Time since last use (of cannabis) will be positively associated with neurocognitive performance, (H3) Recent users (ie, those who reported using cannabis in last 4-weeks) will demonstrate poorer neurocognitive performance than past users, (H4) Frequency of cannabis use in the last 4-weeks and amount of cannabis used per day will be negatively associated with neurocognitive performance.

Methods: We recruited 76 college students who reported recent cannabis use (in last 4-weeks), remote cannabis use (prior to the last 4 weeks), and those who reported no use. We also examined the independent and synergistic effects of comorbid alcohol use. Participants were administered a brief cognitive test battery. Global neurocognitive performance was calculated by averaging T-scores from individual cognitive tests.

Results: Cannabis users performed worse on global cognitive performance ($M = 42.02$; $SD = 13.42$) than non-users ($M = 47.63$; $SD = 7.2$), $F(3, 72) = 12.70$, $p = 0.001$, $\eta^2 = 0.15$. Groups also differed in domains of attention/working memory, $F\ (3, 72) = 9.76$, $p = 0.003$, $\eta^2 = 0.12$; learning and memory, $F(3, 72) = 8.55$, $p = 0.005$, $\eta^2 = 0.11$; information processing speed, $F(3, 72) = 10.23$, $p = 0.002$, $\eta^2 = 0.13$; and executive functioning, $F(3, 72) = 12.94$, $p = 0.001$, $\eta^2 = 0.15$. Multiple regression analyses revealed that after controlling for cannabis use ($r^2 = 0.428$), alcohol ($r^2 = 0.431$) did not significantly predict neurocognitive performance, r^2change = 0.003, $F > (1, 72) = 0.362$, $p = 0.553$. On the other hand, when alcohol use was entered as a control variable, cannabis use significantly added to the prediction of neurocognitive performance, r^2 change = 0.04, $F\ (1, 72) = 5.52$, $p = 0.02$. Results from these analyses suggest that cannabis use was a stronger predictor of neurocognitive performance.

Contrary to study hypotheses, we did not find significant correlations between time since last use and global neurocognitive function, $r\ (43) = 0.105$, $p = 0.502$ or individual cognitive domains. Recent users ($n = 18$) did not demonstrate significant differences in global neurocognitive performance than past users ($n = 25$), $F\ (2, 30) = 0.95$, $p = 0.33$. However, recent users demonstrated worse performance on attention/working memory measures than past users, $F\ (2, 30) = 4.30$, $p = 0.04$, $\eta^2 = 0.09$. Frequency of cannabis use in the last 4-weeks was negatively associated with global neurocognitive performance, $r\ (18) = -0.76$, $p < 0.001$ and cognitive domains of attention/working memory, $r\ (18) = -0.78$, $p < 0.001$, learning/memory, r

$(18) = -0.66$, $p = 0.02$, information processing speed, r $(18) = -0.68$, $p = 0.01$ and executive functioning, r $(18) = -0.76$, $p = 0.004$. Similarly, amount of cannabis use per day was negatively associated with global neurocognitive performance, r $(18) = -0.72$, $p = 0.01$ and cognitive domains of attention/working memory, r $(18) = -0.68$, $p < 0.02$, learning/memory, r $(18) = -0.71$, $p = 0.02$, information processing speed, r $(18) = -0.86$, $p = 0.001$ and executive functioning, r $(18) = -0.74$, $p = 0.009$.

Conclusions: Our results support the widespread adverse effects of acute cannabis use on neurocognitive functioning. Although some of these effects appear to attenuate over time, lower performance on measures of attention and working memory persisted among individuals who reported remote use. With the recent debates over marijuana legalization, it is critical to examine the long-term effects of cannabis on cognition. Although age of onset was not examined in the current study, more studies are needed to determine how age of onset influence the relationship between cannabis and neurocognitive functioning. The current study findings add to the extant literature by clarifying the acute and non-acute effects of cannabis on neurocognition.

Keywords: cannabis, neurocognition, acute effects, non-acute effects.

Disclosures: A. Thames, Nothing to Disclose; N. Arbid, Nothing to Disclose.

W50. Anger in Body and Brain: Elevated Blood Pressure Impedes Reaction Time and Diminishes Neural Activity in Attention and Visual Areas During a Decision Making Task

Sarah N Garfinkel*, Jos Brosschot, Julian Thayer, Hugo D Critchley

Brighton and Sussex Medical School, Sussex, United Kingdom

Background: Emotion and cognition are dynamically coupled with corresponding changes in physiology and neural activity: emotion states reflect and impact brain and physiology, which in turn can affect cognitive function. We explore the neural and physiological basis of anger processing on lexical decisions using a subliminal priming paradigm.

Methods: Two hundred neutral 7-letter strings are devised to form either words ($N = 100$) or non-words ($N = 100$). Participants are instructed to make lexical decisions (ie determine whether these strings form words or non-words) as quickly and as accurately as possible. To investigate the effect of anger, the words ANGER or RELAX are presented subliminally (ie forward and backward masked) prior to the letter-strings. Experimental procedures are accompanied by simultaneous beat-to-beat blood pressure monitoring [$N = 40$, Experiments 1 and 2] and fMRI [$N = 19$, Experiment 2] procedures.

Results: Relax primes facilitate reaction time during lexical decisions, while anger primes impede reaction time, an effect demonstrated in Experiment 1 [$F(1, 20) = 13.031$, $p = 0.002$] and replicated in Experiment 2 [$t(18) = -3.646$, $p = 0.002$]. Blood pressure increased during trials with subliminal presentation of ANGER [$t(18) = 2.5$, $p = 0.02$], and the magnitude of this increase directly correlated with prolonged reaction time following anger primes [$r = -498$, $p = 0.038$]. In the brain, we show neural activation to anger primes, specifically pons activation ([-6 -34 -30], $Z = 3.33$, $K = 10$, $p < 0.001$]). Linking brain activity to behaviour, the facilitated effect of relax primes on decision making corresponds with activation in areas of visual cortex and attention, such as cuneus ([22 -90 6], $Z = 4.31$, $p < 0.001$, $k = 317$) and parietal lobe ([34 -48 54], $Z = 3.87$, $p < 0.001$, $k = 180$).

Conclusions: Implicitly induced emotion can have neural and physiological consequences which impede decision making, while speed of processing can be enhanced by relaxed states through augmented visual processing and attention.

Keywords: systolic blood pressure, stress, anger, parietal cortex, attention.

Disclosures: S. Garfinkel, Nothing to Disclose; J. Brosschot, Nothing to Disclose; J. Thayer, Nothing to Disclose; H. Critchley, Nothing to Disclose.

W51. Input-information Processing During Fear Acquisition in PTSD Using Dynamic Causal Modeling and fMRI

Huijin Song*, Mohammed R Milad

Massachusetts General Hospital, Charlestown, Massachusetts

Background: Previous rodent studies have been investigating fear circuit between regions of prefrontal and subregions of amygdala using fear conditioning. Also, many human fMRI studies have been investigating to find fear circuit using various functional connectivity analyses. However, these approaches couldn't show effective connectivity that may reveal how information is processed from one node to the other within the fear network. In this study, we used dynamic causal modeling (DCM) to investigated effective connectivity between thalamus, amygdala and dorsal anterior cingulate cortex(dACC) and effective connectivity between subregions of amygdala, dACC and ventromedial prefrontal cortex(vmPFC) in healthy controls and in patients diagnosed with post-traumatic stress disorder (PTSD).

Methods: A total of 18 PTSD and 19 trauma-exposed non-PTSD control(TENC) subjects were recruited from the community. fMRI images were acquired during all subjects performed a 2-day fear conditioning and extinction paradigm. In the data to be presented herein, the analyses were focused on the fear acquisition phase. Temporal signal data were extracted from each region of interest (ROI) in the fMRI data of fear conditioning paradigm. ROIs of interest included amygdala subregion (centro medial (CM), laterobasal (LB), and superficial (SF), which were created using cytoarch-tectonic probablility map in Anatomy toolbox. DCM models were specified using extracted data from each ROI. Model expected and exceedance probability were estimated by Baysian model selection (BMS). We tested input-information-processing during fear acquisition in both PTSD and TENC groups. One model tested informa-

tion flow from the thalamus. This model tested if conditioned fear representation would move 'upwards' to the dACC and then to the amygdala or from thalamus to amygdala to dACC. The second model tested information flow from sub-regions of the amygdala as seeds and then 'upwards' to either the vmPFC then the dACC or the dACC then the vmPFC.
Results: Starting with the thalamus as the seed, information processing seem to better-fit the model starting with the thalamus to dACC via amygdala rather than thalamus to amygdala via dACC in both TENC and PTSD group. In the model comparison between subregion of amygdala and prefrontal area, best-fit was the model of CM to vmPFC via dACC and model of LB to vmPFC via dACC were selected by BMS in TENC group. Importantly, effective connectivity of these two amygdala models was decreased in the PTSD group.
Conclusions: Input representation and processing related to fear acquisition across different key nodes of the fear network were tested in this study. Some information flow seem to be intact during fear acquisition with PTSD patients while others may not be. Continuation of this line of investigation is currently being done to understand how these abnormalities may or may not be related to fear extinction as well as to PTSD symptoms.
Keywords: dynamic causal modeling, DCM, fMRI, effective connectivity, PTSD, fear circuit, fear acquisition.
Disclosures: H. Song, Nothing to Disclose; M. Milad, Nothing to Disclose.

W52. Deficits in Reward Prediction Error Signaling in Cocaine Addiction: Evidence from the Feedback Negativity and Relationship to Recency of Cocaine Use

Muhammad Parvaz*, Anna Konova, Jonathan P Dunning, Greg H Proudfit, Pias Malaker, Scott J Moeller, Nelly Alia-Klein, Rita Goldstein

Mount Sinai Hospital, New York, New York

Background: Reward-prediction-errors (RPE) reflect the difference between expected and experienced outcomes, and their computation is vital to reinforcement learning and adaptive behavior. Addiction is a disorder characterized by deficits in fronto-striatal dopaminergic neurotransmission, which contribute to maldaptive RPE computation. Nevertheless, to date, neuroimaging studies have only indirectly shown impaired RPE signaling in drug addiction. We hypothesized that the feedback negativity (FN) component of the event-related potentials (ERP) can be used to directly investigate the RPE impairments in individuals with cocaine use disorder (CUD). Indeed, FN is a reliable electrocortical marker of RPE signaling that reflects neural processes underlying expectation violation.
Methods: Subjects were 26 healthy controls (HC), 30 CUD positive for cocaine on study day based on a urine test (CUD+), and 23 CUD negative for cocaine on study day (CUD−); this urine test indicates use within the past 72 h, separating the groups by frequency of recent cocaine use and abstinence (former is higher and latter is shorter in the CUD+ as compared to CUD−). Participants performed a gambling task comprised of three reward probabilities (25, 50, or 75%). Subjects indicated their expectations about winning or losing on a trial-by-trial basis. Subjects were given feedback regarding the trial outcome on each trial (Win = - green up-arrow; Loss = red down-arrow). Electroencephalography was recorded during the task and averaged ERPs for all 4-conditions (predicted win, unpredicted win, predicted loss, and unpredicted) were computed offline. The difference ERP waveforms were created by subtracting the ERPs observed following wins from the ERPs observed following losses, separately for predicted and unpredicted outcomes. Consistent with previous studies, FN amplitudes were scored at Fz, FC1, FCz, and FC2 electrode sites as the most negative peak in the difference waveform within a window between 200 and 500 ms following feedback.
Results: The FN amplitudes were analyzed using a 2 [*Prediction* (predicted and unpredicted)] 3 [*Group* (HC, CUD+ and CUD−)] repeated measures analysis of variance. Results showed an interaction between prediction and group [$F(2,76) = 3.77$, $p = 0.028$], such that the FN amplitude was larger for unpredicted outcomes compared to predicted outcomes in HC [$t(25) = 2.62$, $p = 0.015$] and CUD+ [$t(29) = 2.46$, $p = 0.020$], but not in CUD− ($p = 0.279$). Moreover, across all CUD, the differential FN amplitude (predicted minus unpredicted) was correlated with days of current abstinence ($r_s = -0.324$, $p = 0.028$) and the frequency of current cocaine use ($r_s = 0.310$, $p = 0.040$), suggesting that attenuated modulation of the FN to unpredicted *vs* predicted outcomes was associated with longer abstinence and less frequent cocaine use.
Conclusions: The current results corroborated previous findings in the HC and indicated that unlike HC and CUD+, CUD− failed to show FN amplitude modulation to unpredicted *vs* predicted reward outcomes. The divergent results between CUD+ and CUD− were further accentuated by the correlations that showed that attenuated RPE-related FN modulation was associated with longer acute abstinence and less frequent current cocaine use in CUD. Thus, using electrocortical markers of reward sensitivity, the current results for the first time demonstrate RPE-related deficits in a subgroup of CUD with less frequent current cocaine use/longer abstinence. These results support the previously-documented 'normalizing' effects of cocaine on cognitive function, extending them to the activity of neural substrates underlying RPE signaling.
Keywords: addiction cocaine EEG/ERP reward prediction error abstinence.
Disclosures: M. Parvaz, Nothing to Disclose; A. Konova, Nothing to Disclose; J. Dunning, Nothing to Disclose; G. Proudfit, Nothing to Disclose; P. Malaker, Nothing to Disclose; S. Moeller, Nothing to Disclose; N. Alia-Klein, Nothing to Disclose; R. Goldstein, Nothing to Disclose.

W53. Genetic Factors Contributing to Body Weight in Anorexia Nervosa and Bulimia Nervosa

Allan S Kaplan*, Zeynep Yilmaz #, Arun K Tiwari, Robert D Levitan, Jo Knight, Sara Piran, Sarah Gagliano, Andrew Bergen, Walter H Kaye, James Kennedy

Center for Addiction and Mental Health, Toronto, Ontario, Canada

Background: Anorexia nervosa (AN) is a serious eating disorder with substantial morbidity and a lifetime of

mortality as high as that associated with any psychiatric illness. Low weight or body mass index (BMI) is the *sine qua non* of AN and the primary target of initial treatment. Low weight and behaviours associated with reaching it, including purging and extreme caloric restriction, are also the primary reason for the high morbidity and mortality in this illness. Bulimia Nervosa is an eating disorder that shares the same psychopathology and disturbed eating behaviors as AN but is not associated with a low body weight and as such does not share the same high mortality as AN. Genetic factors have been purported to play a significant role in weight regulation in humans. The main purpose of this study is to determine the genetic factors that contribute to individuals with AN's susceptibility to reach and sustain an abnormally low body weight compared to individuals with bulimia nervosa (BN), who do not seem able to reach or sustain an abnormally low body weight.

Methods: Only AN probands with no history of BN and BN probands with no history of AN are included in this analysis, reducing phenotypic heterogeneity within groups and possibly amplifying genetic differences between groups. The sample consisted of 745 AN probands with no history of BN, 245 BN probands with no history of AN, and 321 female nonpsychiatric controls. We conducted an analysis of on average two markers with known or putative function per gene among 11 candidate genes with 21 SNPs in total selected from the leptin (*LEPR*, *LEP*, *GHRL*, *HRH1*), melanocortin (*AGRP*, *POMC*, *MC3R*, *MC4R*) and neurotrophin (*BDNF*, *NTRK2*, *NTRK3*) systems known to play a role in the regulation of appetite and/or weight in humans. A combination of Nyholt (gene-based) and Bonferroni (experiment-wise) corrections was applied to control for multiple testing.

Results: Our case-control results suggest that a melanocortin 4 receptor (*MC4R*) genetic variant previously linked to antipsychotic medication-induced weight gain appears to be underrepresented in AN probands compared to nonpsychiatric controls ($p = 0.0027$). We failed to find any statisticallysignificant differences in the selected genes between AN and BN cases. Furthermore, the agouti related protein (*AGRP*) gene was associated with lowest lifetime BMI in AN ($p = 0.0013$), and a neurotrophic tyrosine kinase, receptor, type 2 (*NTRK2*) risk variant was linked to highest lifetime BMI in BN ($p = 0.0018$).

Conclusions: To our knowledge, this is the first study to address the important issue of high crossover rates in eating disorders being a possible confounds in genetic studies. It is also the first study to explore the role of various markers with known or putative function in genetic systems known to regulate appetite and weight in AN and BN. These genetic findings may serve as an important first step toward gaining a better understanding of the regulation of weight, appetite, and energy balance in AN, BN, and healthy populations, including the possible identification of genetic factors that may protect or put at risk individuals for low weight and the possibility of developing eating disorders. These findings on weight regulation in AN and BN also have the potential for developing more effective treatment options and more specifically for providing a highly specific target for the development of novel medications.

Keywords: anorexia nervosa, bulimia nervosa, genetics, weight regulation.

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W54. FKBP5 Moderates Alcohol Withdrawal Severity: Human Genetic Association and Functional Validation in Knockout Mice

Ming-Chyi Huang, Melanie L Schwandt*, Julie A Chester, Aaron M Kirchhoff, Chung-Feng Kao, Tiebing Liang, Jenica Tapocik, Vijay A Ramchandani, David T George, Colin A Hodgkinson, David Goldman, Markus Heilig

NIH, Bethesda, Maryland

Background: Alcohol withdrawal is associated with hypothalamic-pituitary-adrenal (HPA) axis dysfunction. The FKBP5 gene codes for a co-chaperone, FK506-binding protein 5, which regulates glucocorticoid receptor sensitivity and exerts negative feedback on HPA axis function. We investigated the effects of single-nucleotide polymorphisms (SNPs) in the FKBP5 gene in humans, and the effect of Fkbp5 gene deletion in mice, on alcohol withdrawal severity.

Methods: We genotyped FKBP5 gene SNPs (rs3800373, rs9296158, rs3777747, rs9380524, and rs9470080) in 399 inpatients (274 males, 125 females) who had consumed alcohol within 48 h before they were admitted for treatment. All individuals were diagnosed with alcohol dependence according to the Structured Clinical Interview for DSM-IV (SCID). Alcohol withdrawal severity was assessed using the maximum score obtained on the Clinical Institute Withdrawal Assessment-Alcohol revised (CIWA-Ar) during the first four days of the inpatient stay. Genotype data were analyzed using HaploView to determine linkage disequilibrium (LD) and haplotype blocks. Association analyses were conducted using linear regression in PLINK. Fkbp5 gene knockout (KO) and wild type (WT) mice were assessed for alcohol withdrawal using handling induced convulsions (HICs) following both acute and chronic alcohol exposure.

Results: Minor alleles of rs3800373 (G), rs9296158 (A), rs3777747 (A), and rs9470080 (T) were significantly associated with lower CIWA-Ar scores, whereas the minor allele of rs9380524 (A) was associated with higher scores. These effects remained significant when adjusting for potential confounding variables including age, gender, depression and anxiety symptoms, and treatment with benzodiazepines. All five SNPs were found to be in strong LD, and two complementary haplotypes (rs3800373-rs9296158-rs3777747-rs9380524-rs9470080 GAACT and TGGAC) were associated with lower and higher CIWA-Ar scores, respectively. Fkbp5 KO mice showed significantly greater HICs during withdrawal from both acute and chronic alcohol exposure compared to WT controls.

Conclusions: To our knowledge, this study is the first to show a genetic effect of FKBP5 on alcohol withdrawal severity. In humans, several FKBP5 SNPs found to be in high LD were associated with alcohol withdrawal severity,

while in mice the absence of the Fkbp5 gene enhanced sensitivity to alcohol withdrawal. Based on previous reports, we propose that FKBP5 variants might trigger different adaptive changes in HPA axis regulation during alcohol withdrawal by influencing FKBP5 expression levels, with concomitant effects on withdrawal severity.
Keywords: FKBP5 gene, alcohol withdrawal severity, HPA axis, humans, mice.
Disclosures: M. Huang, Nothing to Disclose; M. Schwandt, Nothing to Disclose; J. Chester, Nothing to Disclose; A. Kirchhoff, Nothing to Disclose; C. Kao, Nothing to Disclose; T. Liang, Nothing to Disclose; J. Tapocik, Nothing to Disclose; V. Ramchandani, Nothing to Disclose; D. George, Nothing to Disclose; C. Hodgkinson, Nothing to Disclose; D. Goldman, Nothing to Disclose; M. Heilig, Nothing to Disclose.

W55. Haplotype and SNP Variation in Genes Implicated in GABA Synthesis, Synaptic Transmission and Re-uptake are Predictors for Alcoholism

Mary-Anne Enoch*, Colin A Hodgkinson, Elena Gorodetsky, Cheryl Marietta, Alec Roy, David Goldman

NIAAA, Rockville, Maryland

Background: As the primary inhibitory neurotransmitter in the CNS, GABA has been implicated in the chronic effects of alcohol, including tolerance, dependence and withdrawal. In an earlier study using RNA-Seq we focused on expression changes in alcoholics and cocaine addicts relative to controls in 25 GABAergic pathway genes within the hippocampus that has been shown to be important in drug reinforcement behaviors in animals and craving and relapse in humans. Principal FDR-corrected findings were in genes involved in GABA synthesis (*GAD1*, *GAD2*), synaptic transmission (*GABRG2*, *GPHN*) and re-uptake (*GABBR1*, *SLC6A1*). This RNA-Seq study confirmed the involvement of the GABAergic system in alcoholism but also revealed a hippocampal GABA input in cocaine addiction. The aim of the current study was to determine whether haplotype and SNP variation within these same genes might be vulnerability factors for the development of alcoholism and addiction in general.
Methods: African American men were recruited for this study. Of the participants with DNA available there were 360 treatment-seeking patients with lifetime DSM-IV single and comorbid diagnoses of alcohol, cocaine and heroin dependence, and 187 controls. Haplotype tagging SNPs for *GAD1* ($N=12$), *GAD2* ($N=17$), *GABRG2* ($N=15$) and *GPHN* ($N=23$) were available on an existing addictions array (Illumina GoldenGate platform). This array also included 186 ancestry informative markers. A total of 9 haplotype tagging SNPs spanning *GABBR1* were genotyped using an ABI open array. A functional *SLC6A1* 21 bp promoter insertion polymorphism, unique to African ancestry, was genotyped.
Results: *GAD1:* Of the five haplotypes in a distal 7 SNP haplotype block, one haplotype was more common in the total group of alcoholics compared with controls (0.36 *vs* 0.28) and another haplotype was more common in controls than in alcoholics (0.12 *vs* 0.07) ($p=0.007$). This haplotype association was driven by two SNPs: rs3791850 ($p=0.005$) and rs2058725 ($p=0.027$). *GPHN:* There was strong linkage disequilibrium extending for at least 498 kb across this gene. For ease of data handling, this large haplotype block was divided into three: proximal block: the major haplotype (0.32 in controls) was significantly more common in patients with alcohol dependence (AD) only ($p=0.003$) or cocaine dependence only ($p=0.001$); middle block: the major haplotype (0.32 in controls) was significantly more common in patients with AD only ($p=0.002$) and this association was driven by one SNP rs10135701 ($p=0.005$); distal block: the most common haplotype (0.34 in controls) was significantly more common in patients with AD only ($p=0.02$) but also in all patients with heroin addiction ($p=0.006$) [heroin dependence only ($p=0.012$), alcoholism + heroin addiction ($p=0.038$) and heroin + cocaine addiction ($p=0.033$)]. This association was driven by one SNP rs10141952 ($p=0.002$ –0.008). *GABBR1:* There was a 7 SNP haplotype block within the gene that included 5 haplotypes. Of the two most abundant haplotypes, one was more common in patients with AD only relative to controls (0.48 *vs* 0.37) and the other was more abundant in controls relative to alcoholics (0.35 *vs* 0.27) ($p=0.021$). *SLC6A1:* The insertion polymorphism was significantly more common in controls than in patients with AD only (0.28 *vs* 0.13) ($p=0.028$). *GAD2*, *GABRG2*: There were no haplotype or SNP associations with any addiction phenotype for either gene.
Conclusions: This study has identified associations between alcoholism and *GAD1* that is involved in GABA synthesis for phasic inhibition, *GABBR1* encoding presynaptic GABAB receptors that regulate GABA release, *SLC6A1* that encodes the neuronal GABA transporter GAT-1, and *GPHN*, that encodes gephyrin, a scaffolding protein that anchors GABAA receptors to the postsynaptic skeleton. The results of our study suggest that variation in GABAergic pathway genes may predict vulnerability to the development of alcoholism. There are also indications that *GPHN* might play a broader role in addiction.
Keywords: GABA, GAD1, GPHN, GABBR1, SLC6A1.
Disclosures: M. Enoch, Nothing to Disclose; C. Hodgkinson, Nothing to Disclose; E. Gorodetsky, Nothing to Disclose; C. Marietta, Nothing to Disclose; A. Roy, Nothing to Disclose; D. Goldman, Nothing to Disclose.

W56. Role of Genetic Variation in Host-parasite Interaction Associated with Major Mental Illness

Shinichi Kano*, Colin A Hodgkinson, Lorraine V Jones-Brando, Sharon Eastwood, Koko Ishizuka, Minae Niwa, Alec Roy, Nicola Cascella, Faith Dickerson, Anil Malhotra, David Goldman, Paul J Harrison, Robert Yolken, Akira Sawa

Johns Hopkins School of Medicine, Baltimore, Maryland

Background: Recent clinical studies have reported that patients with major mental illness such as schizophrenia and bipolar disorder have co-morbid physical conditions, suggesting that systemic alterations affecting both brain and peripheral tissues might underlie the disorders. The molecular mechanisms underlying such clinical observa-

tions, however, have rarely been addressed. One of the well-known systemic alterations is the elevated antibody level against *Toxoplasma gondii* (*T. gondii*) in peripheral blood. Here we tested the hypothesis that genetic variations relevant for major mental illness may play a critical role in host immune responses against *T. gondii*.
Methods: Using multidisciplinary approaches of serology, cell biology, gene expression profiling, and genetics, we studied the role of genetic mutations in host immune responses against *T. gondii*.
Results: We found that single nucleotide polymorphisms (SNPs) corresponding to amino acid residue 607 of Disrupted in Schizophrenia 1 (DISC1) protein are involved in host interaction with *T. gondii*. Individuals homozygous for a rare variant Phe607 (Phe/Phe genotype) had elevated levels of serum anti-*T. gondii* antibodies. *In vitro* infection experiments revealed altered host-parasite interactions in DISC1 607 Phe/Phe cells in both gene expression profiling and parasite growth assay. In addition, DISC1 607 Phe/Phe genotype was associated with schizophrenia in African-Americans. Further studies on the effects of DISC1 607 SNP on brain immunity are in progress using animal models and cell culture.
Conclusions: We demonstrate that a genetic variation relevant for mental illness affects host immune responses and thereby alters host-pathogen interaction. Our study provides mechanistic insight into one of the well-replicated serological observations, and suggests that specific combinations of genetic factors and environmental insults may underlie systemic alterations in major mental illness.
Keywords: Toxoplasma, immune, DISC1, SNP, lymphoblastoid cells.
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W57. Genome-wide Association Study of Superior Frontal Volumes in Schizophrenia

Ryota Hashimoto*, Masashi Ikeda, Fumio Yamashita, Kazutaka Ohi, Hedenaga Yamamori, Yuka Yasuda, Michiko Fujimoto, Masaki Fukunaga, Kiyotaka Nemoto, Kiyoto Kasai, Norio Ozaki, Nakao Iwata, Masatoshi Takeda

United Graduate School of Child Development, Osaka University, Suita, Japan

Background: Superior frontal gyrus (SFG), of which gray matter reductions have been repeatedly demonstrated in patients with schizophrenia, is involved in self-awareness and emotion. Self-awareness is a cognitive ability to differentiate between self and non-self cues and is pivotal to understand the behavior of other human beings. Disturbance in self-awareness linked to social cognition is a core feature of schizophrenia. Emotional disturbances including meaningless laughter are often seen in patients with schizophrenia. Meaningless laughter was also seen in unaffected siblings of schizophrenia indicating its heritability and laughter is elicited by electrical stimulation of SFG. Gray matter volumes of bilateral SFG have a strong genetic component with estimated heritability of 76–80%. As there is considerable inter-individual variation in the degree of reduced volume of SFG, it appears that genetic influences play a role in determining the severity of volume reduction of SFG in schizophrenia. Although genome-wide association studies (GWAS) of bilateral hippocampal volume have recently been reported, no study has investigated the other brain structures noted in patients with schizophrenia.
Methods: To identify single-nucleotide polymorphisms (SNP) related to SFG volumes, we conducted GWAS of gray matter volume in right or left SFG of patients with schizophrenia and healthy subjects using the Affymetrix Genome-Wide Human SNP Array 6.0. The participants were 158 patients with schizophrenia (95 males, mean age: 35.3 ± 11.8 years) and 378 healthy subjects (187 males, mean age: 36.5 ± 12.7 years). We performed a multiple linear regression analysis to compare the right or left SFG among genotypes, with diagnosis status, age and gender as covariates, using PLINK 1.07 software.
Results: We observed the associations between five variants on 1p36.12 and the right SFG volume at a widely used benchmark for genome-wide significance ($P < 5.0 \times 10^{-8}$, r^2 among SNPs > 0.8), the strongest association was observed at rs4654899, an intronic SNP in the eukaryotic translation initiation factor 4 gamma, 3 (*EIF4G3*) gene on 1p36.12 ($P = 7.5 \times 10^{-9}$), however, no SNP has been found in the volume of left SFG. The rs4654899 polymorphism was the second significantly associated with left SFG volume ($P = 1.5 \times 10^{-6}$). *Post hoc* analyses separately assessed in patients and controls also revealed slightly reduced associations. Examining potential effects of the rs4654899 on expression levels of genes at genome-wide region, significant effects of the rs3767248 proxy SNP for rs4654899 ($r^2 = 1.0$) were identified in expressions of the heterochromatin protein 1, binding protein 3 (*HP1BP3*) gene ($P = 7.8 \times 10^{-6}$), which lies 3′ to *EIF4G3*, as a cis-acting effect (< 200 kb) and the calpain 14 gene (*CAPN14*) ($P = 6.3 \times 10^{-6}$) as a trans-acting effect (> 200 kb). The both *HP1BP3* and *CAPN14* has moderate to high gene expression throughout the adult human SFG. To date, no study reported associations between these genes and schizophrenia although the chromosomal region (1p36.12) related to risk of schizophrenia has been reported. Our results suggest that there are associations at the $P < 5.0 \times 10^{-8}$ level between SFG and variants in the *EIF4G3* gene, which play important roles in expression of the *HP1BP3* and *CAPN14* genes.
Conclusions: We provide new insights into the genetic architecture of a brain structure closely linked to schizophrenia. Identification of causal variants and their functional effects of these genes may reveal yet unknown players in the neurodevelopment and the pathogenesis of schizophrenia.

Keywords: GWAS, neuroimaging, superior frontal gyrus, schizophrenia, gray matter volume.
Disclosures: R. Hashimoto, Nothing to Disclose; M. Ikeda, Nothing to Disclose; F. Yamashita, Nothing to Disclose; K. Ohi, Nothing to Disclose; H. Yamamori, Nothing to Disclose; Y. Yasuda, Nothing to Disclose; M. Fujimoto, Nothing to Disclose; M. Fukunaga, Nothing to Disclose; K. Nemoto, Nothing to Disclose; K. Kasai, Nothing to Disclose; N. Ozaki, Nothing to Disclose; N. Iwata, Nothing to Disclose; M. Takeda, Nothing to Disclose.

W59. A Trial Matching and Mismatching Ondansetron and Sertraline to 5-HTTLPR Alleles in Non-treatment Seeking Alcohol Dependent Individuals

George Kenna*, Lorenzo Leggio, Robert M Swift, William H Zywiak, John McGeary, James Clifford, Jessica R Shoaff, Samuel R Fricchione, Michael Brickley, Kayla Beaucage, Carolina Haass-Koffler, Cynthia Vuittonet

Brown University, Providence, Rhode Island

Background: Animal and human studies suggest that serotonergic mechanisms are associated with the development and maintenance of alcohol dependence however studies evaluating serotonergic medications in heterogeneous populations of alcohol dependent individuals have produced conflicting clinical efficacy. The significance of this study is underscored by the focus on the 'subtype hypothesis' that proposes response and non- response to ondansetron and sertraline is based on the 5′-HTTLPR polymorphisms LL/SS and SL (Johnson, 2000). Aims: LL-carriers of 5-HTTLPR receiving ondansetron compared to either placebo or sertraline, will result in a significant reduction in alcohol consumption during the 7-day period leading up to and during an alcohol self-administration experiment (ASAE). To evaluate the efficacy of sertraline for reducing drinking in participants who carry either the SL or SS variants of the 5-HTTLPR. Additionally that SL and SS-carriers receiving sertraline compared to either placebo or ondansetron, and LL-carrier receiving ondansetron will result in a significant reduction in alcohol consumption.
Methods: A double-blind placebo controlled 2×2 design human laboratory study randomized 78non-treatment-seeking alcohol dependent persons based on their 5′-HTTLPR variant genotype (LL or SS/SL) into one of two counterbalanced arms: the first arm (LL) received either 200 mg/day of sertraline or ondansetron 0.5 mg/day for 3 weeks followed by an alcohol self-administration experiment (ASAE), then received placebo for 3 weeks followed by a second ASAE. Participants received the second drug for 3 weeks followed by a third ASAE. The second arm (SS/SL) received the same medications in the same balanced fashion.
Results: 55 participants (38% women) were randomized by 5′-HTTLPR genotype, LL (44%) or SS/SL (56%) and completed the medication conditions. TLFB data was used to calculate daily alcohol consumption in SDUs. As assessed by the TLFB, during the 7 days prior to the ASAEs, the gene x order ANCOVA (controlling for gender and baseline drinking) for alcohol use was significant [$F(1, 47) = 8.42$, $p = 0.006$]. Reduced drinking for subjects receiving ondansetron matched to LL alleles for the first ASAE was significant [$F(1, 47) = 7.64$, $p = 0.008$]. A trend for reducing drinking was reported by subjects matching sertraline to SS/SL alleles [$F(1, 26) = 3.87$, $p = 0.06$]. For drinking during the ASAEs the gene x order interaction using ANCOVA for alcohol consumed was not significant.
Conclusions: There was modest support for the 'subtype hypothesis' confirming similar results we previously reported that ondansetron reduces drinking in the 7 day period leading up to the first lab session (Kenna *et al*, 2008). This study adds growing support for the pharmacogenetic approach particularly when using ondansetron in alcohol dependent subjects with LL 5-HTTLPR alleles. None of the authors declare a conflict of interest with the information provided in this abstract. Supported by NIAAA Grant R01-AA016079 (Kenna).
Keywords: alcoholism, 5-HTTLPR, ondansetron, sertraline, genetics.
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W60. Monoamine Polygenic Liability in Health and Cocaine Addiction: Imaging Genetics Study

Scott J Moeller*, Muhammad Parvaz, Elena Shumay, Salina Wu, Nicassia Beebe-Wang, Anna Konova, Michail Misyrlis, Nelly Alia-Klein, Rita Goldstein

Mount Sinai Hospital, New York, New York

Background: Gene polymorphisms that modulate serotonin signaling may increase susceptibility to multiple psychopathologies marked by heightened emotional reactivity and poor affect regulation. These symptoms characterize both drug addiction and depression, highly comorbid psychiatric illnesses that exhibit shared perturbations in brain regions and circuits mediating emotional regulation. Of the candidate serotonin regulator genes that could exacerbate emotional dysregulation in addiction, two genes likely to play prominent roles include those encoding the serotonin transporter (SLC6A4) and monoamine catabolic enzyme monoamine oxidase A (MAOA). Because both gene polymorphisms (when considered individually and collectively) modulate emotional reactivity, including responsiveness to aversive stimuli and experiences in health, we reasoned that these genes may also have implications for addiction. The current imaging genetics study therefore tested the combined effects of 5-HTTLPR and MAOA polymorphisms on reactivity to unpleasant stimuli in individuals with cocaine use disorder (CUD) and healthy controls, and whether such enhanced reactivity relates to higher depression symptomatology.
Methods: Using DNA extracted from peripheral blood, 62 CUD and 57 healthy controls were genotyped for a functional insertion-deletion polymorphism of the SLC6A4 promoter (5-HTTLPR), which produces 'short' (S) and

'long' (L) alleles; and the repeat polymorphism (uVNTR, ie, variable number of tandem repeats) upstream of the MAOA promoter, which produces common alleles with high activity (MAOA-H) and low activity (MAOA-L). Although we examined these genes separately, our primary interest was in creating a monoamine risk-allele profile: individuals with L/L 5-HTTLPR and MAOA-H were coded to have 0 risk variants; individuals with L/S 5-HTTLPR, S/S 5-HTTLPR, *or* MAOA-Low were coded to have 1 risk variant; and individuals with either L/S 5-HTTLPR or S/S 5-HTTLPR *and* MAOA-L were coded to have 2 risk variants. On study day, participants also provided comprehensive diagnostic information [including depression diagnosis and current depression symptoms using the Beck Depression Inventory (BDI)], and underwent event-related potentials (ERPs) and functional magnetic resonance imaging (fMRI). ERPs were collected via electroencephalography (EEG) as participants passively viewed standardized unpleasant and neutral pictures (2000 ms per picture; 30 pictures per picture category). Functional MRI was collected while participants performed an event-related color-word Stroop task (during which they had to press for ink color of color words printed in either their congruent or incongruent fonts). Dependent variables were ERP response to unpleasant stimuli [specifically, the *a priori* defined late positive potential (LPP), thought to index stimulus salience] and fMRI response to errors, negatively valenced task events.

Results: Results of 2 (Diagnosis: CUD, control) $\times$ 3 (Risk Variants: 0, 1, 2) analyses of variance (ANOVAs) revealed that having two risk variants was associated with increasingly greater LPP amplitudes to unpleasant images (*vs* neutral images) [$F(2, 99) = 3.21$, $p = 0.045$] and increasingly greater fMRI reactivity to errors (*vs* correct responses) in the anterior cingulate cortex (peak: $x = 9$, $y = 47$, $z = 16$, $T = 3.67$, corrected $p = 0.038$, 1 voxel). Importantly, only in CUD with two risk variants, the higher the LPP reactivity, the higher the BDI (Spearman $r = 0.61$, $p = 0.005$). When examining these genes separately, however, the only effect to reach significance was the main effect of MAOA on LPPs (L > H) [$F(1, 105) = 5.86$, $p = 0.017$], speaking to the importance of combining these serotonin-related genes.

Conclusions: This study provides novel evidence for additive effects of the 5-HTTLPR and MAOA polymorphisms on unpleasant picture reactivity in health and cocaine addiction; more preliminary, yet complementary evidence using fMRI during error bolstered this conclusion. Another important finding was that uniquely in CUD with two risk variants, heightened unpleasant LPPs tracked depression symptomatology. Thus, beyond a potential impact of these risk alleles to initiate illness, in the presence of disease (eg, addiction) these risk alleles may exacerbate illness by increasing sensitivity to unpleasant cues. Reducing such aversive reactivity could be especially important during early abstinence/detoxification, when difficulties with emotion regulation in addicted individuals are accentuated. Taken together, our results support the important idea that neuroimaging is well-positioned to bridge genetic risk and psychopathology. They also provide support for the implementation of future clinical intervention studies that can aim to leverage the combined power of genetic, neuroimaging, and possibly also clinical symptomatology to investigate long-term outcomes and/or pharmacogenetic therapies in drug addiction and other psychopathologies of emotion dysregulation (eg, anxiety, eating disorders, intermittent explosive disorder, and/or borderline personality).

Keywords: cocaine addiction; imaging genetics; 5-HTTLPR; MAOA; multimodal imaging.

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W61. Brain eQTLs Shared by Multiple Psychiatric Diseases

Chunyu Liu*, Chunling Zhang, Chao Chen, Judith Badner, Ney Alliey-Rodriguez, Elliot Gershon, Eric Gamazon, IOCDF-GC, TSAICG, Nancy J Cox

University of Illinois at Chicago, Chicago, Illinois

Background: Genome-wide association studies (GWASs) have detected some common variants associated with psychiatric diseases. But very few of them impact protein coding. Meanwhile, many common SNPs have been found to be associated with gene expression levels in human brain by expression quantitative trait loci (eQTL) mapping. eQTL SNPs (eSNPs) were reported to be enriched in GWAS signals of multiple psychiatric diseases. Recently cross disease analyses showed that some risk genes might be shared by multiple psychiatric diseases.

Methods: We used brain eSNPs ($p < 0.001$) from cerebellum and parietal cortex to overlap GWAS signals ($p < 0.01$) of bipolar disorder (BD), schizophrenia (SCZ), major depression (MDD), autism (ASD), attention deficit and hyperactivity disorder (ADHD), obsessive compulsive disorder (OCD) and Tourette syndrome (TS) to generate lists of eSNPs that were associated with specific diseases. We further compared lists of eSNPs in disease GWAS signals across two or three diseases to identify eSNPs that are shared across multiple diseases. We used SimpleM to calculate number of linkage-disequilibrium (LD) independent tests, further compute probability of chance sharing across diseases. WTCCC type 2 diabetes (T2D) and blood pressure (BP) GWAS data as control data to evaluate specificity of eSNP sharing for psychiatric diseases.

Results: Different diseases shared different amount of brain eSNPs in their GWAS signals in pairwise comparison, ranging from 0 to 241. Numbers of LD-independent eSNPs shared by two diseases ranged from 0 to 73. SCZ and BD shared the most comparing to other disease combinations. Using eQTL data from cerebellum and parietal data, gene level and exon level analyses showed consistent pattern of sharing. In three-disease comparison, we found one eSNP shared by SCZ, BD and MDD; and eight eSNPs shared by SZ, BD and OCD. Fifteen genes were regulation targets of these SNPs. Most of these genes have their expression levels associated with mouse behavioral phenotypes. Interestingly, T2D and BP, as a control disease or trait, have the least brain eSNPs in its GWAS signals than psychiatric diseases. But the enrichment of brain eSNPs are still significantly higher than chance. One eSNP was shared between T2D and

SCZ at chance level. Diastolic BP and Sistolic BP shared 7 Brain eSNPs.
Conclusions: Our brain eQTL data helped to illustrate functionality of SNPs and their target genes for multiple diseases. Different diseases share different amount of eSNPs that can be captured by GWAS. Multiple novel risk genes were identified as shared risk genes of psychiatric diseases. These findings suggest that genetic foundation of many common diseases could cross diagnosis boundaries. Development of a specific disease might be a product of combination of those disease-risk building blocks.
Keywords: brain eQTL, SNP, cross dissease, association.
Disclosures: C. Liu, Nothing to Disclose; C. Zhang, Nothing to Disclose; C. Chen, Nothing to Disclose; J. Badner, Nothing to Disclose; N. Alliey-Rodriguez, Nothing to Disclose; E. Gershon, Nothing to Disclose; E. Gamazon, Nothing to Disclose; I., Nothing to Disclose; T., Nothing to Disclose; N. Cox, Nothing to Disclose.

W62. Combined Brain Transcriptome Meta-analysis and Genome-wide Association Studies Provide Evidence for Shared Genetic Risk Between Depression and Other Brain Disorders

Etienne Sibille*, Ying Ding, Lun-Ching Chang, Xingbin Wang, Jean-Philippe Guilloux, Jenna Parrish, Hyunjung Oh, David A Lewis, George C Tseng

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: Major depression is a common and severe disorder that is significantly co-morbid with all major psychiatric disorders and often associated with selected medical illnesses, notably metabolic syndrome, cardiovascular disease and immune/inflammation-related diseases. Whether depression itself, or common upstream genetic factors, underlie these reciprocal links with other illnesses, is the subject of investigation, as it has major implications for health management. Using a top-down genetic approach, we tested here the potential of depression-related genes to predict risk for several categories of psychiatric and other medical illnesses.
Methods: (1) MDD-related genes were identified by meta-analysis of eight gene array studies in three corticolimbic brain regions in 101 postmortem brains (50.5% MDD); (2) The biological significance and functional coherence of identified genes was tested by coexpression network and chromosomal localization; (3) Prediction of shared risk was performed through enrichment analysis of MDD-related genes in gene sets identified by nearby genetic variants in the $\sim$1400 genome-wide association studies (GWAS) from the Catalog of Published GWAS and sorted by disease categories.
Results: MDD-related genes ($n = 308$ at 20% false discovery rate (FDR); 566 at 25%FDR) include novel and previously-implicated neuroplasticity- and stress-related genes, encompass multiple synaptic and signaling pathways, and suggests complex changes in cell structure and function. MDD-related genes display low connectivity and hubness in coexpression networks and are evenly distributed throughout the genome. Genetic variations nearby MDD-related genes are associated with greater risk for neuropsychiatric disorders ($p < 0.0004$), neurological disorders ($p = 0.0058$) and age-related phenotypes (brain changes and disease age of onset; $p = 0.0043$), but not for metabolic syndrome, cardiovascular disease, immune and inflammation-related disorders, multiple cancer, and other multiple medical illnesses (all $p > 0.25$).
Conclusions: The set of identified MDD-related genes provides firm leads for investigating the polygenic heterogeneous biology of MDD, and implicates altered neuroplasticity and stress-related factors. The genetic prediction analysis provides evidence for a genetic structure that independently and potentially directly confers risk for multiple other brain disorders. The risk associated with multiple medical illnesses appears independent of the genetic liability for brain disorders. Together these findings contribute to delineating pathways for depression-related biological risks across illnesses.
Keywords: depression, gene array, transcriptome, postmortem, cingulate, amygdala, genetic, psychiatric disorders, cardiovascular disease, coexpression network, comorbidioty.
Disclosures: E. Sibille, Nothing to Disclose; Y. Ding, Nothing to Disclose; L. Chang, Nothing to Disclose; X. Wang, Nothing to Disclose; J. Guilloux, Nothing to Disclose; J. Parrish, Nothing to Disclose; H. Oh, Nothing to Disclose; D. Lewis, Nothing to Disclose; G. Tseng, Nothing to Disclose.

W63. A Candidate Gene Analysis of Acoustic Startle Latency and Psychosis

Lauren Gensler, Tanja Jovanovic, Alicia K Smith, Lynn Almli, Seth D Norrholm, Ebony Glover, Kerry J Ressler, Bekh Bradley, Erica Duncan*

Atlanta VA Medical Center, Decatur, Georgia

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 required 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). We previously reported that startle latency is highly heritable (90%) among a sample of SCZ and CON subjects and their first-degree relatives (1). For this study, we undertook an analysis of selected single nucleotide polymorphisms (SNPs) to determine potential genetic association with latency as a potential inroad into genetically based vulnerability to psychosis.
Methods: The subjects were 245 individuals tested as part of the Grady Trauma Project (PI: K Ressler). Startle testing was conducted by means of a Biopac M150 system according to published methods from our group (2). Data on startle latency were extracted from a session assessing startle magnitude, fear potentiation of startle, and startle latency.

Subjects received diagnostic and symptom ratings, from which data they were dichotomously classified as having a history of a psychotic disorder *vs* no history. DNA was obtained from salivary samples for a genome-wide association study of post-traumatic stress disorder. Genotyping was performed with the Illumina Human Omni1-Quad and OmniExpress BeadChips. Candidate SNPs were selected from 12 genes associated with startle, startle latency and/or SCZ in the animal or human literature. All SNPs had call rates >95% and were consistent with Hardy-Weinberg proportions ($p > 0.001$). For associations between SNPs and log(latency), separate linear regressions were conducted using an additive model (eg homozygote 1 *vs* heterozygote *vs* homozygote 2), controlling for the following covariates: age, sex, chip type, and the top ten principal components (to correct for population substructure). If the minor allele in the additive model contained less than 10 subjects we followed up with a dominant model by combining the minor homozygote with the heterozygote. Follow up logistic regressions were run on the dichotomous history of psychosis variable, controlling for age, sex, chip type, and the top ten principal components.

Results: Of 73 SNPs tested, eight predicted slowing of latency ($p < 0.05$). The SNP rs1912718 in the *GRID2* gene significantly predicted log(latency) in an additive model (Beta = −0.263, $p = 0.004$), indicating that subjects with the AA genotype had slower mean latency (81.7 ± 34.7 msec) than subjects with CA (69.2 ± 20.9 msec) or CC (69.8 ± 19.6 msec) genotypes. For this same SNP the AA homozygotes were more frequent in the psychosis group than in the group without a history of psychosis (Beta = −0.73, $p = 0.004$) after controlling for other variables in the model. These results are not likely to be simply due to sample size: when we ran similar dominant models by collapsing the minor allele with the heterozygote the results were concordant for latency and for history of psychosis. The SNP rs980989 in the *DISC1* gene had too few subjects with the minor allele for the additive model regression on latency to be informative. A regression on log(latency) indicated that genotype significantly predicted latency: the GG subjects had longer mean latency (75.0 ± 26.9 msec) than the mean latency of the TG and TT subjects (63.2 ± 16.8 msec; Beta = −0.42, $p = 0.009$).

Conclusions: Latency of the acoustic startle response, an index of neural processing speed, is a potential endophenotype for SCZ: latency is prolonged (ie slower) in SCZ and highly heritable. A candidate gene approach yielded potentially promising SNPs that predicted slowing of latency, particularly rs1912718 (*GRID2*) and rs980989 (*DISC1*) as well as several others. For rs1912718 the association of genotype with latency was concordant with the association of genotype and psychosis. 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 psychotic disorders. Supported by NIMH (MH071537 and MH096764 to K.J.R. and MH085806 to A.K.S).

(1) Hasenkamp W, Epstein MP, Green A, Wilcox L, Boshoven W, Lewison B, Duncan E (2010) Heritability of acoustic startle magnitude, prepulse inhibition and startle latency in schizophrenia and control families. Psychiatry Res 178(2):236–43 (2) Jovanovic T, Blanding NQ, Norrholm SD, Duncan E, Bradley B, Ressler KJ (2009) Childhood abuse is associated with increased startle reactivity in adulthood. Depression and Anxiety 26:1018–26.

Keywords: acoustic startle, latency, psychosis, genetics, single nucleotide polymorphisms.

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W64. DSM-IV and DSM-5 Alcohol, Cannabis, and Methamphetamine Use Disorders: Rates, Heritability, and Co-morbidity in an American Indian Community Sample

David Gilder*, Ian Gizer, cindy L Ehlers

The Scripps Research Institute, La Jolla, California

Background: DSM-IV Alcohol Use Disorder (AUD), Cannabis Use Disorder (CUD), and Amphetamine Use Disorder (MUD) defined as abuse or dependence are substantially heritable and co-morbid with anxiety, affective, ASPD, and substance use disorders in samples drawn from the general U.S. population. Some American Indian tribes have high rates of DSM-IV AUD, CUD, and MUD. Little is known about to what extent DSM-5 AUD, CUD, and MUD are heritable and co-morbid with psychiatric and other substance use disorders in any population, including high risk American Indian samples.

Methods: This study examined rates of DSM-IV and DSM-5 AUD, CUD, and MUD (considered without one year clustering) and heritability of those disorders with age and gender as covariates in a sample of 847 (352 male, 495 female) adult American Indians living on contiguous reservations. DSM-IV and DSM-5 diagnoses were obtained using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA). Heritability analyses were conducted using SOLAR, which uses a variance components approach to estimate heritability. Because the proposed DSM-5 substance use disorder diagnosis, which requires ⩾2 symptom criteria, also includes a specifier for the severity of the disorder based on the number of use disorder criteria (2–3 criteria, mild; 4–5 criteria, moderate; and ⩾6 criteria, severe), we assessed heritability of DSM-5 AUD, CUD, and MUD considered as ⩾2 criteria, ⩾4 criteria; and ⩾6 criteria *vs* no diagnosis. We also assessed whether there was an association (OR, *p*-value) of DSM-IV anxiety and affective and cannabis and methamphetamine use disorders with DSM-IV and DSM-5 AUD in the presence of potential covariates, age, ASPD, and age of first alcohol intoxication, in men and women separately.

Results: The rates of use disorder diagnoses (men, women) were: DSM-IV AUD: 76.1%, 64.1%; CUD: 48.6%, 30.8%; MUD: 36.4%, 35.3%. The rates of DSM-5 use disorders (men, women) were: with ⩾2 criteria: AUD: 75.6%, 65.5%; CUD: 51.1%, 30.4%; MUD: 35.5%, 35.3%; with ⩾4 criteria: AUD: 59.7%, 45.2%; CUD: 32.7%, 20.6%; MUD: 32.1%, 31.7%; and with ⩾6 criteria: AUD: 40.9%, 32.1%; CUD: 19.6%, 14.3%; MUD: 27.3%, 27.2%. Heritability (*p*-value) of DSM-IV use disorders were: AUD: 0.13 (0.12); CUD: 0.26

(0.01); MUD: 0.23 (0.02). Heritability (p-value) of DSM-5 use disorders were: with $\geqslant 2$ criteria: AUD: 0.03 (0.40); CUD: 0.32 (0.01); MUD: 0.28 (0.01); with $\geqslant 4$ criteria, AUD: 0.29 (0.01), CUD: 0.19 (0.08), MUD: 0.38 (0.001); and with $\geqslant 6$ criteria: AUD: 0.43 (<0.001), CUD: 0.09 (0.31), MUD: 0.49 (<0.001). DSM-IV ASPD and both drug use disorders were highly co-morbid (4.84–16.99, all $\leqslant 0.01$) with both DSM-IV and DSM-5 AUD in men and women. In men, no anxiety or affective disorder was co-morbid with DSM-IV or DSM-5 AUD. In women, only any anxiety disorder was co-morbid with DSM-IV AUD individually (2.12, <0.001) and in the presence of covariates (2.40, 0.011). However, any anxiety disorder (1.93, 0.010), any affective disorder (1.72, <0.001), and major depressive disorder (1.56, 0.030) were all individually co-morbid with DSM-5 AUD. In the presence of covariates, any anxiety disorder (2.05, 0.032), any affective disorder (2.00, 0.010), and major depressive disorder (1.81, 0.034) were co-morbid with DSM-5 AUD.

Conclusions: The rates of DSM-IV and DSM-5 AUD, CUD, and MUD in this American Indian community sample are substantially higher than those reported for the general U.S. population. In this sample, the heritability of DSM-IV use disorders is intermediate between DSM-5 low and moderate severity diagnoses for AUD and CUD and below that of DSM-5 low severity diagnosis for MUD. Heritability of DSM-5 AUD and MUD diagnoses increases as severity increases, whereas heritability of DSM-5 CUD decreases as severity increases. Differences in heritability, as well as differences in co-morbidity of anxiety and affective disorders with AUD in women assessed using DSM-5 as opposed to DSM-IV, may result from the inclusion of diagnostic orphans and/or inclusion of craving and exclusion of legal problems as diagnostic criteria in DSM-5 and may identify gender differences in underlying heritable risk factors. Differences in patterns of heritability for different substances using DSM-5 severity criteria are consistent with the notion that environmental as well as underlying genetic risks may vary for different substances within the same population. Requiring one year clustering for DSM-5 diagnoses may alter heritability and co-morbidity findings for these diagnoses. This study suggests that the use of the new DSM-5 as compared to the previous DSM-IV diagnostic system may influence the rates, heritability, and co-morbidity of substance use disorders, at least in the high risk population from which this sample was drawn (supported by AA10201 and NIMHD).

Keywords: DSM-IV and DSM-5, substance use disorders, American Indian.

Disclosures: D. Gilder; I. Gizer, Nothing to Disclose; c. Ehlers, **Part 1:** I am a consultant for Neurocrine Biosciences Inc (biotechnology company), 12780 El Camino real, San Diego, CA.

W65. A Second Large-scale Candidate Gene Analysis of Endophenotypes for Schizophrenia Further Implicates the Glutamate and Neuregulin-ErbB4 Signaling Pathways

Tiffany A Greenwood*, Gregory A Light, Neal R Swerdlow, David L Braff

University of California San Diego, La Jolla, California

Background: We developed a second custom single nucleotide polymorphism (SNP) array consisting of 1536 SNPs within 64 candidate genes for schizophrenia and related endophenotypes. Genes were selected based on the results of our first custom array, originally developed for use with the family-based sample collected by the Consortium on the Genetics of Schizophrenia (the COGS chip), which implicated neuregulin-ErbB4 signaling and genes in the glutamate and GABA pathways as being of particular relevance to schizophrenia. Genes implicated in recent genome-wide association and copy number variation studies of schizophrenia were also included. We utilized this second custom SNP array to conduct association analyses of the same 127 schizophrenia patients and 92 controls of European ancestry collected locally by the UCSD Schizophrenia Research Program for whom we have previously published data for the COGS chip.

Methods: In addition to schizophrenia diagnosis, ten neurophysiological and neurocognitive endophenotypes that tap into critical schizophrenia-related domains and clearly discriminate between patients and controls were selected for analysis. These included prepulse inhibition (PPI), startle habituation, P50 S1 amplitude, the antisaccade task, the Letter-Number Span (LNS) forward and re-ordered, the California Verbal Learning Test (CVLT-II) immediate and delayed recall, and the Wisconsin Card Sort Test (WCST) perseverative responses and categories complete. Of the 1536 SNPs, 1403 remained for analysis following elimination based on quality control thresholds for call rate, cluster separation, and marker informativity. Association analyses were conducted using linear or logistic regression for the endophenotypes and diagnosis, respectively, covarying for age and/or sex included as necessary based on significant correlations with the endophenotypes. The first two multidimensional scaling principal components were also used as covariates in all analyses to correct for residual population stratification, and label-switching permutation procedures were utilized to estimate the empirical significance of the results.

Results: A total of 29 genes were found to be associated with at least one endophenotype or schizophrenia at the $p<0.01$ level or less. The most significant finding in these analyses was the association of *TCF4* with WCST perseverative responses ($p=8.7\times10^{-5}$). Many of the associated genes interact on a molecular level and, in combination with the results from our initial custom chip, provide strong evidence for the involvement of the glutamate and neuregulin-ErbB4 signaling pathways in schizophrenia and related endophenotypes. Additionally, 14 genes displayed evidence for pleiotropy, revealing significant associations with two or more endophenotypes. Among these genes were *ANK3*, *CACNA1C*, *NOS1*, *NRXN1*, and *TCF4*, further supporting a role for these genes in mediating schizophrenia susceptibility. We will expand on these results through the on-going analysis of an additional sample of more than 350 patients and controls for both custom arrays.

Conclusions: These data extend our knowledge of the genetic basis of schizophrenia and related endophenotypes. Detailed analyses of the genes associated with each endophenotype are needed to elucidate the underlying genetic pathways involved in schizophrenia susceptibility. Identifying the causal genetic variants and elaborating their molecular interactions will ultimately facilitate the early

identification of schizophrenia and the development of individualized treatment strategies.
Keywords: schizophrenia endophenotype genetics association.
Disclosures: T. Greenwood, Nothing to Disclose; G. Light, Nothing to Disclose; N. Swerdlow, **Part 1:** I have received unrelated compensation for consulting services from Neurocrine.; D. Braff, Nothing to Disclose.

W66. An Interactive Effect of the Two SNPs in the Catechol-O-Methyltransferase (COMT) Gene on Dopamine Concentration in the Prefrontal Cortex

Elena Shumay*, Joanna Fowler, Nora D Volkow

Brookhaven National Laboratory, Upton, New York

Background: COMT enzyme degrades dopamine (DA) regulating DA levels in prefrontal brain regions (PFC), therefore the *COMT* gene is of substantial interest to neuroscience and biological psychiatry. Numerous studies have examined the association of the functional Val158Met polymorphism (rs4680) with conditions of disrupted prefrontal activity including psychiatric disorders (schizophrenia and addiction) and age-related cognitive decline. It remains unclear, however, whether the COMT genotype affects DA concentration in PFC. Here we explored the relationships between rs4680 and a neighboring SNP (rs4818) that was previously reported as a modulator of the COMT mRNA stability using high resolution sequencing of the population samples. We than tested the effect of the two SNPs on baseline DRD2 availability in the PFC measured by PET with 11C raclopride as single genetic markers and in their combination.
Methods: All subjects were originally recruited to participate in imaging studies at BNL Imaging Center and agreed to provide a genetic sample for the analysis. Genotyping of the 400 population samples was performed by PCR with in house flanking primers and optimized conditions of amplification. Sequencing of the amplicons was performed using an ABI 3130xl (Applied Biosystems). After the initial editing with the Sequencher software program (Gene Codes), individual sequences were aligned against the reference genomic sequence (hg18) using CLUSTALW program (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Individual genotypes were assigned based on the amplicon reads. About 10% of the samples were analyzed as duplicates to ensure the reads quality. 11Craclopride images of 68 African-American individuals (AA) who had participated as healthy controls for studies evaluating DA D2 receptor availability were used to assess the effect of the COMT genotypes on raclopride binding potential at preselected ROIs in cortical and subcortical brain regions.
Results: In the population, the frequencies of the rs4680 alleles were close to those predicted by the HWE ($\chi 2 = 0.03$), while we observed more occurrences of the rare G-allele of rs4818 than expected (8 *vs* 5.6). SPSS analysis (GLM) revealed that although the main effect of both SNPs does not statistical significance, their interaction was associated with baseline DRD2 availability in dorsal PFC (BA 13: $F(2, 59) = 4.45$, $p = 0.016$); anterior cingulate gyrus (BA 32: $F(2, 59) = 3.82$, $p = 0.028$); and inferior PFC (BA 45: $F(2, 59) = 4.4$, $p = 0.017$).
Conclusions: Our preliminary data are in line with previous finding, suggesting that the combinatorial effect of two SNPs influences the local structure of the resulting mRNA transcript and subsequently altering its translational efficacy which manifests in variable levels of local COMT enzymatic activity.
Keywords: COMT gene, 11Craclopride imaging, PFC, DRD2 receptor.
Disclosures: E. Shumay, Nothing to Disclose; J. Fowler, Nothing to Disclose; N. Volkow, Nothing to Disclose.

W67. Differential Allelic Expression and cis-Regulatory Sites at Human Neuronal Genes

Qiaoping Yuan*, Seungeun Yeo, Zhifeng Zhou, Colin A Hodgkinson, David Goldman

NIAAA, Bethesda, Maryland

Background: Unknown *cis*-acting regulatory variants contribute to most heritable traits, including neuropsychiatric diseases and behavioral differences. RNA sequencing (RNAseq) coupled with genome is a powerful tools to detect differential allele expression and can target the search for corresponding *cis*- acting regulatory sites at which there is functional genetic variation. Recently, GEUVADIS (Genetic European Variation in Disease, A European Medical Sequencing Consortium) sequenced mRNAs isolated from lymphoblastoid cell lines of 462 humans whose genomes had been sequenced in the 1000 Genomes Project. Using the GEUVADIS RNAseq data along with phased genotypes from the 1000 Genome Project, we evaluated human neuronal genes for differential allele expression and bioinformatically identified potential *cis*-acting regulatory variants in core promoter regions. Initially 21 977 genes with heterozygous exonic SNPs were evaluated, and genes expressed both in lymphoblastoid cell lines and neuronal cells are the main focus of this report.
Methods: The RNA sequencing (RNAseq) data for 462 human lymphoblastoid cell lines from CEPH (CEU), Finn (FIN), British (GBR), Toscani (TSI) and Yoruba (YRI) samples in the 1000 Genomes sample collection was obtained from GEUVADIS (Genetic European Variation in Disease, A European Medical Sequencing Consortium). For each sample, poly A + RNA had been sequenced by GEUVADIS on Illumina HiSeq instruments to a minimum depth of 20 million mapped reads. The RNAseq reads that had been mapped using GEM mapper v 1.349 were downloaded as BAM files from ArrayExpress (ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/GEUV/E-GEUV-1/processed/). Phased genotypes from autosomal chromosomes were downloaded from ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/GEUV/E-GEUV-1/genotypes/ and chrX from ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/release/20110521/. To quantitate differential allele expression genome-wide, RNAseq reads containing the reference base and alternative base in the BAM files were counted at each genotypically heterozygous

location within RefGene exonic regions. RNAseq allele-specific reads ratios on both phased chromosomes were calculated for each gene and *p*-values based on the binomial model were calculated with the read counts at each heterozygous exonic site. Candidate genes with deferential allelic expression were selected based on RNAseq reads ratios, *p*-values and different FDR cutoffs.

Results: From the 462 human cell lines studied by GEUVADIS, a total of 549 368 heterozygous exonic SNP sites at 21 977 refGenes were surveyed. There were 281 982 heterozygous exonic SNP sites at 14 955 genes with RNAseq read coverage of $>=5\times$ in at least one sample, representing the genes at which significant differential allele expression might be detected (for example, a 5/0 allele expression ratio). 138 533 heterozygous exonic SNPs in 13 491 genes had a *p*-value $<=0.05$ in at least one sample and 98 960 SNPs survived 10% FDR cutoff. 268 of these were at sites identified for 169 diseases/traits in the NHGRI catalog of genome-wide association studies (GWAS). 27 of them were significant variants identified in the GWAS for neuropsych phenotypes, such as alcohol dependence and bipolar disorder. We were able to identify potential *cis* acting regulatory sites at many of the differentially expressed selected genes based on correlation between the differentially expressed alleles and bioinformatically functional alleles in the phased gene promoters and upstream 1 Kb regions. At neuronal genes that the focus of further analysis and verification we were able to show that several known *cis*-acting regulatory sites including rs16147 in the promoter region of NPY were detectable by this genomic approach, and rs16147 had a effects on gene regulation as we had observed previously in lymphoblastoid cell lines, brain and a reporter construct expressed in a neuronal cell line.

Conclusions: Because gene expression and regulation are altered by tissue and cell type specific factors, the detection of *cis*-acting loci altering gene expression in brain investigation ideally would use neuronal tissues or cells. Although it is difficult to obtain such samples from large numbers of people, neurons derived from induced pluripotent stem cells may soon be used to capture effects of cell-specific factors. Meanwhile we have taken advantage of an existing resource of human RNASeq data derived from peripheral lymphocytes, identifying evidence of *cis*-acting regulatory elements in genome wide fashion, finding genetically correlated variants predicted to be regulatory at many of the genes that are differentially expressed, and confirming several. In several instances, including NPY, HTTLPR, and MAOA, regulatory variants altering gene expression in brain have previously been shown to have parallel effects in readily accessible lymphoblastoid cells. The genome wide detection of differential expression and correlation with potential *cis*-regulatory variants identifies a panel of genes and polymorphisms that can be further evaluated by studies on brain tissue, neuronal cells and reporter constructs.

Keywords: human, expression, regulation, genetics.

Disclosures: Q. Yuan, Nothing to Disclose; S. Yeo, Nothing to Disclose; Z. Zhou, Nothing to Disclose; C. Hodgkinson, Nothing to Disclose; D. Goldman, Nothing to Disclose.

W68. Application of Sequencing, Fatty Acid Profiling, and Metabolomics Investigations to Explore Pathogenesis and Treatment Strategy for Anorexia Nervosa

Pei-an Betty Shih*, Jun Yang, Christophe Morisseau, Ashley Van Zeeland, Toni-Kim Clarke, Andrew W Bergen, Pierre Magistretti, Katherine Ann Halmi, Wade Berrettini, Nicholas Schork, Walter H Kaye, Bruce D Hammock

University of California, San Diego, California

Background: Individuals with Anorexia Nervosa (AN) restrict eating and become emaciated. They tend to have an aversion to foods rich in fat. We have identified a novel AN susceptibility gene, Epoxide Hydrolase 2 (*EPHX2*), through a series of complementary genetic study designs (GWAS, exon-based sequencing, single-locus association and replication studies) in 1205 AN and 1948 controls ($p=0.0004$–0.00000016) (*Molecular Psychiatry*, *2013*). To assess the mechanisms by which *EPHX2* influences AN risk, here we applied a multi-disciplinary approach using lipidomics, metabolomics, and *ex vivo* techniques to evaluate the biological functions of *EPHX2*.

Methods: *EPHX2* codes for soluble epoxide hydrolase (sEH) which binds to specific epoxides and converts them to the corresponding diols; thereby it plays a major role in the metabolism of endogenous lipid epoxides, such as the epoxyeicosatrienoic acids (EETs), a derivative of arachidonic acid (ARA). We measured polyunsaturated fatty acids (PUFAs) and eicosanoids (bioactive lipid mediators that are derived from the metabolism of PUFAs) in 20 female AN cases and 17 age-, gender- and race-matched controls using the GC/MS and LC/MS/MS systems. EET-to-DHETs ratios were calculated as proxy markers of *in vivo* sEH activity, whereas *ex vivo* sEH activity was directly measured in 36 controls.

Results: Omega 6 PUFAs (DGLA, ARA, and Osbond acids) and omega 3 PUFAs (ALA, SDA, EPA, and DHA) were significantly elevated in ANs compared to controls ($p=0.0003$–0.00004). Controlling for effects of age and BMI, the 8.9.EpETrE of ARA and 8.9.EET-to-Diol ratio were significantly higher in ANs compared to controls ($p<0.0001$) whereas the eicosanoids markers of LA, another PUFA substrate of sEH, were not significantly different. The *ex vivo* sEH activity measured in 13 controls showed marginal association with 8.9.EET-to- Diol ratio ($p=0.07$), suggesting 8.9.EpETrE of ARA may be a sensitive activity target of sEH. Variant allele carriers of an AN-associated *EPHX2* SNP in the 3′-UTR region showed significant association with 11,12- EET-to-Diol ratio ($p=0.02$) after controlling for the effects of age, BMI, and disease status, further providing evidence for *EPHX2* variation's influence on sEH activity and the subsequent effects on PUFA metabolism and eicosanoid activity.

Conclusions: This study suggests that *EPHX2* influences AN risk through biological interaction with the PUFA pathway, specifically the ARA. It demonstrates that an application of multiple genetic designs interrogating common and rare variation is an effective approach to identify otherwise unsuspected risk genes in AN; that joint investigations of

genetic mechanisms with their biological non-genetic partners (eg: diet, stress) may lead to improved understanding of pathophysiology, and new treatment strategies for AN.

Keywords: anorexia nervosa, arachidonic acid, epoxide hydrolase, polyunsaturated fatty acids, eicosanoids.

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W69. Evidence for Epistatic Interactions Between ANK3 and Voltage Gated Ion Channels in Influencing Schizophrenia Risk

Rebecca Birnbaum*, Fengyu Zhang, Daniel Weinberger

Lieber Institute for Brain Development, Baltimore, Maryland

Background: The ANK3 gene, which encodes the ankyrin G scaffolding protein, has been implicated as a susceptibility gene for both bipolar disorder and schizophrenia in genome-wide association studies. The interactions of ankyrin G with voltage-gated ion channels is thought to be critically important to neuronal structure and function, for the recruitment and localization of sodium and potassium channels to the axon initial segment, and for neurons to initiate and propagate action potentials. Interestingly, many of the ion channels that are putative ankyrin G interactors have also been implicated as susceptibility genes for neuropsychiatric disorders, namely SCN2A implicated in autism spectrum disorders which encodes the sodium $Na_v1.2$ channel, and KCNQ2 implicated in bipolar disorder and schizophrenia, which encodes a subunit of the M potassium channel. Data also suggest that ankyrin G may interact with CACNA1C, a susceptibility gene for both bipolar disorder and schizophrenia, which encodes a subunit of a voltage gated calcium channel. KCNH2, a schizophrenia susceptibility gene which encodes the brain selective potassium HERG channel, might also be considered a potential interactor. We hypothesized that given the biological interaction of ankyrin G with ion channels, that ANK3 variants interacting with variants at other genes would influence its clinical association. In the present analysis, we aimed to identify statistically significant interactions of SNPs in ANK3 with SNPs at any of four genes encoding ion channels (KCNH2, KCNQ2, SCN2A, CACNA1C) that influence schizophrenia risk in multiple case-control cohorts. We then aimed to validate the two-locus statistical epistasis in a biological model, demonstrating ANK3 genetic epistasis with ion channels, at the level of gene transcription.

Methods: Criteria for inclusion were SNPs within the ANK3 region or regions of the 4 genes encoding ion channels, with evidence of association in published schizophrenia GWAS studies, or with evidence of single SNP cis-eQTL association in the BrainCloud expression database (Colantuoni *et al*, Nature 2011). A total of 84 independent SNPs (with $r2 < 0.3$) were selected, including 11 ANK3 SNPs, and 13 SCN2A SNPs, 10 KCNH2 SNPs, 44 CACNA1C SNPs, and 6 KCNQ2 SNPs. Four schizophrenia case-control cohorts were used in the clinical analysis: (1) LIBD/CBDB (Lieber Institute for Brain Development/Clinical Brain Disorders branch, NIMH) (413 cases, 2432 controls) (2) GAIN (Genetic Association Information Network) (936 cases, 1190 controls) (3) Scotland cohort (537 cases, 540 controls) (4) Germany cohort (518 cases, 517 controls). Clinical association was evaluated by regressing case status against SNP1 (in ANK3), SNP2 (in ion channel gene), and the interaction SNP1*SNP2. Using logistic regression, each SNP and each SNP pairwise interaction was evaluated in schizophrenia case-control cohorts individually and then in a meta-analysis. SNP pairwise interactions that were statistically significant in the clinical datasets were then examined for their association with gene expression in postmortem DLPFC (dorsolateral prefrontal cortex) samples, using the BrainCloud expression database. The association was evaluated using linear regression, modeling gene expression at a microarray probe (that mapped to the ANK3 or the ion channel gene transcript) as a function of SNP1, SNP2, and SNP1*SNP2.

Results: 5 pairwise interactions between ANK3 SNPs and SNPs in ion channel genes were significant for schizophrenia risk association, within the 4 case-control cohort meta-analysis ($p < 0.01$), including 3 ANK3-SNC2A interactions, 1 ANK3-KCNH2 interaction, and 1 ANK3-KCNQ2 interaction. An ANK3-CACNA1C interaction was nearly significant with a combined p-value for association in the meta-analysis of 0.01 Analysis of the same pairwise interactions using the BrainCloud expression database validated epistasis for ANK3-SCN2A, ANK3-KCNQ2, and ANK3-CACNA1C. ANK3 transcripts sampled by a probe in the microarray with no main effect when regressed against the single ANK3 SNP, rs1938526, showed significant epistatic association when combined with SCN2A rs3769931 (Interaction Coeff $= -0.3$, $p = 5 \times 10{-}3$). Similarly, ANK3 demonstrated no cis-association with the single ANK3 SNP rs10994253 but showed significant association when combined with KCNQ2 rs4603829 (Interaction Coeff $= 0.51$, $p = 1.53 \times 10{-}2$). Finally, the ANK3 SNP rs7902905, though not showing ANK3 cis- association, revealed an interaction with CACNA1C rs7971903 on ANK3 expression (Interaction Coeff $= 0.61$, $p = 9.29 \times 10{-}3$).

Conclusions: The present analysis suggests interactions of ANK3 with ion channel genes (SCN2A, KCNQ2, and CACNA1C) in influencing schizophrenia susceptibility. That gene products in a potential etiological causal pathway are acting epistatically to influence risk, and that the non-synonymous risk variants are affecting gene expression, parallels findings of epistasis at other loci that may influence schizophrenia risk (eg NRG1-ERBB4-PI3K). While

none of the clinical epistatic associations would survive agnostic statistical correction for all tests performed, these interactions are predicted by the basic biology of ANK3 and their confirmation at the level of gene expression support their validity. Ongoing work includes analysis of each interaction, and structural analysis of ANK3, via RNA Sequencing, to quantify gene expression and identify novel exons and transcripts that may help elucidate schizophrenia risk.

Keywords: schizophrenia, ANK3, EPISTASIS.

Disclosures: R. Birnbaum, Nothing to Disclose; F. Zhang, Nothing to Disclose; D. Weinberger, Nothing to Disclose.

W70. Suicidal Ideation and Suicidality in an American Indian Community: Comorbidity with Trauma Exposure, ASPD, Affective Disorders and Drug Dependence

Cindy L Ehlers*, David Gilder

The Scripps Research Institute, La Jolla, California

Background: American Indians appear to experience a higher rate of trauma and suicide than what has been reported in general population surveys. American Indians also suffer higher alcohol related death rates than any other U.S. ethnic group in the U.S. population. Therefore efforts to delineate factors that may uniquely contribute to increased likelihood of suicide and substance use disorders (SUD) over the lifetime in American Indians are important because of the high burden of morbidity and mortality that they pose to American Indian communities. Therefore, the aims of the present study were: (1) to document the range of suicidal behaviors reported in an American Indian community; (2) to study the relationship between suicidal behaviors and demographic/psychosocial characteristics including trauma exposure; (3) to determine the comorbidity of suicidal behaviors with substance dependence, affective disorder, and conduct disorder/antisocial personality disorder.

Methods: Participants were 715 American Indians recruited from reservations who were assessed with the semi-structured assessment for the genetics of alcoholism (SSAGA), data were collected on suicidal ideation, suicide plans, suicide attempts, and completed suicides. Multivariate ANOVA and logistic regression were used to evaluate the data.

Results: Of the 715 participants, 15% of the population reported suicidal ideation only, 5% had reported creating a plan but had not attempted, 15% had attempted but survived and 3% had a fatal suicide attempt. There were no differences in age between those who experienced any of the levels of suicidal thoughts/behaviors and those who did not. Equal numbers of men and women reported ideation/plans, more women had made non-fatal attempts, where as more men had fatal suicides. There was no association between levels of suicidal thoughts/behaviors and measures of cultural identification or frequency of thoughts of historical trauma. A comparison of those individuals with no suicidal thoughts/behaviors to those with ideation only revealed that family history of alcoholism (FHA), having experienced assaultive trauma, having a lifetime diagnoses of any affective or anxiety disorder (ANY AXAF), post traumatic stress disorder (PTSD), Alcohol dependence (ALCDEP4) and Marijuana dependence (MJ4) and symptoms of antisocial personality disorder/conduct disorder (ASPD/CD) were all significantly associated with suicidal ideation. Factors associated with having created a plan were: FHA, ANYAXAF, ALCDEP4, and ASPD/CD. Suicide attempts were associated with: gender, assaultive trauma, PTSD, ANYAXAF, ALCDEP4, MJDEP4, stimulant dependence (STIMDEP4), education and ASPD/CD. Fatal suicides were associated with: gender, Native American heritage (NAH), economic level, ALCDEP4, ASPD/CD and sleep quality as assessed by the PSQI.

Conclusions: These studies suggest that suicidal thoughts and behaviors are highly prevalent this American Indian community and that they are associated with similar risk factors to what are seen in the general population, namely, affective and anxiety disorders and substance dependence. Additionally, sleep quality may be an important predictive measure of level of suicidality. These findings further suggest that suicide prevention programs for American Indians may benefit from addressing issues related to trauma and substance dependence and their associated symptomatology.

Keywords: suicide, native Americans, alcohol use disorders, affective disorders, sleep disorders.

Disclosures: C. Ehlers, **Part 1:** consulting neurocrine corporation; D. Gilder, **Part 1:** consulting neurocrine corporation.

W71. Whole Genome Sequencing of Schizophrenia in a Founder Population

Todd Lencz*, Semanti Mukherjee, Shai Carmi, Anil Malhotra, Itsik Pe'er, Ariel Darvasi

Zucker Hillside Hospital, Glen Oaks, New York

Background: Amongst the most well-replicated and robust findings in the schizophrenia literature are the high heritability ($\sim$80%) and elevated siblingrecurrence of the disorder, indicating a strong genetic component. Nevertheless, the identification of susceptibility genes for SZ has proven challenging. While genomewide association studies (GWAS) have begun to yield replicable susceptibility loci, effect sizes are small and leave the majority of variance unexplained by common genetic variants. With the advent of affordable whole genome sequencing (WGS), it is now possible to fully interrogate rare genetic variation as well. However, the examination of rare variation is potentially confounded by subtle population stratification and random inter-individual variation. The present study is designed to reduce these confounds by examination of samples from a homogeneous founder population, the Ashkenazi Jewish (AJ) population, which is marked by an extreme population bottleneck in the relatively recent past; this bottleneck results in a marked reduction of intrapopulation variance. A small number of schizophrenia cases and controls from the AJ population were sequenced with WGS, and then candidate variants were subsequently genotyped in a larger cohort of AJ cases and controls.

Methods: We performed whole genome sequencing of $n = 40$ AJ SZ cases and $n = 128$ AJ controls using the

Complete Genomics, Inc (CGI) platform. Median depth was 55×, with >96% of the genome called, resulting in ~3.4 M single nucleotide variants per sample, with 99.9% consistency with SNP array data. The transition/transversion (Ti/Tv) ratio of detected variants was ~2.14 (±0.003) and was consistent across the entire genome, independent of local depth of coverage or minor allele frequency, indicating consistent high-quality of variant calls and lack of GC or other bias. Moreover, 98.8% of the exome was called at ⩾10× depth, indicating that the CGI platform lacks the hybridization biases associated with exome sequencing.
Results: Despite the small sample size of our WGS pilot, we have identified a number of highly promising candidates which are currently undergoing follow-up genotyping in our larger AJ cohort ($n \sim 1000$ cases and 2000 controls). First, we utilized a method of detecting identity-by-descent in our previously collected GWAS data ($n = 904$ cases and 1640 controls) to identify extended haplotypes shared by ⩾7 cases (and 0 controls). For each such segment, we sequenced 2–3 carriers, and then interrogated the sequence data for variants shared exclusively in those samples. Using this method, we found a missense variant, designated by B-SIFT as activating (gain-of-function) in EPB41L3, a strongly brain-expressed gene that has three roles relevant to SZ pathophysiology: (1) it is critical to myelination; (2) it is essential to recruitment of NMDA receptors to the membrane of hippocampal neurons; and (3) it binds D2/D3 dopamine receptors to the membrane. Additionally, we identified several functional exonic variants, significantly over-represented in cases *vs* controls, that were also reported as candidates in recent SZ sequencing studies.
Conclusions: WGS conducted in an ethnically homogeneous founder population has great potential to identify rare variants associated with risk for schizophrenia. Additional sequencing and follow-up genotyping are currently underway, and updated results will be presented at the time of the meeting.
Keywords: schizophrenia; whole genome sequencing; rare variants.
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W72. A Genome-wide Association Study on Antipsychotic-induced Body Weight Gain Dissecting the CATIE Sample

Daniel J Mueller*, Eva J Brandl, Arun K Tiwari, Clement C Zai, Nabilah I Chowdhury, Tamara Arenovich, Jiangshan J Shen, James L Kennedy

Centre for Addiction and Mental Health, Toronto, Ontario, Canada

Background: Antipsychotic medications are widely used across major psychiatric disorders irrespective of classification criteria for mental disorders. However, the marked observed inter-individual variability with respect to response and side effects often require lengthy treatment trials until the right medication is found for the right patient. This variability is thought to largely depend on genetic factors and where pharmacogenetic effects are supposedly larger and thus easier to replicate compared to disease genetics. We have identified several important gene variants in the past years which could be successfully replicated in independent samples investigating the genetics of antipsychotic-induced weight gain (AIWG). AIWG is a severe and common side effect often leading to patient non-compliance and increased morbidity and mortality due to metabolic syndrome and cardiovascular events. Beyond these negative effects for the patients, medical conditions caused by AIWG lead to increased health care utilization and costs. Genetic predictors would be extremely helpful to identify subjects at risk prior to antipsychotic exposure and first genetic tests are now becoming commercially available. However, more research is required and the vast majority of previous studies have not used hypothesis-free genome wide strategies such as genome-wide association studies (GWAS). The Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study is one of the largest clinical trials conducted for comparing the effectiveness of several antipsychotic drugs and where genome-wide data is available. Notably, in a subset of the CATIE samples ($n = 738$) a GWAS has been conducted to assess association of single nucleotide polymorphisms (SNPs) with metabolic side effects (Adkins *et al*, 2011). However, the study sample used in that GWAS analysis was limited by several important factors such as use of patients with different ethnicities and medications with different propensities to cause weight gain. This prompted us to conduct a new set of analyses using rigorous inclusion criteria in order to obtain a more homogeneous sub-sample derived from the CATIE-GWAS data.
Methods: Our refined subsample of patients consisted exclusively of individuals who were not exposed to high risk medication for weight gain prior to study inclusion, who did not show marked obesity (BMI > 40) at baseline (T0) or were exposed to low risk medication for weight gain during the CATIE trial (eg ziprasidone). This refined sample ($n = 358$) eventually consisted of 251 individuals of European and 107 individuals of African-American ancestry with well-documented information on weight changes available throughout the study. Exclusion criteria included (a) related individuals, (b) available genotype rate of less than 95%, (c) mismatch between genetic markers and assigned sex status and (d) heterozygosity of more than four standard deviations from the mean. SNPs with minor allele frequencies of less than 5%, and genotypes which deviated significantly from Hardy-Weinberg Equilibrium ($p < 0.001$) were removed. A series of mixed models was used including all potentially relevant covariates (eg, baseline BMI) and change in BMI over a period of three months as the outcome variable. In addition, interactions between time and all other predictors/covariates were considered. The final model included a random intercept and slope associated with time, study medication group and baseline BMI. Standard quality control workflow was applied to the genotype data. After quality control, we analyzed 328 733 SNPs in each individual of our sample. In order to rule out the effect of population stratification, we plotted the MDS components and selected patients within the cluster corresponding to European ancestry as the largest cohort. The GWAS analysis presented here was conducted on 189

individuals treated with risperidone, quetiepine, or olanzapine. The SNP *vs* BMI association analysis was carried out using the R package: 'nlme'.

Results: None of the SNPs was significant at the genome-wide threshold of $p = 5 \times 10^{-8}$. The top hit of the GWAS was rs12924003 ($p = 1.06 \times 10^{-5}$) located downstream of the SAL-1 gene on chromosome 16. The sal-like-1 gene functions as a zinc finger domain containing transcriptional repressor and is associated with developmental syndromes. The second hit, SNP rs4771655 ($p = 1.91 \times 10^{-5}$) is $\sim$194 kb upstream of IRS2 gene (insulin receptor substrate 2). IRS2 mediates effects of insulin and several cytokines and has been associated with insulin resistance, coronary artery disease and cancer in the general population. The third hit, rs4751427 ($p = 2.4 \times 10^{-5}$) is located $\sim$59 kb upstream of the Neuropeptide S gene. The 20 amino acid peptide coded by this gene has been shown to influence food intake, anxiety, locomotion, memory, and drug addiction.

Conclusions: Our analyses did not detect an association when considering the commonly applied genome-wide correction threshold for multiple testing. However, our analysis presented here using stringent inclusion and exclusion criteria on the CATIE GWAS data has revealed interesting new genes that may be associated with antipsychotic induced weight gain. Two of our top hits, IRS2 and NPS, were previously shown to be involved in regulation of insulin sensitivity and food intake in other populations. Direct functional effects of the identified SNPs are yet unknown and functional studies as well as replication in independent samples are required. Beside the main limitations given by the relatively small sample size, other limitations include previous antipsychotic exposure in most patients and heterogeneous medication. Nonetheless, our findings are an important contribution to understanding genetic mechanisms of AIWG by using a genome-wide approach.

Keywords: antipsychotics, weight gain, GWAS, CATIE, genetics.

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W73. Pharmacogenetics of Obsessive-Compulsive Disorder Candidate Genes and Antidepressant Response

Gwyneth Zai*, Clement C Zai, Vanessa Goncalves, Eva J Brandl, Karen Wigg, James L Kennedy, Peggy MA Richter

Centre for Addiction and Mental Health, Toronto, Ontario, Canada

Background: Obsessive-compulsive disorder (OCD) is a chronic and debilitating psychiatric disorder with a strong genetic etiology. Genetic associations between OCD and several candidate genes including the glutamate transporter (SLC1A1), monoamine oxidase (MAOA), glutamate NMDA receptor 2B (GRIN2B), serotonin 2A receptor (5HTR2A), serotonin transporter (SLC6A4), brain-derived neurotrophic factor (BDNF), and catecholamine-O-methyl transferase (COMT) genes have previously been reported. Pharmacogenetics represents a valuable alternative strategy to define subtypes of OCD and to define clinically useful inter-individual genetic variation in drug response.

Methods: We investigated 14 genes including those mentioned above and serotonin 1B receptor (HTR1B) as well as top hit genes from a recent OCD genome-wide association study: Disks Large (drosophila) homolog-associated protein 1 (DLGAP1), BTB (POZ) domain containing 3 (BTBD3), SLIT and NTRK-like family (SLITRK5), Fas apoptotic inhibitory molecule 2 (FAIM2), glutamate receptor, ionotropic, kainite 2 (GRIK2), and fucosyl-transferase 2 (FUT2). We examined a total of 32 single nucleotide polymorphisms across these candidate genes and their regulatory regions using a custom-made 32-SNP OpenArray® chip and genotyping was performed using the QuantStudio™ 12K Flex Real-Time PCR System in 222 OCD patients with retrospective response data on multiple serotonin reuptake inhibitor (SRI) trials. Individuals were grouped into those who improved following an adequate trial of one or more serotonin reuptake inhibitors [SRI(s)] as compared with those who reported 'minimal', 'no change', or 'worsening'. Genotypes and response data were examined on a combined selective SRI (SSRI) and SRI basis.

Results: Interesting associations ($P = 0.005$–0.04) were detected for DLGAP1, SLITRK5, BTBD3, HTR1B, and SLC1A1 in SSRI/SRI response.

Conclusions: These results suggest that genetic variants may play a role in SRI response to OCD. Combination of these variants may be clinically useful in predicting treatment resistance *vs* response in OCD.

Keywords: obsessive-compulsive disorder; pharmacogenetics; antidepressant response.

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W74. The Genetic Basis of Neurocognitive Decline and Reduced White-matter Integrity in Normal Human Brain Aging

David C Glahn*, Jack Kent, Rene L Olvera, Laura Almasy, Peter Kochunov, Ravi Duggirala, John Blangero

Yale University, Hartford, Connecticut

Background: Identification of genes associated with brain aging should markedly improve our understanding of the biological processes that govern normal age-related decline. However, challenges to identifying genes that facilitate successful brain aging are considerable including a lack of established phenotypes and difficulties modeling the effects of aging *per se*, rather than genes that influence the underlying trait.

Methods: In a large cohort of randomly selected pedigrees ($n = 1246$ subjects), we documented profound aging effects from young adulthood to old age (18–97 years) on

neurocognitive ability and diffusion-based white-matter measures.
Results: Despite significant phenotypic correlation between white-matter integrity and tests of processing speed, working memory, declarative memory and intelligence, no evidence for pleiotropy between these classes of phenotypes was observed. Applying an advanced quantitative gene by environment interaction analysis where age is treated as an environmental factor, we demonstrate a heritable basis for neurocognitive deterioration as a function of age. Furthermore, by decomposing gene by aging ($G \times A$) interactions, we infer that different genes influence some neurocognitive traits as a function of age, while other neurocognitive traits are influenced by the same genes, but to differential levels, from young adulthood to old age. In contrast, increasing white-matter incoherence with age appears to be non-genetic.
Conclusions: These results clearly demonstrate that traits sensitive to the genetic influences on brain aging can be identified, a critical first step in delineating the biological mechanisms of successful aging.
Keywords: aging, gene by environment interaction, neurocognition, white matter.
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W75. Variation in the ZNF804A Gene is Associated with Striatal Presynaptic Dopamine Function

Catherine E Hegarty*, Daniel P Eisenberg, Philip D Kohn, Daniel R Weinberger, Joseph C Masdeu, Karen F Berman

Brown University, Providence, Rhode Island

Background: The rs1344706 single nucleotide polymorphism (SNP) in the *ZNF804A* gene has been associated with risk for schizophrenia in a large GWAS study (O'Donovan *et al*, 2008), a finding supported by some subsequent investigations (International Schizophrenia Consortium *et al*, 2009; Shi *et al*, 2009; Riley *et al*, 2010; Steinberg *et al*, 2011; Zhang *et al*, 2011) but not others (Stefansson *et al*, 2009; Rietschel *et al*, 2012). The function of the *ZNF804A* protein remains incompletely understood, and therefore candidate mechanisms underlying the gene's contribution to illness risk are poorly defined. However, efforts to elucidate the gene's role in illness have been made. For example, one study has shown that enhanced *ZNF804A* expression modulates transcription of several putative schizophrenia risk genes, including the dopamine related genes *COMT* and *DRD2* in rat cortical progenitor cells (Girgenti *et al*, 2012). The risk allele (A) has also been shown to be associated with increased white matter volume, reduced gray matter volume and impaired neurocognitive performance, all of which are phenotypes that have been associated with schizophrenia (eg, Lencz *et al*, 2010; Thurin *et al*, 2012). Furthermore, neuroimaging association studies in humans have linked *ZNF804A* genotype to fMRI-measured functional coupling of the prefrontal cortex to paralimbic structures (Rasetti *et al*, 2011; Esslinger *et al*, 2009), which is another phenotype that has been linked to schizophrenia and is hypothesized to rely in part on dopaminergic transmission (Meyer-Lindenberg and Weinberger, 2006). To more directly test the effect of *ZNF804A* on the dopamine system, and in light of reports that schizophrenia is associated with exaggerated striatal presynaptic dopamine, we used positron emission tomography (PET) in healthy volunteers to assess the relationship between variation in *ZNF804A* and striatal dopamine synthesis.
Methods: DNA was obtained from 103 healthy controls under 55 years of age (mean age $= 36.32 \pm 11.21$; 46 females) who were genotyped for the *ZNF804A* rs1344706 SNP and who were also studied with the ^{18}F-FDOPA PET technique for measuring presynaptic dopamine synthesis and storage. One hour prior to the PET scan, volunteers were administered carbidopa (200 mg) by mouth to decrease peripheral tracer decarboxylation. ^{18}F-FDOPA (8–16 mCi) was then administered intravenously, and a 90 min dynamic emission scan on a GE Advance PET scanner was performed. Specific uptake levels (K_i) of ^{18}F-FDOPA for the caudate, putamen and nucleus accumbens were calculated using the Patlak-Gjedde method with a cerebellar reference region. Ki values from each region were entered into a univariate general linear model with genotype and sex as fixed factors and age as a covariate. *Post-hoc* pairwise tests were performed to determine the differences between individual genotype groups. A whole brain, voxel-wise analysis for the main effect of *ZNF804A* genotype on Ki value was also performed using SPM5 (http://www.fil.ion.ucl.ac.uk/spm/software/spm5).
Results: The sample of 103 healthy volunteers contained 38 with the AA genotype, 52 AC heterozygotes, and 13 CC individuals. ROI analysis of extracted Ki values revealed higher Ki levels in risk allele carriers (AA & AC, combined) than in non-carriers (CC) in both the putamen and caudate, but not in the nucleus accumbens. While the effect in the caudate survived correction for multiple comparisons, the effect in the putamen did not reach a significance of $p < 0.05$ after a Bonferroni correction. Pairwise *post-hoc* analyses of the three groups revealed that this effect was most pronounced in heterozygotes, who showed a significantly higher Ki than the non-carriers ($p = 0.035$, corrected). Voxel-wise analysis supported our findings from the univariate ROI analysis, whereby a main effect of *ZNF804A* was seen in the caudate and putamen ($p < 0.005$). There were no regions in which the CC group had higher Ki levels than risk-allele carriers.
Conclusions: The putative schizophrenia risk variant of *ZNF804A* is associated with the illness phenotype of elevated striatal dopamine synthetic capacity, suggesting that this variant, or one in close linkage disequilibrium with it, may impact dopamine regulation in humans. This finding was supported by both a univariate ROI analysis and voxel-wise findings. Though these findings in healthy individuals cannot directly inform *ZNF804A*'s role in risk for schizophrenia, they do provide *in vivo* support for further investigation of potential dopaminergic mechanisms underlying *ZNF804A*'s clinical associations.
Keywords: ZNF804A, schizophrenia, dopamine, F-FDOPA, striatum.

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W76. Pharmacoepigenetics of Depression—A Role of Monoamine Oxidase A DNA Hypomethylation?

Katharina Domschke*, Nicola Tidow, Jürgen Deckert, Volker Arolt, Peter Zwanzger, Bernhard Baune

University of Wuerzburg, Wuerzburg, Germany

Background: The monoamine oxidase A (*MAO-A*) gene (Xp11.4-p11.3) has been suggested to be involved in the pathogenesis as well as the pharmacological treatment of Major Depression: The more active alleles of a VNTR polymorphism in the *MAO-A* promoter have been found to be associated with major depression and impaired antidepressant treatment response particularly in female patients [1,2]. Recently, *MAO-A* DNA hypomethylation, presumably leading to increased MAO-A expression, was reported to be associated with depression in female patients [3]. In the present study, for the first time a pharmacoepigenetic approach was applied by investigating the influence of DNA methylation patterns in the *MAO-A* regulatory and exon1/intron1 region on antidepressant treatment response.
Methods: Ninety-four Caucasian patients with a Major Depressive Episode (f = 61, m = 33) were analyzed for DNA methylation status at 43 *MAO-A* CpG sites in the promoter region as well as exon 1 and parts of intron 1 via direct sequencing of sodium bisulfate treated DNA extracted from blood cells. The obtained sequences were quantitatively analyzed by determining relative peak heights (C/C + T) from the normalized sequence trace files using Epigenetic Sequencing Methylation analysis software (ESME) [cf. 3]. Patients were additionally genotyped for the functional *MAO-A* VNTR. The clinical response to antidepressant pharmacological treatment with escitalopram (with individual co-medication) was assessed by weekly changes of HAM-D-21 scores relative to HAM-D at week 1 over the six weeks study period adjusted for age, smoking status, lifetime duration of depression, lifetime hospitalisations, lifetime number of MDD episodes and co-medication with neuroleptics and mood stabilizers.
Results: In female patients, lower methylation at three CpG sites in the *MAO-A* promoter region (positions 43.514.063, 43.514.574, 43.514.684; UCSC Human Genome Browser; Feb 2009; GRCh37/hg19) was associated with a significantly worse response under antidepressant treatment after weeks 4, 5 and 6 ($p < 0.04$–0.001). After applying Bonferroni correction for multiple testing yielding a corrected p-value of $\leqslant 0.001$, the result for CpG 43.514.684 in amplicon B remained significant for response at week 5. *MAO-A* VNTR genotype did not influence *MAO-A* methylation status. Male subjects showed no or only very minor methylation across all CpG sites, which was not associated with antidepressant treatment response.
Conclusions: The present pilot data suggest that MAO-A gene hypomethylation—possibly via consecutively decreased serotonin and/or norepinephrine availability—negatively influences antidepressant treatment response in female patients. These findings are in line with MAO-A hypomethylation being associated with depression [2] and the more active MAO-A VNTR alleles conferring impaired antidepressant treatment response [1]. Future studies are warranted to replicate the present results and to clarify the functional consequence of MAO-A DNA hypomethylation on an mRNA and protein level. A pharmacoepigenetic approach as applied in the present study might eventually contribute to the development of a more individualized treatment concept of major depression based on epigenetic information, eg suggesting a potentially beneficial use of MAO-A inhibitors as an adjunct treatment in patients displaying MAO-A DNA hypomethylation.
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Keywords: MAO-A; epigenetics; methylation; depression; gender.
Disclosures: K. Domschke, Nothing to Disclose; N. Tidow, Nothing to Disclose; J. Deckert, Nothing to Disclose; V. Arolt, Nothing to Disclose; P. Zwanzger, Nothing to Disclose; B. Baune, Nothing to Disclose.

W78. High Transcriptional Plasticity of the AKT1 Gene is Revealed by RNA Sequencing Analysis in the Brain

Gianluca Ursini*, Joo Heon Shin, Bin Xie, Giovanna Punzi, Yuan Gao, Joel E Kleinman, Thomas M Hyde, Keri Martinowich, Daniel R Weinberger

Lieber Institute for Brain Development, Baltimore, Maryland

Background: AKT1 signaling is a target of psychotropic drugs and is altered in brain of individuals with schizophrenia. Although it has been reported that AKT1 protein levels are decreased in frontal cortex of patients with schizophrenia, it is unclear which are the specific molecular mechanisms that lead to an altered AKT1 signal. Here, we use RNA sequencing to analyze *AKT1* RNA transcription in dorsolateral prefrontal cortex (DLPFC) of individuals with schizophrenia and controls subjects.
Methods: PolyA enriched RNA was extracted from postmortem human DLPFC grey matter ($n = 107$ controls, $n = 107$ patients with schizophrenia) and then purified and enriched with PCR to create a final cDNA library for

high throughput sequencing using the Illumina HiSeq2000. The Illumina Real Time Analysis (RTA) module was used to perform image analysis and base calling, followed by use of BCL Converter (CASAVA v1.8.2) to generate FASTQ files containing sequence reads. Pair-end reads of cDNA sequences obtained by the HiSeq2000 were aligned to the human genome reference (UCSC hg19) by splice-read mapper (TopHat v2.0.4), providing known transcripts from Ensembl Build GRCh37.67. To quantify abundance at the gene-level and at the level of all expressed sequences, we used htseq-count v0.5.3 (with intersection-strict mode) to count the properly-paired and mapped reads and RPKM (Reads Per Kilobase per Million mapped reads) was calculated. Expression level was compared between patients and controls by ANCOVA with age, sex, race and RIN as covariates. All novel expressed sequences were validated by PCR, with subsequent sequencing of the PCR products inserted into a plasmid vector for Sanger sequencing.

Results: Surprisingly, *AKT1* mRNA expression as RPKM is greater in patients, compared with controls ($N = 214$; $F_{1,205} = 3.7$; $p = 0.05$). However, no significant relationship was found between expression of known exons of *AKT1* and diagnosis ($p > 0.25$), thus suggesting that the greater expression of *AKT1* in patients could be related to new transcripts. New splicing events were detected in the region of *AKT1* gene, and are predicted to alter translation. Three potential novel 5′ exons are spliced with exons 2 or 3. Two transcripts show novel exons spliced with exons 3–4, and exons 4–5, while both exons 5 and 6 are partially deleted in another novel transcript. Exons 5, 7, 9 and 10 show extensions of 20, 32, 60 and 16 bp respectively, providing another source of novel transcripts. An additional transcript is characterized by a deletion starting at the rs1130233 SNP and spanning the adjacent exon 9. A novel exon overlaps with the central part of exon 11, while many splicing events were also detected in the 3′end of the gene. Further, we found a transcript with a deletion of 52 bp in the 3′UTR, two transcripts with a truncated 3′ exon spliced with exon 14 or with a novel exon, a transcript with a junction between the 3′ exon and a new exon overlapping the central part of exon 14. Finally, different long non-coding RNAs span a large region of Chr 14 (104972633–105361511), which include *AKT1*, one of which includes exon 6 of *AKT1*.

Conclusions: Our RNA sequencing analysis shows multiple novel expressed sequences in the *AKT1* locus in human brain, demonstrating exceptionally complex transcriptional regulation of *AKT1*. The high transcriptional plasticity of *AKT1* might be related with its increased expression in patients with schizophrenia, given that many novel transcripts are predicted to alter translation or represent non-coding RNAs. Further analyses are necessary to clarify the function of each transcript.

Keywords: AKT1, RNA sequencing, schizophrenia, prefrontal cortex, transcription.

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W79. Oxytocin and Vasopressin Peptide Gene Region: Associations with Autism Related Phenotypes

Sunday Francis, Emily Kistner-Griffin, Guter Stephen, Edwin H Cook, Suma Jacob*

University of Minnesota, Minneapolis, Minnesota

Background: There has been growing literature on the oxytocin genes and associations with human social behaviors that originated with studies of the oxytocin receptor in autism spectrum disorders (ASD). Few studies have focused on the peptide producing oxytocin gene (OXT) and its evolutionarily related, vasopressin (AVP) gene. The human OXT and AVP are closely linked on chromosome 20p13 and are positioned in opposite transcriptional orientations, while separated by only 12 kb of DNA. We aimed to examine associations between single nucleotide polymorphisms (SNPs) of the OXT and AVP genes with ASD diagnosis and intermediate, quantitative phenotypes.

Methods: 209 probands, including 157 trios and 52 single parent families, were assessed for ASD using the Autism Diagnostic Interview-Revised, Autism Diagnostic Observation Schedule and clinical DSM-IV-TR criteria. A European American subsample was based on self-reported race/ethnicity and confirmed by Genetic Array Ancestry with 121 ASD probands. Ten Tag-SNPs covering the adjacent OT and AVP gene regions were genotyped using TaqMan. We examined associations between the SNPs and ASD diagnosis as well as IQ measures. Given our previous findings about the relationship between blood oxytocin and serotonin levels in ASD and within related animal models (Hammock *et al*, 2012), we also examined how these SNPs related to whole blood serotonin levels. *P*-values that are reported here are uncorrected for multiple comparisons, so these results will need to be replicated in larger samples of ASD families.

Results: With the entire study sample, family based association testing found an association with rs6084258 ($p = 0.001$) in the 5′ OXT region. In the EA ancestry subgroup, the rs6084258 association ($p = 0.005$) suggests that this SNP with a minor allele frequency 0.366 may be associated in Western European populations. With Full-Scale IQ examined as a quantitative phenotype within ASD probands, rs6133010 ($p = 0.008$) was associated. With non-verbal IQ as a quantitative phenotype, both rs6133010 ($p = 0.01$) and rs6084258 ($p = 0.03$) were associated. This replicates previously published data showing SNPs in the 5′ OXT region, such as rs6133010 to be associated with ASD diagnosis overall or within OXT haplotypes using PBAT-mFBAT analyses models with IQ and the Vineland Adaptive Behavior Scale (Ebstein, 2009). A subsample also has OT blood levels measured, and associations with OT-AVP SNPs will be examined and reported. When whole blood serotonin was examined as a quantitative phenotype in the EA subgroup with ASD, rs4813625 ($p = 0.03$) and rs877172 ($p = 0.04$) were associated with serotonin levels and are also in the 5′ OXT region ($N = 98$ probands).

Conclusions: Our results suggest that polymorphisms near and in the 5′ promoter region of the OXT gene may be associated with the diagnosis of ASD and related inter-

mediate phenotypes. Autism is heterogeneous disorder with many genes and pathways that contribute to its development. Only a few published studies have looked at OXT genes in social behavior and other disorders, although there are more studies published on the receptor gene (OXTR) and effects of intranasal oxytocin drug administration. Our genetic findings need to be replicated larger samples, but suggest that IQ and quantitative blood biomarkers need to be further studied to examine how this particular region of OXT may be related to phenotypes found in neurodevelopmental disorders like ASD.

Keywords: autism; ASD; oxytocin; vasopressin; quantitative phenotypes.

Disclosures: S. Francis, Nothing to Disclose; E. Kistner-Griffin, Nothing to Disclose; G. Stephen, Nothing to Disclose; E. Cook, **Part 1:** Seaside Therapeutics—Consultant and site investigator for clinical trial (with support to conduct the trial), **Part 4:** Seaside Therapeutics—sponsored clinical trial mentioned in 2 above; S. Jacob, Nothing to Disclose.

W80. Association of SCN2A Variants with Cognitive Ability in Schizophrenia, and Additional Support from Analyses of Unaffected Siblings, Independent Schizophrenia Samples, fMRI, and mRNA Expression in Brain

Dwight Dickinson*, Richard Straub, Joey W Trampush, Yuan Gao, Ningping Feng, Gianluca Ursini, Kristin Bigos, Bhaskar Kolachana, Ryota Hashimoto, Masatoshi Takeda, Dan Rujescu, Joseph H Callicott, Thomas M Hyde, Karen F Berman, Joel E Kleinman, Daniel R Weinberger

NIMH, Bethesda, Maryland

Background: Schizophrenia is a heritable neurodevelopmental disorder characterized by disturbed patterns of behavior and abnormalities of brain function. Genome-wide association studies (GWAS) are beginning to yield insights into the genetic architecture of schizophrenia, although effect sizes for individual genes and markers are modest. However, few GWAS have examined behavioral or biological traits associated with the disorder, which may reflect more penetrant effects of common genetic variation. Broad cognitive impairment is common in schizophrenia. Subtle cognitive differences are often measurable years before psychotic symptoms or exposure to medications, and impairment is seen in attenuated form in unaffected relatives, suggesting that impaired cognition is an intermediate phenotype related to genetic risk for schizophrenia. We sought to identify single nucleotide polymorphisms associated with general cognitive ability ('*g*') in people with schizophrenia and controls. Analyses identified a genome-wide association of *SCN2A* SNPs rs10174400 and rs10182570 to general cognitive ability ('*g*') in a Caucasian schizophrenia sample from the NIMH/CBDB Genetic Study of Schizophrenia. *SCN2A* encodes part of a sodium ion channel that is widely expressed in the brain and involved in the initiation and propagation of action potentials. *SCN2A* has been associated previously with epilepsy, intellectual disability and autism.

Methods: Initial GWAS analyses were conducted in a discovery cohort of 339 people with schizophrenia and 363 controls. Follow-up work sought to support and extend initial findings. We tested the *SCN2A* rs10174400 genotype association to g and related variables in unaffected siblings and independent schizophrenia samples. BOLD fMRI was used to examine differences in prefrontal cortex activation during 'Nback' performance as a function of *SCN2A* genotype in cases and controls. RNA sequencing data from post-mortem PFC samples allowed examination of genotype-related differences in *SCN2A* mRNA expression in schizophrenia and comparison samples.

Results: In 334 Caucasians with schizophrenia, the *SCN2A* SNP rs10174400 association to *g* surpassed the genome-wide significance threshold ($p = 9.3e^{-10}$). *SCN2A* SNP rs10182570, showed a similar magnitude of association. Controls showed a trend for *g*/genotype association with reversed allelic directionality. The genotype-by-group interaction was also GWAS-significant ($p = 1.75 \times 10^{-9}$). In unaffected siblings, there was a genotype association with *g* ($t[146] = -2.245$, $P = 0.026$), accounting for 3.4% of *g* variance and an association with education completed ($t[146] = -3.001$, $p = 0.003$), accounting for 5.7% of education variance. In independent schizophrenia samples from the USA ($n = 279$) and Japan ($n = 95$), tests of a proxy SNP replicated the main *g* finding (respectively: $t[275] = -2.115$, $p = 0.035$, 1.5% variance; $t[94] = -2.217$, $p = 0.029$, 4.8% variance, recessive model). In 397 community controls and 87 schizophrenia cases studied with fMRI during working memory, rs10174400 was differentially associated with PFC efficiency (MNI coordinates: −36 27 33, interaction $p = 0.02$ FWE-corrected). Analysis of RNA sequencing data from post-mortem PFC grey matter tissue samples showed significantly reduced expression of *SCN2A* mRNA in the schizophrenia sample relative to controls for two of three RefSeq transcripts and significant genotype effects and interactions for these two transcripts that paralleled the directionality of the associations with cognition.

Conclusions: We have identified common variants in a gene that, in the context of schizophrenia and risk for schizophrenia, show substantial associations with broad cognitive performance, educational attainment, brain physiology, and mRNA expression in the brain. The findings are plausible, both biologically and in terms of known clinical associations. *SCN2A* encodes the pore-forming, α2 subunit of a voltage-gated sodium ion channel that is widely expressed in the brain. The ion channel contributes to the initiation and propagation of action potentials. Na(v)1.2 (the protein encoded by *SCN2A*) is abundant in parvalbumin-positive GABAergic inhibitory interneurons, at least in hippocampus and temporal lobe. GABA system abnormalities have been a particular focus of cognitive impairment research in schizophrenia. Multiple mutations in *SCN2A*, including several identified in recent exome-sequencing studies, have been associated with childhood epilepsies, intellectual disability and/or autism-like syndromes, and antiepileptic medications that block sodium channels (eg, topiramate) have adverse cognitive effects. In sum, current findings point to novel mechanisms of cognitive impairment in schizophrenia, highlight the importance of genetic context in shaping variant associations with complex traits, and suggest new avenues for treatment development.

Keywords: SCN2A, schizophrenia, cognition, GWAS, fMRI, RNA sequencing.
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W81. Variation in the Williams Syndrome GTF2i Gene and Anxiety-proneness Interactively Predict DLPFC Response to Aversive Social Stimuli in Humans

Mbemba Jabbi*, Qiang Chen, Nicholas Turner, Michael White, J Shane Kippenhan, Philip Kohn, Dwight Dickinson, Bhaskar Kolachana, Venkata Mattay, Daniel R Weinberger, Karen Berman

NIH, Bethesda, Maryland

Background: The general transcription factor gene, *GTF2i*, located in the 'Williams syndrome (WS) region' on chromosome 7q11.23, is hemizygously deleted in WS (MIM 194050) and duplicated in the recently discovered 7q11.23 duplication syndrome (dup7q11.23; MIM 609757). Persons with these two different 7q11.23 copy number variants (1 copy *vs* multiple copies of affected genes, respectively) have contrasting anxiety phenotypes, and mouse models constructed to have one, two, or three copies of *Gtf2i* have demonstrated relationships between anxiety and *Gtf2i* copy number that parallel observations in these two human syndromes (Mervis *et al*, 2012). In light of the heritability of human anxiety (Smoller *et al*, 2008), these results suggest a role for *GTF2i* in the etiology of human anxiety, but the impact of this gene on neurobehavioral correlates of state and trait anxiety remains largely unknown. Here, we examined the role of rs2527367, a common intronic single nucleotide polymorphism (SNP) in the *GTF2i* gene, and tested for its association with trait anxiety and with neural response to anxiety-laden social stimuli in the general population.
Methods: 260 healthy participants (115 homozygous for the major allele, 118 heterozygotes, and 27 homozygous for the minor allele) completed fMRI and measures of the tridimensional personality subscale of harm avoidance (HA; a trait measure of anxiety-proneness [Cloninger, 1987]). During fMRI, participants were shown a trio of faces and were asked to match a target face to one of the other two faces on the basis of emotional expression (angry or fearful). Blocks of these events were compared to blocks of a control condition involving the matching of a target geometric shape to one of two other shapes. This emotion discrimination task promotes implicit processing of aversive social stimuli and taps into the neural substrates of innate social anxiety (Hariri *et al*, 2003).
After preprocessing & 1st-level analysis (aversive evaluation > shape evaluation), a second-level 1-sample *t*-test assessing neural response to aversive evaluation and its association with HA was carried out. A flexible factorial design (3-levels of genotype) using HA as regressors, and sex, age and IQ as covariates of no interest was adopted to examine the interactions of these factors on brain response to angry and fearful faces.
Results: We found no main or interaction effects between *GTF2i* genotype and HA, age, sex or IQ. A main effect of aversive content relative to control was found in the amygdala, the DLPFC region implicated in top-down guidance of attention and thought (Arnsten and Rubia, 2012), and in fusiform/visual areas at $p<0.05$, corrected. However, using HA scores as predictors of whole-brain response to aversive social content, we found that the DLPFC locus responding to aversive content as a main effect of task was modulated by HA ($p<0.005$ uncorrected). Given that genes influence human temperament and risk for neuropsychiatric disorders by acting upon brain circuits mediating behavior (Hamer, 2002), we next tested for an interaction between individuals' HA scores and variations of *GTF2i* genotype on brain response to aversive social cognition. Using a flexible factorial design with HA scores as predictors, we found that variation in *GTF2i* was associated with DLPFC response to aversive social cues (in the same anatomical region predicted by HA) at $p<0.0001$. To further examine this combined effect of HA and *GTF2i* genotype on the DLPFC response, we extracted each individual's mean signal value from the response cluster and, for each genotype group separately, performed a regression analysis using HA scores as predictors. We found a stepwise genotype-related relationship such that individuals homozygous for the major allele showed a negative association between percent signal change in DLPFC response and their HA scores ($t=-2.589$, $p=0.0109$), the heterozygotes showed no association between DLPFC response and HA ($t=1.252$, $p=0.213$), and individuals homozygous for the minor allele showed a significant positive association ($t=2.905$, $p=0.00758$).
Conclusions: Here, we demonstrated an interactive effect of *GTF2i* genotype and degree of HA (a trait measure of anxiety-proneness) on right DLPFC responsivity to aversive social stimuli in a large adult cohort. The hemideletion of *GTF2i* in WS, and its duplication in dup7q11.23 have been implicated in the expression of anxiety (Mervis *et al*, 2012) and in autism spectrum disorders (Sanders *et al*, 2011). By demonstrating that sequence variation at rs2527367, a common intronic SNP in *GTF2i*, affects the relationship between an anxiety trait measure and response to aversive stimuli in DLPFC, a brain region known to guide attention and thought (Arnsten and Rubia, 2012), these results may suggest a possible neurogenetic contribution to individual difference in anxiety. Further characterization of these findings in healthy individuals and in pathological conditions may provide information to guide the search for new pharmacotherapeutic targets for anxiety.
Keywords: genetics, anxiety proneness, GTF2i, DLPFC response.
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W82. Generation of Serotonin Transporter Knock-in Mice Carrying Ile425Val Coding Variant Associated with Obsessive-Compulsive Disorder and Tourette Disorder

Sammanda Ramamoorthy*, Padmanabhan Mannangatti, Kamalakkannan Naidu, Lankupalle Jayanthi, Dennis L Murphy

Virginia Commonwealth University, Richmond, Virginia

Background: Obsessive-Compulsive Disorder (OCD) is a complex neuropsychiatric disorder. Neuroimaging studies have indicated that OCD individuals have decreased serotonin transporter (SERT) expression in the midbrain and brainstem. Moreover, Serotonin Selective Reuptake Inhibitors (SSRIs) are currently the only clinically effective medications for the treatment of OCD. A rare single nucleotide polymorphism (SNP), isoleucine to valine at amino acid 425 (I425V) in SERT has been implicated in the pathogenesis of OCD and Tourette Disorder (TD). Expression of I425V in heterologous cell models exhibits higher 5-HT transport with higher Vmax and decreased Km. Our additional studies revealed that I425V alters the normal regulation by Protein Kinase G-mediated upregulation and phosphorylation. However, the physiological effects of V425-SERT in serotonergic signaling and whether V425-linked dysregulation of SERT imposes behaviors contributing to OCD, TD and possibly to other psychiatric disorders are unknown.
Methods: To investigate the impact of V425-SERT in a physiological context, we replaced isoleucine to valine at position 425 of exon 9 in the SERT gene (Slc6a4). The V425-SERT knock-in mutation was generated by homologous recombination in the C57BL/6 background mice. Both female and male V425 homozygous mice were used for behavioral and neuro-biochemical assays and compared with wild type littermate controls.
Results: The mutation was identified by PCR using primers specific for either wild type or the mutant allele and the presence of the V425 mutation in SERT was confirmed by sequencing. V425-SERT mice have a birth rate consistent with Mendelian inheritance ratios, indicating that the V425 mutation in SERT did not create a trans-dominant lethal phenotype. Both female and male homozygous mice were viable, displayed no major developmental abnormalities and exhibit normal size and growth. Results from neurochemical analysis, brain regional distribution of SERT activity, expression, phosphorylation, PKG/p38 MAPK mediated regulation and anxiety related behaviors are currently under evaluation.
Conclusions: Given the value of SERT offering a pharmacological target in the treatment of OCD and the association of SNP V425 in SERT with OCD and TD, *in vivo* studies using the SERT-V425 knock-in animal model may provide insights to our understanding of neuropsychiatric disorders and other disease states resulting from aberrant 5-HT transmission and an opportunity for the development of potential pharmacotherapeutic strategies for the treatment of such disorders.
Keywords: obsessive-compulsive disorder, tourette disorder, serotonin transporter, regulation, human coding varients.
Disclosures: S. Ramamoorthy, Nothing to Disclose; P. Mannangatti, Nothing to Disclose; K. Naidu, Nothing to Disclose; L. Jayanthi, Nothing to Disclose; D. Murphy, Nothing to Disclose.

W83. New Insight into Genetic Mechanism Underlying the Treatment Effect of Obsessive-Compulsive Disorder Using SSRIs

Haide Qin*, Jack Samuels, Ying Wang, Gerald Nestadt, Yin Yao

NIH, Bethesda, Maryland

Background: Selective serotonin reuptake inhibitors (SSRIs) are first line medications for the treatment of obsessive-compulsive disorder (OCD). Although SSRIs are currently the most frequently used drug therapy for OCD, approximately 30% of OCD patients show limited or no response to these medications, and $>7\%$ cannot tolerate side effects. Genetic predictors for OCD treatment responsiveness are in demand. We hypothesized that genetic variations in genes expressed in human brain may influence SSRI response.
Methods: To test the hypothesis, we conducted a genome-wide association study on SSRI drug effect in 1668 OCD cases from a family-based GWAS study. We used a powerful Quasi-Likelihood Score Test, namely, MQLStest, to conduct association test correcting for the relatedness coefficients (based on identity-by-descent, IBD).
Results: The top significant SNPs are rs17162912, with $P = 1.44 \times 10^{-8}$. The SNP rs17162912 is an intergenic variation near *DISP1* gene on 1q41-q42, a microdeletion region related to mental retardation. The 51 other significant variants are located in 20 genes, including *IRAK3*, *PRKCH*, *GPC6*, *NTM*, *LIN7A*, *ENTPD5*, *SLC9A4*, and *RPS19*. Interestingly, most of these genes are expressed in human brain and have significant biological relevance to the mechanisms of psychiatric disorders, pharmacogenomics, or neurological development, implying the essential roles of genetic alterations in SSRI response in OCD patients.
Conclusions: The importance of identification of drug response loci is for the development of 'personalized medical treatment' of OCD patients treated with SSRIs. The potential results would provide new targets for developing novel drugs for the treatment of non-responders. More well-designed case-control studies with larger sample size with next-generation sequencing data are needed to investigate the role of causal exonic variations for the most significant genes.
Keywords: selective serotonin reuptake inhibitors (SSRIs) obsessive-compulsive disorder (OCD) pharmacogenomics GWAS study family-based design.
Disclosures: H. Qin, Nothing to Disclose; J. Samuels, Nothing to Disclose; Y. Wang, Nothing to Disclose; G. Nestadt, Nothing to Disclose; Y. Yao, Nothing to Disclose.

W84. The Utility of DNA Extracted from Saliva for Methylation Studies of Psychiatric Traits

Alicia K Smith*, Varun Kilaru, Torsten Klengel, Kristina B Mercer, Karen Conneely, Kerry J Ressler, Elisabeth Binder

Emory University, Atlanta, Georgia

Background: DNA methylation has become increasingly recognized in the etiology of psychiatric disorders. Because

brain tissue is not accessible in living humans, epigenetic studies are most often conducted in blood. Thus, numerous studies have been published associating specific DNA methylation patterns with psychiatric conditions affecting adults. For example, epigenetic correlates of child abuse have been identified in adult samples and animal models, but comparable research is lacking in children because even a blood draw can be considered too invasive. Saliva is readily collectable, but the proportion of epithelial cells and leukocytes varies between individuals and represents a significant barrier to conducting studies in children. The goal of this study is to evaluate whether DNA isolated from saliva is comparable to DNA isolated from blood for outcomes relevant to psychopathology, using child abuse as an example.

Methods: Saliva and blood samples were collected from 64 African American participants in the Grady Trauma Project (Atlanta, GA). Child abuse was assessed using the Childhood Trauma Questionnaire (CTQ) and operationalized as a report of moderate to severe sexual, emotional or physical abuse. DNA methylation was interrogated for each sample using the HumanMethylation450 BeadChip (Illumina). The method described by Houseman and colleagues was used to estimate the proportion of epithelial cells in saliva DNA and the proportion of lymphocytes and neutrophils in blood DNA. We examined the association of each CpG site with child abuse using a linear model that adjusted confounding factors such as age, sex, race, batch effects and cellular heterogeneity. We also used linear models that adjusted for batch effects and cellular heterogeneity to test whether the DNA methylation levels of saliva predicted those of blood. For all analyses, the false discovery rate was controlled at 5%.

Results: Tissue-specific DNA methylation patterns were clearly indicated by hierarchical clustering of methylation levels across the genome, which segregated blood samples from saliva samples. DNA methylation from saliva predicted that of blood in only 24% of CpG sites ($2.3 \times 10^{-61} < p < 3.8 \times 10^{-3}$) though there were correlated CpG sites in almost every gene in the genome. Correlated CpG sites were more likely to occur in between genes or in CpG island shores ($p < 2.2 \times 10–16$), an area that is more likely to harbor disease-related variation. While no individual CpG site remained associated with child abuse after adjustment for multiple testing, the test statistics for comparable analyses of saliva and blood were moderately correlated ($r = 0.43$).

Conclusions: This analysis suggests that tissue-specific differences are more prominent than those related to individual genetic or environmental factors. DNA derived from saliva may be informative for research questions that can be assessed in blood, though only a small fraction of CpG sites can be considered correlative across tissues. These results have applications for longitudinal and biomarker studies as well as large-scale DNA methylation studies of childhood psychiatric disorders. Further studies will be necessary to quantify the correlation between saliva and brain methylation patterns.

Keywords: epigenetic, DNA methylation, child abuse, trauma.

Disclosures: A. Smith, Nothing to Disclose; V. Kilaru, Nothing to Disclose; T. Klengel, Nothing to Disclose; K. Mercer, Nothing to Disclose; K. Conneely, Nothing to Disclose; K. Ressler, Nothing to Disclose; E. Binder, Nothing to Disclose.

W85. ADRB2 Gene Polymorphism Interacts with Childhood Trauma in Conveying Risk for Adult Posttraumatic Stress Disorder (PTSD)

Anthony P King*, Kerry J Ressler, Lynn Almli, Greg Cohen, Marijo Tamburrino, Sandro Galea, Joseph R Calabrese, Israel Liberzon

University of Michigan, Ann Arbor, Michigan

Background: Posttraumatic stress disorder (PTSD) is a debilitating and highly prevalent (7.6%) consequence of trauma exposure, of unknown etiology. However, only a subset of trauma-exposed individuals develops PTSD, and heritable factors, in interaction with environmental exposure (trauma), have been implicated by twin and family studies. Targeted molecular genetic studies have identified a number of candidate genes in PTSD vulnerability, however large studies containing replication cohorts and detailed phenotypic data are needed to examine gene by environment interaction (G x E) models. Using two independent trauma-exposed cohorts, we report novel findings of beta 2 adreno-receptor gene (ADRB2) SNP's conveying risk for PTSD in interaction with trauma exposure (childhood and lifetime).

Methods: Participants wereselected from two independent samples. The discovery sample is a prospective longitudinal study of Ohio National Guard soldiers, primarily European-American males, recruited while in training for deployment to Iraq and Afghanistan (total $N = 2616$, genotype data for this analysis $N = 715$ European ancestry by PCA). The replication sample ($N = 2083$) was from the Grady Trauma study, a study of predominantly African-American females with low income and high levels of trauma exposures. PTSD symptoms, childhood adversity, and lifetime adult trauma exposures were assessed by diagnostic interviews and self-report measures. Genotyping was performed in the discovery sample using a custom 3755 SNP Illumina Infinium genotyping array, and in the replication sample using Illumina HumanOmniExpress BeadChip. Association analyses were performed in PLINK. Correction for admixture and population structure was performed by PCA of 1500 markers in the discovery sample and genomewide in the replication samples; Bonferroni correction for multiple comparisons (threshold $1.33 \times 10–5$) was applied to the discovery sample.

Results: We identified a SNP within the promoter region of ADRB2 gene associated PTSD symptoms in interaction with childhood trauma (rs2400707, $p = 1.02 \times 10–5$, additive genotype relative risk ~ 1.5), controlling for level of lifetime trauma exposure which was significant after Bonferoni correction. Association of rs2400707 with PTSD in interaction with childhood adversity was confirmed in an independent, predominantly female, African American cohort (Grady Trauma Project $N = 2083$, rs2400707 x childhood trauma interaction $p = 5.01 \times 10–4$).

Conclusions: Altered adrenergic/noradrenergic function has been long believed to play a key etiologic role in PTSD

development, however direct evidence to this link has been missing. The rs2400707 polymorphism has been linked to function of the adrenergic system6 and to the development of chronic pain, however this is the first report linking the ADRB2 gene to PTSD or any psychiatric disorders. These findings have important implication PTSD etiology, chronic pain and stress related comorbidity as well as for both primary prevention and treatment strategies.
Keywords: PTSD, ADRB2, beta-adrenergic receptor, gene x environment iinteraction, childhood trauma.
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W86. Variation in the Human Fatty Acid Amide Hydroxylase (FAAH) Gene and Threat Processing

Francisco J Amador*, Andrew Holmes, Carmen L Cadilla, Mohammed R Milad, Karen G Martinez, Gregory J Quirk

University of Puerto Rico, San Juan, Puerto Rico

Background: FAAH (fatty acid amide hydroxylase) breaks down the endogenous endocannabinoid anandamide. A common genetic variation (*FAAHC385A*, A-allele) results in 50% less enzyme activity and therefore increased endocannabinoid levels (Chiang *et al*, 2004). In humans, the A-allele is associated with increased substance abuse (Sipe *et al*, 2002). A recent publication reports that healthy Caucasian A-allele carriers, compared to C-allele (*FAAHC385A*, non-carriers), showed faster amygdala reactivity habituation during threat (Hariri *et al*, 2009 and Gunduz-Cinar *et al*, 2012). Our aim was to investigate A-allele carriers in fear conditioning/extinction, together with threat processing.
Methods: Forty-eight consenting healthy Hispanic adults (31 female, mean age = 32) were screened with the Structural Clinical Interview for DSM-IV, matched by demographics, and grouped as A-allele or C-allele from saliva samples. Subjects completed the NEO Five Factor Inventory & State-Trait Anxiety Inventory (STAI) questionnaires, performed the Emotional Stroop Test (EST) and the Multisource Interference task (MSIT). They were then given fear conditioning and extinction, using a visual CS+, CS− and mild shock US (Milad *et al*, 2005b), and returned the following day to assess recall of extinction. Skin conductance responses (SCR) was used as a measure of fear.
Results: Our sample showed a high A-allele frequency (A = 0.40, C = 0.60, Hardy-Weinberg Equilibrium $x2 = 1.2$, $p = 0.297$). There were no significant differences between C-allele & A-allele subjects in their SCR to conditioned stimuli in any phase of testing (habituation, conditioning, extinction, recall of extinction, renewal). ANOVA showed no significant main effect of genotype allele or interaction. Matching for sex or age did not reveal any differences. Differential learning (CS+ minus CS−) also did not differ between groups. A-allele carriers, however, showed less of a difference in response times to threat *vs* neutral words in the EST (differential: −4 ms (A) *vs* −26 ms (C); $p = 0.024$), and longer response times to non-congruent trials in the MSIT (non-congruent; 889 ms (C) *vs* 967 ms (A); $p = 0.020$). Compared to C-allele, A-allele carriers showed higher neuroticism in the NEO (52 *vs* 43; $p = 0.005$), and chose significantly lower shock levels for fear conditioning (1.9 *vs* 2.7 mA; $p = 0.014$). There were no group differences in state or trait anxiety (STAI).
Conclusions: Our findings from the EST and MSIT suggest that A-allele carriers are slower to respond to conflict. Moreover, their higher neuroticism and choice of lower shock levels suggests increased anticipation of threat. However, they did not show increased physiological fear responses, suggesting that healthy A-allele subjects show behavioral rather than autonomic anticipation of threat.
Keywords: FAAH threat fear extinction endocannabinoid.
Disclosures: F. Amador, Nothing to Disclose; A. Holmes, Nothing to Disclose; C. Cadilla, Nothing to Disclose; M. Milad, Nothing to Disclose; K. Martinez, Nothing to Disclose; G. Quirk, Nothing to Disclose.

W87. Telomere Length Measurements in Post-mortem Human Brain in Major Depressive Disorder

Firoza Mamdani*, Brandi Rollins, William E Bunney, Richard M Myers, Jack David Barchas, Alan F Schatzberg, Stanley J Watson, Huda Akil, Marquis P Vawter, Pedro A Sequeira

University of California, Irvine, California

Background: Major depressive disorder (MDD) is a debilitating and chronic condition that affects between 5 and 10% of the general population. Several studies have shown that stressful life events, both during early childhood and during adulthood, are risk factors for psychiatric disorders. Telomeres, which are special sequences of DNA located at the end of chromosomes that preserve DNA integrity, naturally shrink with age and eventually can lead to cell death. Stress has been shown to be associated with telomere shortening in peripheral tissue, and there is evidence for decreased telomere length in mood disorders and schizophrenia. Telomere shortening occurs normally in cells undergoing mitosis, although this also occurs in cells not undergoing active mitosis when exposed to stress. To date the majority of investigations of telomere length in MDD have been performed in peripheral blood mononuclear cells (PBMCs), with only one study carried out using post mortem occipital cortex samples, however, in both cases no differences were found between MDDs and controls. The purpose of this study was to survey telomere length in the brain, by first determining if differences in length could be observed between MDD-associated brain regions, and second to ascertain if telomere length shortening can be observed in MDD subjects.
Methods: In this study we measured telomere length across five brain regions (dorsolateral prefrontal cortex (DLPFC), hippocampus (HC), amygdala (AMY), nucleus accumbens (NAC), and substantia nigra (SN)) in both MDDs and controls ($N = 10$, each group). Postmortem brains were obtained through the UCI Brain Bank. Telomere length was determined with qPCR using two genomic assays, one for the single copy human albumin gene and the other with primers specific to the repetitive telomeric sequence. A ratio

of the relative quantities was then used as a quantitative measure of telomere length.
Results: Statistically significant differences were observed in telomere length between brain regions ($p<0.001$), with SN having the longest telomeres and DLPFC the shortest. We observed no significant difference between groups for telomere length in the DLPFC, AMY, NAC, or SN. However, in the HC significantly lower telomere length was observed in MDDs compared to controls ($p=0.004$). We also observed as expected, a negative correlation between age and telomere length, but the decreased telomere length remained significant even after controlling for age.
Conclusions: Our results revealed regional differences in telomere length in the human brain and a significant decrease of telomere length in the hippocampus of MDDs. These results provide further evidence linking stress to MDD and might help explain the reduction in hippocampal volume observed in MDD.
Keywords: major depressive disorder, post-mortem human brain, stress, telomeres.
Disclosures: F. Mamdani, Nothing to Disclose; B. Rollins, **Part 4:** Pritzker Neuropsychiatric Disorders Research Consortium; W. Bunney, **Part 4:** Pritzker Neuropsychiatric Disorders Research Consortium; R. Myers, **Part 4:** Pritzker Neuropsychiatric Disorders Research Consortium; J. Barchas, **Part 4:** Pritzker Neuropsychiatric Disorders Research Consortium; A. Schatzberg, **Part 4:** Pritzker Neuropsychiatric Disorders Research Consortium; S. Watson, **Part 4:** Pritzker Neuropsychiatric Disorders Research Consortium; H. Akil, **Part 4:** Pritzker Neuropsychiatric Disorders Research Consortium; M. Vawter, **Part 4:** Pritzker Neuropsychiatric Disorders Research Consortium; P. Sequeira, **Part 4:** Pritzker Neuropsychiatric Disorders Research Consortium.

W88. Dynamics of Transcriptional Coexpression Brain Networks Over the Human Lifespan

Claudia C Wehrspaun*, Wilfried Haerty, Danielle Bassett, Joo Heon Shin, Daniel R Weinberger, Chris Ponting

Oxford University, Oxford, United Kingdom

Background: Brain development depends on the successful function of many complex biological networks. Genetic interaction networks are important for programmed development and plasticity, especially during critical developmental stages. We therefore hypothesized that transcriptional coexpression networks would change across lifespan. In early developmental stages, transcriptional networks may be more tightly controlled and therefore exhibit changes that are more consequential to the developing organism, compared to increasingly random, and less consequential, change over later adult stages. Second, we hypothesized that the networks would differ between females and males, due to gender differences in hormonal balance.
Methods: Using RNA sequencing data from 200 human postmortem brain samples (prefrontal cortex), we tested our hypotheses of change across the lifespan by generating gene regulatory networks (GRNs) from fetal to adult age (−0.52 weeks to 83 years). Gender comparison was done using samples of females and age-matched males (each $n=55$). We probed network dynamics across the human lifespan by generating GRNs ($n=100$) from fetal to adult age. We then used different network algorithms to identify (i) temporal changes in network metrics and (ii) network modules within different age groups. We applied various network metrics, including mean correlation, a measure of network cohesiveness, and modularity, a measure of network segregation. Secondly, we generated multilayer networks across the lifespan to analyze which genes formed the stable temporal core of the networks. The multilayer network allowed coupling of networks along a time axis—so that we could assess individual genes, specifically to query which genes formed the stable 'temporal core' and which genes switched module assignment most often ('flexible periphery') in a time dependent manner.
Results: Younger and older age groups' GRNs exhibited higher modularity than those of middle age. However, only network modules in the younger groups showed strong evidence for functional enrichment (GO-terms and KEGG-pathways at $\alpha=0.05$; Bonferroni corrected), which likely indicates the tight control of gene co-expression during early development. More specifically, modules whose eigengenes (first principal component) correlated positively with age (18 modules in total) were enriched for neuronal signaling (36 non-redundant GO-terms); while modules negatively correlated with age (199 modules in total) were mainly enriched for splicing regulation (27 non-redundant GO-terms). Comparison of gender differences across the lifespan revealed similar modules in both genders during early age: Two main modules were detected in the younger groups, both showing strong enrichment, which decayed over the lifespan. Differences became obvious during older age when an additional module was detected only in males, enriched for neurodegenerative disease-association. Correspondingly, the temporal core of the network was similar in females and males and mainly consisted of the module enriched for neuronal signaling. In contrast, the genes with highest flexibility, which switched module assignment most often during the lifespan (highest 5% of flexibility), showed strong gender differences. Only in females, the genes with highest flexibility were enriched for terms linked to neurodegenerative diseases.
Conclusions: Our results suggest that splicing regulation is important during early development. In contrast, modules detected in older age groups show weaker enrichment of splicing regulation which could indicate less stringent control of splicing and transcript abundance. The decay in enrichment was observed for females and males, although it was slightly stronger in males. In addition to the weaker enrichment in later age, gender differences increased with age. Whereby the modules detected in both females and males were highly similar in young age, they differed more strongly, in terms of size, number and enrichment, in the older age groups. The comparison of temporal core and flexible periphery of networks across time indicates that genes involved in neurodevelopment form a relatively stable interaction pattern which is highly regulated in early life. This is true for both females and males. In contrast, genes associated with neurodegenerative diseases exhibit strong gender differences, assembling to form an additional module in late life in the male samples but not in the female samples. In contrast, genes enriched for neuro-

degenerative disease-association are the most flexible in the female sample. Future work will aim to identify gene subnetworks driving the changes in the described network metrics.

Keywords: networks, neurodevelopment, gene expression, genetic interactions.

Disclosures: C. Wehrspaun, Nothing to Disclose; W. Haerty, Nothing to Disclose; D. Bassett, Nothing to Disclose; J. Shin, Nothing to Disclose; D. Weinberger, Nothing to Disclose; C. Ponting, Nothing to Disclose.

W89. Large-scale RNA-sequencing of Schizophrenia Brains by the CommonMind Consortium

Pamela Sklar*

Mount Sinai School of Medicine, New York, New York

Background: Schizophrenia is a severe psychiatric disease with ~1% lifetime prevalence in the general population. Although it is a highly heritable disease, a picture of the genetics is only now emerging from large-scale studies that is nowhere near complete. The specific genes and pathways involved are still largely unknown, and no individual variant explains even a moderate fraction of the total genetic variance. Thus, two things have become increasingly clear: (i) large-scale genetic studies are needed due to disease heterogeneity and polygenicity, and (ii) study of any single level of biology (genetics, expression, imaging, etc.) will be insufficient to fully unravel the biology of disease, but rather information must be collected and combined across multiple levels. The CommonMind Consortium (CMC, http://commonmind.org) is a public-private pre-competitive consortium that brings together disease area expertise, large and well-curated brain sample collections, and data management and analysis expertise. The goal of the CMC is to generate and analyze large scale data from human subjects with neuropsychiatric and neurodevelopmental disorders and to make this data and the analytical results broadly available to the public as a free resource. This is based on the belief that development of new therapies is best met by pooling resources and by sharing data with the entire research community. The consortium consists of four academic groups (Icahn School of Medicine at Mount Sinai, University of Pennsylvania, University of Pittsburgh, and University of Texas Southwestern), two pharmaceutical companies (Takeda Pharmaceutical Company, and F. Hoffman-La Roche LTD), one non-profit group (Sage Bionetworks) and the NIMH.

Methods: Phase I of the CMC project is generating whole-genome transcriptome data via RNA sequencing on the prefrontal cortex as well as high-density SNP genotypes from ~600 postmortem brain samples from schizophrenia and control tissue collections. Currently it is envisaged that this data will become available to the public through Synapse (www.synapse.org) in 2014. Subsequent phases of the project will expand the molecular data for the brain collection to include new brain regions and new types of information beyond transcriptomic data (exome sequencing, epigenetic marks, etc.). Here we describe this Phase I large-scale study of RNA-sequencing-based gene expression in prefrontal cortex in brains of individuals with schizophrenia, as compared to control subjects. The full sample is composed of post-mortem brain tissue of schizophrenia patients ($N \sim 300$) and controls ($N \sim 300$). The utility of the data being generated here, beyond the large size of the sample, is that the data will be processed concurrently at the same site (Icahn Medical School at Mount Sinai), which permits randomization of case and control samples into batches for sample preparation and sequencing (eg, across different sequencing lanes, etc.). Having a uniform data generation and analysis pipeline from start to finish will allow us to be as highly powered as possible to draw conclusions about functional changes at the molecular level in the brains of individuals with schizophrenia.

Results: This project will generate a gene expression dataset for schizophrenia far larger than any currently available to date. RNA sequencing is currently underway and expected to be completed late 2013, so we present preliminary results on the first ~300 cases, ~300 controls). We performed extensive QC of pilot samples ($N = 14$ controls), including 5 samples for which RNA was prepared using both polyA isolation and RiboZero depletion of ribosomal RNA, in order to calibrate the amount of sequencing necessary to detect both low- and high-expression transcripts. Similarly, we carried out an analysis of power for differential expression to inform our decision of how deeply to sequence. The analysis of the expression data will aim to identify particular genes and pathways with differential expression between cases and controls, while correcting for available clinical (age at onset, medications) and technical (post-mortem interval, collection site) covariates. We will also explicitly search for more homogeneous subsets of affected individuals, as compared to controls. Moreover, we will curate a catalogue of brain-expressed genes and their splice forms in case and control individuals.

Conclusions: The CommonMind Consortium (CMC) is poised to expose signals of functional changes in the expression levels of genes in the human brain as related to schizophrenia. Leveraging a large and uniformly processed and analyzed dataset that will also be made public, the schizophrenia research community will be empowered to make novel discoveries relating neurobiology to risk of disease.

Keywords: schizophrenia gene expression RNA sequencing bipolar disorder genetic.

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W90. Pharmacogenetics of Growth Effects Complicating ADHD Treatment

Erika L Nurmi*, Allyson Mallya, Karyn S Mallya, Gerhard S Hellemann, James McGough, Sandra K Loo, Robert M Bilder, James T McCracken

University of California, Los Angeles, California

Background: Pharmacogenetics matches individuals with optimal treatment based on genetic background and can identify individuals at risk for adverse medication events. However, psychiatric pharmacogenetics lags behind other

areas of medicine. Previously we reported monoaminergic variants influencing treatment response to methylphenidate and guanfacine in children and adolescents with ADHD in the UCLA Translational Research to Enhance Cognitive Control (TRECC) sample. We now report pharmacogenetic influences on stimulant-mediated growth slowing. Growth effects of ADHD pharmacotherapies are important, treatment-limiting side effects. Due to monoamine and acetylcholine involvement in growth hormone regulation and energy balance, we examined genetic variation in these signaling pathways for association with growth effects.

Methods: 202 subjects between 7–14 years of age were recruited for the acute phase of a randomized, double-blind, placebo-controlled trial of dexmethylphenidate (d-MPH) and guanfacine for pediatric ADHD. Three treatment groups included guanfacine monotherapy, d-MPH monotherapy, and combination guanfacine plus d-MPH. Medication responders continued in the trial for approximately 14 months ($n = 99$) and height and weight were tracked regularly in addition to ADHD symptoms. We tested association of genetic variation in monoamine and energy balance candidate systems with height and BMI changes during medication exposure. Complete common variation was captured across dopaminergic (DRD1–5), adrenergic (ADRA2A, SLC6A2, HCN1), serotonergic (SLC6A4, HTR2C), cholinergic (CHRNA3, A4, A5, A7, CHRNB2), monoamine catabolic enzyme (MAO-A, MAO-B), and energy balance (FTO, LEP, NPY, MC3R, MC4R, CNR1) pathways. A generalized linear mixed model (GLMM) was used to analyze the three-way interaction of treatment group by genotype by time on change in height (*Z*-score) and BMI (*Z*-score). Since small non-Caucasian group sizes could produce unstable effects, we limited our analyses to the Caucasian subset ($n = 147$). Due to the large number of markers tested ($n = 95$), we only report results meeting genomewide significance.

Results: In our sample, guanfacine monotherapy was associated with height and BMI acceleration compared to that predicted from CDC growth charts (*Z*-score increase of 0.14 for height and 0.41 for BMI). Both d-MPH monotherapy (*Z*-score decrease of −0.12 for height and −0.84 for BMI) and combination treatment (*Z*-score decrease of −0.19 for height and −0.83 for BMI) were associated growth slowing. Overall, variation in treatment group trajectories for height ($p = 6.47 \times 10–15$) and BMI ($p = 5.58 \times 10–34$) between were highly significant. Variants in the cannabinoid receptor CNR1 (rs806378 $p = 2.68 \times 10–9$); the norepinephrine transporter SLC6A2 (rs36021 $p = 2.29 \times 10–15$); the serotonin transporter SLC6A4 (rs4251417 $p = 3.75 \times 10–9$); the dopamine DRD2 receptor (rs1079596 $p = 1.49 \times 10–9$, rs6277 $p = 1.96 \times 10–9$); and cholinergic receptors CHRNA4 (rs2236196 $p = 5 \times 10–8$), CHRNA5 (rs16969968 $p = 1.96 \times 10–19$) and CHRNA7 (rs6494212 $p = 2.51 \times 10–8$, rs711 $p = 6.00 \times 10–9$) were associated with height but not BMI change across all 3 treatment conditions. Effects specific to treatment condition were apparent. Individuals homozygous for the common allele of DRD4 rs3758653 treated with guanfacine monotherapy showed a 0.6 increase in height *z*-score compared to minor allele carriers ($p = 5.54 \times 10–9$), while no genetic effects were present in MPH + groups. Conversely, in the MPH-only condition, DRD3 rs3732790 minor allele homozygotes showed a 2.0 greater *z*-score weight loss then common allele carriers ($p = 1.09 \times 10–8$), while no genetic effects were present in guanfacine + groups. Surprisingly, minor allele homozygotes at DRD1 rs4867798 demonstrated greater height gain than predicted in both MPH + conditions, but not the guanfacine monotherapy group ($p = 8.32 \times 10–21$). Homozygotes for the common allele at ADRA2A rs3750625 ($p = 1.62 \times 10–8$) and the rare allele at DRD3 rs2134655 ($p = 2.18 \times 10–9$) revealed opposite height effects in guanfacine and MPH conditions. Negative effects of multiple NPY alleles on height change were predominantly seen in the combination condition (rs16147 $p = 9.67 \times 10–10$, rs16141 $p = 4.02 \times 10–10$, rs3905497 $p = 7.33 \times 10–17$, rs16148 $p = 3.98 \times 10–14$).

Conclusions: The power afforded by a repeated measures model across 16 months of visits would allow us to detect even small effects of guanfacine and stimulant treatment on growth. However, effect sizes between alleles are consistently in the moderate to strong range. Rare variants with extreme height effects in CHRNA4, CHRNA5, and DRD3 certainly require replication in larger samples, but are supported by functional data for variant effects on receptor signaling. Interestingly, the alleles associated with treatment response in our prior analyses were also associated with growth effects here, supporting a hypothesis of allele-specific increases in drug sensitivity. We are currently exploring methods for combining genetic factors to produce clinically actionable pharmacogenetic-based treatment recommendations. The results presented here suggest that genetic factors contribute significantly to stimulant-mediated growth slowing and are able to identify patients likely to be affected. Choice of optimal treatment in the future can be guided by both response and side effect pharmacogenetics.

Keywords: pharmacogenetics, ADHD, methylphenidate, guanfacine, growth.

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W91. Obsessive-Compulsive Traits in Children and Adolescents from the General Population: A Genome-wide Association Study

Paul D Arnold*, Christine Burton, Laura Park, Bingbin Li, S-M Shaheen, Vanessa Sinopoli, Annie Dupuis, Andrew Paterson, Jennifer Crosbie, Russell Schachar

The Hospital for Sick Children, Toronto, Ontario, Canada

Background: Obsessive-Compulsive disorder (OCD) is a common (1–2% lifetime prevalence), debilitating and phenotypically heterogeneous neuropsychiatric disorder. There is growing evidence that OCD is a multidimensional syndrome consisting of four to six underlying quantitative traits (Bloch *et al*, 2008) that are temporally stable and differ from one another in clinical course and neuroanatomical

correlates. Twin studies have demonstrated that both OC traits (Van Groothest *et al*, 2005) and its component dimensions (Iervolino *et al*, 2012) are highly heritable, particularly when symptoms begin in childhood or adolescence. Although genetic studies, including a recent genome-wide association study (Stewart *et al*, 2013) have yielded suggestive findings, no genetic associations have been conclusively identified. One possible reason for the lack of clear genetic findings to date is that previous studies have ignored the quantitative and multidimensional nature of OCD, which may have reduced the power of association studies, particularly if they included controls who were unscreened for psychiatric traits. We therefore set out to conduct a genome-wide study to identify variants associated with quantitative OC traits measured in a large, community-based sample of children.

Methods: The sample consisted of 16 380 children and adolescents (mean age 11.0 years, 51% male) recruited from a science museum in Toronto, Ontario, Canada. A subset of 220 twin pairs (60 MZ and 160 DZ) were collected and used for heritability analysis. Saliva DNA and information regarding obsessive-compulsive (OC) symptoms was collected from all participants. To measure OC traits we used both the obsessive-compulsive scale of the Child Behavior Checklist (CBCL-OCS) and a 21-item measure we developed to measure OC traits in the general population. Trait information was obtained from parents (80% of cases) and/or youth themselves (20% of cases). Principal components factor analysis was used to identify OC trait dimensions from these measures. For the genetic study, we selected 5940 European Caucasian individuals and genotyped them using the Illumina HumanCoreExome beadchip. Quality control analyses were conducted using PLINK, including multi-dimensional scaling (MDS) for population structure and the Cochrane-Armitage trend test for detection of association.

Results: The two quantitative OC trait measures were moderately correlated ($r = 0.6$). Factor analyses resulted in six factors consistent with previous studies of OCD and OC traits. Twin analyses revealed that OC traits were 65% heritable, with the remainder of the variance mostly accounted for by non-shared environment. The GWAS is ongoing and results will be available at the time of the presentation. Quality control analyses on an initial set of 475 individuals resulted in 9/475 exclusions due to sex discrepancy ($n = 5$) and non-European ancestry based on MDS plots ($n = 4$). Overall call rates were 99.9%.

Conclusions: OC traits in the general child population are continuously distributed, heritable and have a dimensional structure consistent with that seen in clinical studies of OCD. Our study is the first that demonstrates the utility of performing a genome-wide study of quantitative OC traits in children. Genome-wide association analysis, currently in progress promises to identify candidate variants for future biological investigation. We predict that a population-based approach will increase power for genetic studies of OCD and accelerate the identification of genetic risk factors for OCD and for other neuropsychiatric disorders (eg autism spectrum disorders) with prominent obsessive-compulsive features.

Keywords: genome-wide association study, obsessive-compulsive traits, dimensional traits, child psychiatric disorders.

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W92. Leveraging Hyperserotonemia and Whole Exome Sequencing in Autism Families to Identify Genetic Risk Factors

James S Sutcliffe, Nicholas Campbell, Emily L Crawford, Bingshan Li, Lea K Davis, Nancy J Cox, Edwin H Cook*

University of Illinois, Chicago, Illinois

Background: Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by deficiencies in communication, reciprocal social interaction, and restricted and repetitive behaviors such as insistence on sameness (IS) and other rigid-compulsive behaviors (RCBs). Numerous lines of investigation implicate serotonergic dysfunction as an element of ASD etiology. Prime examples include hyperserotonemia, or significantly elevated levels of platelet serotonin (5-HT) in ~35% of cases, and (2) efficacy of selective serotonin reuptake inhibitors in ameliorating anxiety and irritability related to IS and RCBs in ASD. While ASD demonstrates a predominantly genetic etiology, it is complex and extremely heterogeneous. Common variants individually show small and thus far non-reproducible effects on risk, yet in aggregate, common genetic variation contributes significantly to heritability. Both CNV and whole exome (WES) and whole genome (WGS) sequencing pointing to rare variants, an *de novo* variation as a major class of autism risk in 6–8% or more of ASD cases. Additionally, careful examination of inherited rare variants from WES studies has shown that compound heterozygous, or two-hit, loss-of-function (LOF) mutations contribute significantly to ASD risk (Lim *et al*, 2013). We hypothesize that examination of rare and common variation in autism families with hyperserotonemic probands will reduce genetic heterogeneity and offer insight into ASD risk factors and those specifically relevant to serotonergic dysfunction.

Methods: To tease out genetic factors related to RCBs and in particular hyperserotonemia as a heritable biomarker in ASD, we conducted WES of families selected on the basis of these traits. While most families were parent-child trios, one was a large 4-generation, multiplex family with several affected members across generations. In addition to filtering WES data for *de novo* mutations (DNMs), partially reported in Neale *et al*, 2012, we also filtered phased family exome data to identify autosomal or X-linked (in females) genes harboring inherited compound heterozygous (CH; '2-hit'), or X-lined hemizygous (in males) 'functional' variants (missense, nonsense, consensus splice site and read-through). Application of various algorithms to multiple databases (KEGG, GO, Reactome, InWeb, etc.) were used to explore pathway, network or functional enrichment. Additionally, a gene-collapsing transmission disequilibrium test (TDT) was employed to explore potential over-

transmission of variants across genes in this biomarker-selected sample.
Results: A number of loci were identified to harbor 'functional' CH genotypes and DNMs. Various analyses indicated an enrichment of genes in pathways related to cell adhesion, extracellular matrix proteins and genes previously implicated in ASD, intellectual disability or other neurological phenotypes. Analysis of the multiplex family revealed twelve 'functional' variants shared across all affected members and obligate ('unaffected') carriers. As expected, there is a bias towards larger genes harboring such variants, but pathway enrichment nevertheless suggested empirical significance TDT analysis revealed an expected over-representation of under-transmitted functional variants, reflecting false negative calls in probands or false positive calls in parents. We continue to explore the application of consensus calling pipelines that employ multiple algorithms to call genotypes from WES data to dramatically reduce such biases, and to integrate functional rare variants with CNV data. Our findings from DNMs and CH-harboring genes continue to provide interesting clues into specific gene sets and functional pathways.
Conclusions: We have used a hyperserotonemic and RC-selected subset of ASD to identify potential risk factors in ASD and have found numerous genes harboring 'functional' *de novo* mutations and inherited two-hit, compound heterozygous variants that will contribute to our growing body of knowledge regarding risk factors in ASD.
Keywords: autism spectrum disorder, whole exome sequencing, serotonin, rare variants, *de novo* mutation.
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W93. A Genome-wide Association Study Identifies a Genetic Locus in the GRB10-Amino Acid Decarboxylase Region of 7p12.2 Associated with Caucasian Treatment Resistant Schizophrenia Patients

Herbert Y Meltzer*, Jiang Li

Northwestern Feinberg School of Medicine, Chicago, Illinois

Background: Approximately 30% of schizophrenia patients have persistent moderate-to-severe positive symptoms after treatment with standard doses of typical or atypical antipsychotic drugs (APD), and are considered treatment resistant (TR). The remainder are considered non-TR (non-TRS). Once identified as TRS, the APD of choice is clozapine, which is effective in ameliorating positive symptoms in 50–60% of TRS. The dose of clozapine which is effective in such patients is higher than that for non-TRS. Some TRS patients respond within the usual time frame for APD response, 2–6 weeks, but more require 3–6 month treatment. A further differentiation of patients with TRS is whether they are TR from the first episode or only develop TR subsequently. Thus, it is reasonable to suppose that TRS, based on course of illness and treatment response, let alone neurobiology, is non-homgeneous. A biomarker for at least some TRS patients would be of value to more rapidly initiate a trial with clozapine and to identify a subgroup for studying the neurobiological basis for their refractoriness.
Methods: We report here a genome-wide association study (GWAS) of Caucasian schizophrenic patients, diagnosed by DSM-IV criteria. Genome-wde SNP genotyhping was performed using Illumina 619K quad BeadChip®. Taqman® assay for the top hits was performed to validate the GWAS data. All GWAS association testing was conducted with PLINK 1.0.7 software. The GWAS sample consisted of 79 TR and 95 non-TR cases diagnosed prospectively by Kane *et al*, (AGP, 1988) criteria.
Results: Although the effect size was small, five SNPs with significant association ($p < 10–5$) with TRS were identified based on chi-square analysis, supported by a Manhattan plot. No SNP associated with TRS survived Bonferroni correction. Rs2237457, the top hit associated with TRS, is located in a genomic region of 7p12.2 with an imprinted inheritance. Haploview disclosed a 30 kb haplotype block flanking this SNP at intron 4 of GRB10. Growth factor receptor-bound protein (Grb)10 is a protein that interacts with the IGF-I receptor and may thus regulate IGF-I which is critical for normal human somatic growth and development. Grb10 is located 70 kb upstream of amino-acid decarboxylase (AADC), an enzyme required for the synthesis of dopamine (DA) and, although not usually considered to be rate limiting. Nevertheless, it has been implicated in schizophrenia and the actions of APDs. We reanalyzed the GWAS data after excluding the heterozygous genotypes of this SNP because of unclear inheritance of the risk allele. The association with TRS was significantly enhanced: $p < 10–11$. This marker survived Bonferroni correction amd FDR-BH correction. TT, the homozygous risk allele of rs2237457, in the 103 homozybous schizophrenia patients provided sensitivity of 48% and specificity of 94%'. 88.89% positive predictive value of 88.9%value and 94.34% specificity. Rs2237457 identified 48% of TRS, just less than half of the TRS in this sample Those so identified may be a subgroup of TRS. Braincloud gene expression data suggested that this SNP and related haplotype serves as an exclusive *cis*-acting eQTL for the gene expression of AADD in dorsolateral prefrontal cortex of normal controls.
Conclusions: The risk allele for TRS leaded to significantly lower level of expression in AADD, indicating lesser ability to respond to antipsychotic drug-induced upregulation of AADD. Several studies of the effect of acute and chronic treatment with typical neuroleptic drugs and clozapine have shown that both lead to upregulation of the mRNA and increased expression of AADD protein and activity in striatum without affecting tyrosine hydroxylase, which is the rate limiting enzyme in the synthesis of DA. Using selective ligands. Neff *et al* (JPET, 2006) reported that D4, 5-HT1A, and 5-HT2A D1, D2 and D3 antagonists augmented AADD activity in rat striatum. An increase in AADD activity might also affect levels of serotonin (5-HT), which might impact on stimulation of 5-HT receptors important to antipsychotic drug action, or influence the levels of kynurenic pathway tryptophan metabolites that affect drug response and psychopathology. The other four SNPs and closest genes we found to be most strongly associated with TRS were rs10825879 (ZWINT), rs1421321 (CNPY1), rs6763175 (MRPS35P10 and rs6500291 (HEATR3). In summary, we have identified a novel genetic signature for

some TRS patients and a genetic marker in 7p12.2 region, which may provide an initial screening test to identify some TR Caucasian schizophrenia. Replication studies are needed.
Keywords: schizophrenia, genetic, clozapine, treatment resistant.
Disclosures: H. Meltzer, **Part 1:** SureGene, Teva, Sunovion, Dainippon Sumitomo, Alkermes, Lundbeck, EnVivo, Takeda, Janssen, Reviva, **Part 2:** SureGene, **Part 3:** SureGene, **Part 4:** Teva, Sunovion, Dainippon Sumitomo, Alkermes, Lundbeck, EnVivo, Takeda, Janssen, Reviva, **Part 5:** NOne; J. Li, Nothing to Disclose.

W94. Characterization of Autism-associated *De Novo* Mutations Impacting Integrin Receptor Subunit Genes

James S Sutcliffe*, Emily L Crawford, Keaton Wadzinski, Ana Carneiro

Vanderbilt University, Nashville, Tennessee

Background: Autism spectrum disorder (ASD) is characterized by a triad of impairments affecting development of language and communication, social deficits, restricted interests and behavioral inflexibility. The high incidence of ASD demands further studies on the biological processes underlying the pathology. While ASD has a strong genetic component, studies are further complicated by the genetic heterogeneity evident from numerous genetic studies. Gene enrichment studies reveal that rare variants can be clustered into conserved biological processes, including cell adhesion and motility. Cell adhesion is modulated by the expression of cell surface receptors and signaling pathways, many of which have been identified based on harboring *de novo* variants in ASD families. Examples of such ASD-associated focal adhesion genes include *CTNNB1*, *ITGB3*, *FN1*, *ROCK1*, *ITGA2*, *LAMB2*, *ITGA5*, *PTEN*, *ITGA11* and *RELN*. Having noted several *de novo* mutations in integrin receptor subunits and their ligands, we hypothesize that integrin signaling is a conserved molecular pathway associated with ASD. Integrins are heterodimeric plasma membrane receptors, composed of one α and one β subunit, that interact with the extracellular matrix (ECM). In both mice and humans there are 26 integrin genes: 18 genes encoding α-subunits, and eight genes that encode β-subunits. Early in development, integrin signaling supports developmental events such as neuronal migration and synaptic differentiation. Here we studied the cellular mechanisms by which *de novo* mutations influence integrin function *in vitro* models.
Methods: Integrin variants were expressed in heterologous cells to determine cell adhesion, survival and migratory properties. Attachment specificity was determined by cell adhesion assays using different ECM proteins (laminin, vitronectin, fibronectin). ECM concentration curves (10–500 mg/ml) revealed changes in receptor affinity. We treated cells with protein kinase C activator β-PMA, a known modulator of cell migration and attachment to determine whether adhesion changes result from altered sensitivity to growth-receptor mediated activation. Cell adhesion relative strength was determined by attaching cells and inducing detachment by centrifugation, where the number of cells attached under increasing *g* forces is proportional to the strength of the adhesion.
Results: Exome sequencing studies conducted by the NIH ARRA Sequencing Consortium identified a *de novo* nonsense mutation in *ITGA5* (E885*), and similar studies conducted by groups working on the Simon Simplex Collection revealed a missense mutation in *ITGB3* (R378H), previously implicated in ASD and in regulation of the serotonin transporter. These are but two of other integrin or integrin-related mutations observed. The *ITGA5* mutation leads to a truncation in the extracellular membrane-proximal domain of the integrin a5 protein, likely resulting in a non-functional protein. We expressed the truncated *vs*, wiltype a5 in CHO cells to determine the influence of the truncated a5 on the endogenous $\alpha5\beta1$ function. CHO cells overexpressing the WT $\alpha5$ show a time-dependent increase in attachment, while the truncated isoform inhibits this increase. Receptors with truncated $\alpha5$ inhibit both MnCl2- and PKC-mediated attachment of the endogenous $\alpha5\beta1$. The signaling properties of the endogenous $\alpha5\beta1$ were also modified by co-expression of the truncated $\alpha5$, as measured by shear stress-induced cell death (anoikis). The co-expression of the truncated $\alpha5$ inhibits cell death measured as reduction of cells attached over time. We also examined a coding variant in *ITGB3*. We found no significant changes in basal adhesion, suggesting that this mutation does not alter the conformation of αvb3. We observed, however, a blunted response to β-PMA by the *ITGB3* variant.
Conclusions: The transition between neuronal migration, axonal formation and synaptogenesis depends on the differential expression of integrin subunits together with the extracellular guidance cues. The interaction between migrating neurons and radial glia, and the timely detachment of neurons at the end of migration involves $\alpha3\beta1$ integrin. The expression of extracellular matrix proteins, such as reelin, laminin and fibronectin modulate the fate of axons and synapses. Both reelin (*RLN*), which binds $\alpha3\beta1$ and fibronectin (*FN1*), which binds both $\alpha5\beta1$ and $\alpha V\beta3$ are expressed during development. For this study, we focused on fibronectin-binding receptors and identified significantreductions in cell adhesion in both variants studied. The significance of this study extends from unpublished genetic data to characterize a conserved biological pathway modified in ASD.
Keywords: integrins, autism, *de novo* mutation, whole exome sequencing, cell adhesion.
Disclosures: J. Sutcliffe, Nothing to Disclose; E. Crawford, Nothing to Disclose; K. Wadzinski, Nothing to Disclose; A. Carneiro, Nothing to Disclose.

W95. RNA Editing Levels of 5-HT2C and GluA2 are Increased in Suicides with Major Depression

Monsheel Sodhi*, Thomas M Hyde, Stefan Green, Joel E Kleinman

University of Illinois, Chicago, Illinois

Background: A completed suicide occurs every 14 min in the United States. Many individuals who complete suicide have previously been diagnosed with major depression. Accumulating data indicate that a process known as 'RNA editing' may contribute to the pathophysiology of

suicide. RNA editing is a post-transcriptional process that creates sequence changes in RNA. The most common form of RNA editing in the human brain is catalyzed by the ADAR enzymes, ADARs1–3. This form of RNA editing creates sequence changes of adenosine to inosine in numerous RNA targets. The most important RNA edited targets characterized in the mammalian brain to date include the 5-HT2C, AMPA and kainate glutamate receptors. The physiological functions of these receptors are profoundly altered by the RNA editing process (reviewed by Nishikura, 2011). Some but not all reports have found increased 5-HT2C RNA editing in the prefrontal cortex of postmortem subjects with mood disorders and in suicide cases. At least in part, these discrepancies may reflect small sample sizes. We have recently reported increased ADAR1 expression in the dorsolateral prefrontal cortex of major depressive suicide cases (Simmons *et al*, 2010), indicating that increased RNA editing in these patients may be more generalized and not restricted to 5-HT2C receptors. We now present data from analyses of all three ADAR enzymes, 5-HT2C RNA editing and GluA2 RNA editing in a much larger cohort of postmortem subjects ($n = 114$) to test the hypothesis that generalized increases in RNA editing occur in suicides with major depression.

Methods: RNA was extracted from the gray matter of the DLPFC of 80 postmortem major depressive suicide cases and 34 control subjects. All postmortem subjects included in these analyses were tested for the presence of antidepressants using toxicology screens, and were diagnosed by psychological autopsy (for details see Lipska *et al*, 2006). Expression analyses were conducted by quantitative polymerase chain reaction (QPCR). QPCR data were normalized to the expression of three housekeeping genes and analyzed using the relative standard curve method. Subsequently, levels of RNA editing of the 5-HT2C receptor were measured using tagged next generation sequencing in these subjects. GluA2 RNA editing was assessed using RT-PCR followed by restriction enzyme analysis (RFLP). Data were analyzed by ANCOVA, covarying for age at death, postmortem interval and postmortem pH of the brain. When we detected association between the expression of the gene being analyzed and the presence of antidepressants or other drugs, the subjects testing positive for those drugs were excluded from the analyses.

Results: ADAR expression and 5-HT2C RNA editing but not GluA2 RNA editing were associated with the presence of antidepressants. Therefore the antidepressant positive subjects were excluded from analyses of ADAR expression and 5-HT2C RNA editing. In the antidepressant free MDD suicides, there were increased levels of 5-HT2C RNA editing, resulting in increased expression of the 5-HT2C-VDV isoform in this subject group ($F2, 73 = 4.3$, $p = 0.01$)and increased levels of RNA editing at the 5-HT2C 'B' site ($F2, 73 = 3.8$, $p = 0.02$). These increases in RNA editing were not observed in the entire MDD group compared with the controls. The levels of RNA editing of GluA2 and 5-HT2C are indices of ADAR activity. GluA2 RNA editing at the R/G site was measured using QPCR followed by RFLP. The expression of all three ADAR enzymes was correlated with GluA2 RNA editing ($F3, 74 = 24.6$, $p < 10–10$) and also 5-HT2C RNA editing at the B site ($F3, 74 = 6.2$, $p = 0.001$). *Post-hoc* tests showed that GluA2 RNA editing was also increased in the major depressive suicide group ($F2, 112 = 5.1$, $p = 0.008$). Multivariate analyses revealed a gender by diagnosis effect of all three ADAR enzymes with major depression ($F3, 68 = 10.4$, $p < 10–4$).

Conclusions: We have found that both 5-HT2C and GluA2 RNA editing are increased in major depressive suicides relative to major depression non- suicide cases and the control group. These results were generated from analyses of antidepressant negative patients, since 5-HT2C RNA editing and ADAR expression was significantly associated with the presence of antidepressants on toxicology screens. RNA editing of both 5-HT2C and GluA2 was strongly correlated with the expression of all three ADAR enzymes. In addition, a gender by diagnosis interaction was detected between the expression of the three ADAR enzymes and major depression. Taken together with our previous studies, these data indicate that the RNA editing of 5-HT2C and GluA2 are increased in major depressive suicide cases. Increases in the editing of these RNA targets would be predicted to lead to reduced 5-HT2C signal transduction (Niswender *et al*, 1999) and also reduced cell surface trafficking and altered conductance of the AMPA receptor ion channel (Greger *et al*, 2006). Therefore RNA editing may contribute to the pathophysiology of suicide in major depression. In addition to identifying potential biomarkers of suicide, these molecular changes may also facilitate the development of improved antidepressant drugs.

Keywords: gene expression, serotonin, AMPA receptor, ADAR, dorsolateral prefrontal cortex.

Disclosures: M. Sodhi, Nothing to Disclose; T. Hyde, Nothing to Disclose; S. Green, Nothing to Disclose; J. Kleinman, Nothing to Disclose.

W96. Deep RNA Seq Characterization of Novel Transcripts in CACNA1C

Joo Heon Shin*, Dewey Kim, Joshua Hurtado, Bin Xie, Thomas M Hyde, Joel E Kleinman, Daniel R Weinberger

Lieber Institute for Brain Development, Baltimore, Maryland

Background: The L-type calcium channel gene CACNA1C has been identified by several independent studies as a risk gene for bipolar disorder and schizophrenia. It has also been shown that healthy subjects who carry the CACNA1C risk-associated genotype exhibit patterns of brain activity that are associated with mental illness. Further, the risk associated genotype is associated with increased mRNA expression of a fetally expressed transcript of CACNA1C in the prefrontal cortex of human post-mortem brain samples based on an oligonucleotide array expression analysis. In order to probe the function of CACNA1C in mental disease and the molecular mechanism of the clinical associations, a detailed characterization of transcript processing of CACNA1C is necessary.

Methods: We analyzed RNA sequencing data from postmortem dorsolateral prefrontal cortex (DLPFC) of 556 brain samples (Control, Schizophrenia, MDD, Bipolar, Autism).

RNA-seq reads are achieved by two different library construction procedures, Poly-A selection or Ribosomal RNA depletion (RiboZero) with strand specific protocols followed by high-throughput sequencing. After sequencing run on the HiSeq 2000, the Illumina Real Time Analysis (RTA) module to perform image analysis, base calling, and the BCL Converter (CASAVA v1.8.2) were used to generate the sequence reads. The sequencing depth is 100 bp paired-end 80–100 fold coverage of mapable sequencing reads which are aligned back to the human genome by the spliced-read mapper (Tophat v2.0.4) based on known transcripts of Ensembl Build GRCh37.67. Then, relative abundances of genes, exons, or transcript are estimated. To enrich for novel transcript signals in CACNA1C, we selected the reads aligned to CACNA1C using samtools (v0.1.18) and combined all the reads across all the samples. Then, for each junction read, we annotated it with the known transcripts of Ensembl to check whether either end of a junction falls into a known transcript. After that, all the novel expressed sequences, ie those not mapping to known transcripts, were validated by PCR, with subsequent Sanger sequencing of the PCR products inserted into a plasmid vector. To check whether or not any of the novel expressed sequences are brain-specific, we compared it with stem cell RNA-seq data.

Results: Two novel transcription start sites (TSS) were discovered within the first canonical intron of CACNA1C: one in a CpG island, with low transcriptional abundance, and another 152 bp downstream from the CpG island, with relatively high transcriptional abundance. The two novel signals were confirmed by PCR using pooled adult and fetal brain samples. Both of these signals have been confirmed in the adult and fetal samples, but the CpG-island associated TSS is absent in the fetal samples. To confirm the tissue-specificity of the novel TSS, we used 9 stem-cell RNA-seq samples using the same protocol and analysis pipelines. There was no evidence of transcription within these two novel regions.

Conclusions: We describe the discovery of two potentially brain-specific TSS sites in CACNA1C. We have also discovered similar novel TSS signals in other genes of the CACNA1 family (CACNA1A and CACNA1E) in the RNA-seq data that have yet to be confirmed by PCR. CACNA1B does not have a novel TSS signal, but that may be due to truncation of the CACNA1B 5′ end compared to its ancestral sequence—there is a CpG island upstream from CACNA1B that may be an analog to the CpG island that is being targeted in CACNA1A and CACNA1C. To explore the possible relevance to psychiatric illness and genetic risk for psychiatric illness, we are quantifying expression of CACNA1c known and novel transcripts across diagnostic groups using both the RNA seq data and Taq-man qPCR techniques and examining the associations with GWAS positive risk genotypes.

Keywords: CACNA1C, psychiatric illness, RNA-Seq, TSS, brain-specific.

Disclosures: J. Shin, Nothing to Disclose; D. Kim, Nothing to Disclose; J. Hurtado, Nothing to Disclose; B. Xie, Nothing to Disclose; T. Hyde, Nothing to Disclose; J. Kleinman, Nothing to Disclose; D. Weinberger, Nothing to Disclose.

W97. Genetic Pathway Analyses of the Endocannabinoid System in a Sample of Social Drinkers and Treatment-Seeking Alcoholics

Jia Yan*, Bethany L Stangl, Mark A Reimers, Melanie L Schwandt, Hui Sun, Colin A Hodgkinson, David Goldman, Daniel W Hommer, David T George, Kenneth S Kendler, Markus Heilig, Vijay A Ramchandani

NIH, Bethesda, Maryland

Background: The endocannabinoid system has been implicated in a range of substance use and related phenotypes in both animal and human models. In particular, single nucleotide polymorphisms (SNPs) in the cannabinoid receptor 1 (*CNR1*) and fatty acid amide hydrolase (*FAAH*) genes have been reported to be associated with a number of addiction disorders, including cannabis, cocaine, heroin, nicotine, and alcohol dependence. Previous findings in our group suggested that rs2023239, a functional polymorphism in *CNR1*, may be involved in the severity of alcohol withdrawal. In a sample of treatment-seeking individuals, carriers of the C allele of rs2023239 had a lower mean peak alcohol withdrawal, as measured by the Clinical Institute Withdrawal Assessment-Alcohol Revised (CIWA-Ar) tool, compared with noncarriers. Here, we expand our investigation into the role of the endocannabinoid system in alcohol dependence by using a pathway-based approach to examine the joint effect of polymorphisms in key endocannabinoid system genes. Genetic studies of alcohol dependence and other complex phenotypes have increasingly supported a polygenic model of risk in which individual genetic variants account for only a small proportion of the variance in the trait. Pathway analysis has been developed as a systematic, targeted approach to analyze functional gene sets. Based on the notion that variants conferring small perturbations along a biological pathway could in concert have a larger influence on disease risk, testing variants as a single statistical unit could increase power to detect association with complex disease phenotypes. In our analyses, we assessed four genes encoding proteins that play roles in the function of the endocannabinoid system in the central nervous system: *CNR1*, *FAAH*, transient receptor vanilloid 1 (*TRPV1*), encoding a receptor for the endogenous cannabinoid, anandamide, and the gene encoding N-acetyl-phosphatidylethanolamine-hydrolysing phospholipase D (*NAPEPLD*), which is involved in the biosynthesis of anandamide.

Methods: The sample in this study consisted of 551 treatment-seeking alcoholics and 405 non-treatment seeking participants enrolled in studies at the National Institute on Alcohol Abuse and Alcoholism Laboratory of Clinical and Translational Studies. All samples were genotyped using the Illumina OmniExpress BeadChip. We selected SNPs genotyped on this array within a 50-kb window of *CNR1*, *FAAH*, *TRPV1*, and *NAPEPLD*. SNPs with call rates less than 90%, minor allele frequency less than 1%, and deviation from Hardy Weinberg Equilibrium ($p<0.0001$) and samples missing more than 10% of the SNPs of interest were excluded from the analyses, resulting in a final set of 142 SNPs. The association between the endocannabinoid pathway and alcohol disorder, determined by DSM-IV criteria for current or past alcohol abuse or dependence ($n=707$ cases, 287 controls), was assessed using logistic

regression with sex and self-reported African, European, or Asian ancestry as covariates. Pathway analysis was performed using the PLINK v1.07 set-based test. For this test, single-SNP analyses are first performed within the set to select a list of SNPs that individually meet a nominal *p*-value threshold ($p<0.05$) and an LD threshold ($r2<0.50$) for selection. The selected set of SNPs is then used to create a test statistic based on the mean of single-SNP tests in the selected set. The entire procedure is then repeated for simulated datasets generated by shuffling the phenotype labels in the dataset. An empirical *p*-value is generated based on the proportion of SNP sets from simulated data with test statistics exceeding that of the observed data. In our analyses, we selected a maximum of 5 SNPs for the set, and repeated the procedure using 10 000 permutations to generate an empirical *p*-value. We further assessed the effects of the pathway within a subset of social drinkers who participated in an intravenous alcohol self-administration study using the computer-assisted self-infusion of ethanol (CASE) open bar paradigm ($n=80$).

Results: Set-based pathway analysis showed a nominal association of the endocannabinoid pathway with alcohol disorder in the sample of treatment-seeking alcoholics and non-treatment seeking participants (empirical *p*-value = 0.07169). The optimal set of SNPs meeting all thresholds consisted of rs1861726 (*NAPEPLD*), rs9911213 (*TRPV1*), and rs224546 (*TRPV1*). Analysis of the same pathway in the CASE self-administration paradigm did not result in significant association with study session measures such as peak and average breath alcohol content.

Conclusions: Pathway analysis in a sample of social drinkers and individuals seeking treatment for alcohol dependence provided nominal support for the importance of the endocannabinoid system in alcohol abuse and dependence. The lack of association within the subset of social drinkers participating in the CASE paradigm suggests variability in influence on alcohol administration. These results emphasize the need for further investigation into the biological mechanisms by which subtle genetic effects confer changes in endocannabinoid function and subsequent risk for alcohol use disorders.

Keywords: alcohol dependence, pathway analysis, endocannabinoid system, genetics.

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W98. Clinical and Genetic Predictors of Length of Sobriety in Alcoholics Treated with Acamprosate

Joanna Biernacka*, Jennifer Geske, Gregory Jenkins, Mark A Frye, Doo-Sup Choi, Daniel Hall-Flavin, Terry Schneekloth, Falk Kiefer, Karl F Mann, Victor Karpyak

Mayo Clinic, Rochester, Minnesota

Background: Acamprosate supports abstinence in some alcoholics. It is believed to counteract the 'relief craving' associated with increased brain glutamate levels. Experimental findings also indicate that glycine neurotransmission, which modulates glutamate effects, may contribute to acamprosate response. However, predictors of inter-individual differences in acamprosate response are unknown. We investigated associations between acamprosate response and genetic variation in pathways responsible for glycine and glutamate neurotransmission, as well as candidate genes previously implicated in acamprosate response.

Methods: 518 tag SNPs covering 40 candidate genes and 28 ancestry informative markers were genotyped in 225 alcoholics treated with acamprosate and followed-up for 3 months (discovery sample). Cox proportional hazard models were used to test for association of genotype with the length of abstinence (time to first alcohol use) during the first 3 months of acamprosate treatment. Data from 164 German male alcoholics treated with acamprosate or placebo in the PREDICT study was used for replication of the top SNP association findings. Baseline demographic, clinical and consumption variables were also tested for association with treatment outcome in both samples, and relevant covariates were accounted for in statistical models used for testing pharmacogenetic effects.

Results: In the discovery sample, increased intensity of craving was associated with shorter abstinence, while increased number of days since last drink before starting acamprosate was associated with longer abstinence. After adjustment for these variables and recruitment site, shorter abstinence was found to be associated with *GRIN2B* rs2058878 T ($p=4.6\times10–5$, Bonferroni corrected $p=0.024$) and *GLRB* rs17035723 A ($p=1.2\times10–4$, Bonferroni corrected $p=0.064$) alleles. In the replication sample, increased intensity of craving, depressive symptoms, and higher alcohol consumption were associated with shorter abstinence. After adjustment for these covariates, *GRIN2B* rs2300272 G (proxy for rs2058878 A, $r2=0.9$) was associated with shorter abstinence ($p=0.024$).

Conclusions: Our findings indicate that abstinence length in acamprosate-treated alcoholics is associated with the intensity of craving and depressive symptoms as well as the *GRIN2B* rs2058878 variant and its proxy rs2300272. Future research should focus on replication of these findings and investigation of the potential functional role of the identified *GRIN2B* variants or other SNPs in linkage disequilibrium. These findings may help predict response to acamprosate treatment, leading to improved individualized strategies for treating alcohol dependence.

Keywords: pharmacogenetics, acamprosate, alcohol dependence, abstinence, glutamate.

Disclosures: J. Biernacka, Nothing to Disclose; J. Geske, Nothing to Disclose; G. Jenkins, Nothing to Disclose; M. Frye, **Part 1:** Allergan, Merck, Myriad, Sanofi-Aventis, Sunovion, Takeda Global Research, Teva Pharmaceuticals, United Biosource Corporation, Mitsubishi Tanabe Pharma Corporation, **Part 4:** Dr Frye has had grant support from Pfizer and Myriad.; D. Choi, Nothing to Disclose; D. Hall-Flavin, Nothing to Disclose; T. Schneekloth, Nothing to Disclose; F. Kiefer, **Part 1:** Falk Kiefer received honoraria from Pfizer and Desitin, and is a consultant to Lundbeck, AWD.pharma and Roche.; K. Mann, **Part 1:** Karl Mann was a consultant to Alkermes (injectable naltrexone) and received honoraria from Desitin (naltrexone). He is a consultant to Lundbeck (Nalmefene)., **Part 4:** Karl Mann

received research grants from Schering Plough and Merck.; V. Karpyak, Nothing to Disclose.

W99. Alterations of BDNF Signaling and Splicing in Violent Suicide

Giovanna Punzi*, Gianluca Ursini, Kristen Maynard, Joo Heon Shin, Bin Xie, Yuan Gao, Joel E Kleinman, Thomas M Hyde, Keri Martinowich, Daniel R Weinberger

Lieber Institute for Brain Development, Baltimore, Maryland

Background: Impulsivity is frequently concomitant of violent suicide attempts and is associated with higher lethality. Psychotic patients tend to choose more violent and impulsive methods of suicide. Previous evidence suggests that independent of psychiatric diagnosis, BDNF signaling may be altered in individuals who commit suicide. The promoter of BDNF exon 1 contains a binding site for NPAS4, a transcription factor that has been involved in emotional dysregulation, suggesting that it might be a link between BNDF and suicide. We analyzed BDNF expression in post-mortem human brains of individuals who died of suicide and assessed aggressive behavior in a transgenic model of altered BDNF signaling to explore relationships between specific alterations of BDNF signaling with violent suicide and impulsivity.
Methods: We utilized a standard resident-intruder paradigm to assess aggressive behavior in mice engineered to disrupt BDNF expression generated specifically from *Bdnf* promoter I (BDNF-e1 mice) to determine whether deletion of BDNF protein derived only from BDNF exon I containing transcripts was sufficient to cause aggressive behavior. We analyzed RNA sequencing data from post-mortem dorsolateral prefrontal cortex (DLPFC) of 106 patients with schizophrenia (77 non-suicide, 13 non-violent suicide, 16 violent suicide). PolyA enriched RNA was extracted and then purified and enriched with PCR to create a final cDNA library for high throughput sequencing using the Illumina HiSeq2000. The Illumina Real Time Analysis (RTA) module was used to perform image analysis and base calling, followed by BCL Converter (CASAVA v1.8.2) to generate FASTQ files containing sequence reads. Pair-end reads of cDNA sequences obtained by the HiSeq2000 were aligned to the human genome reference (UCSC hg19) by splice-read mapper (TopHat v2.0.4), providing known transcripts from Ensembl Build GRCh37.67. To quantify the gene-level and the exon-level expression, we used htseq-count v0.5.3 (with intersection-strict mode) to count the properly-paired and mapped reads and RPKM (Reads Per Kilobase per Million mapped reads) was calculated. We used IGV software to visualize RNA seq data and to search for novel transcripts of the BDNF gene. All the novel findings were validated by PCR, with subsequent sequencing of the PCR products inserted into a plasmid vector. ANCOVA with age, sex, race and RIN as covariates, was performed to analyze the relationship between gene expression and manner of death.
Results: BDNF-e1 mice ($n=12$ wild-type and $n=12$ BDNF-e1, age 10 weeks), showed highly aggressive behavior in a resident intruder paradigm compared to wild-type littermates. BDNF-e1 mice showed decreased latency to attack ($P<0.001$), increased number of fights ($p<0.001$), increased number of tail rattles ($p<0.001$), and abnormal aggressive tendencies including targeting vulnerable regions of opponents. We therefore analyzed RNA seq data focusing on novel transcripts in the BDNF exon 1 region, potentially relevant for violent suicide. New splicing events were detected in the region of BDNF exon 1. Specifically, a new exon overlapping with the central part of exon 1 (1truncated, 1t) is spliced with different exons, which have not been previously described, also providing a source of novel transcripts. The expression of this novel exon 1t is increased in violent suicide (VS), compared with non suicide (NS), with non violent suicide (NV) being intermediate ($N=106$; $F2,99=3.754$; $p=0.02$; *post hoc* with Fisher's least significant difference: NS < VS, $p<0.01$; NS < NV, $p=0.31$; NV < VS, $p<0.01$), consistently with experiments in mice showing an effect of exon 1 deletion on aggressive behavior. No significant difference was found in the expression of the region of exon 1, known to be spliced with the coding exon of BDNF ($p>0.1$). Moreover, we compared BDNF expression in VS, NS and NV, and we found that manner of death is related to BDNF expression as RPKM ($N=106$; $F2,99=7.235$; $p<0.01$). Specifically, BDNF expression is greater in VS and lower in NS, with NV being intermediate (*post hoc* with Fisher's least significant difference: NS < VS, $p<0.01$; NS < NV, $p=0.05$; NV < VS, $p<0.01$). Consistently, manner of death is also related to expression of genes involved in BDNF/WNT signaling, including GSK3B ($N=106$; $F2,99=2.973$; $p=0.05$) and CTNNB1 ($N=106$; $F2,99=7.306$; $p<0.01$).
Conclusions: Detection of specific markers for short-term suicidal risk is a potentially important insight for the development of high impact prevention strategies. We demonstrate that transcription at the BDNF locus is altered in DLPFC of violent suicides. Specifically, our experiment in mice and our RNA sequencing analysis in humans are consistent in demonstrating a role of BDNF exon 1 alteration in aggressive behavior in mice and violent suicide in humans. The gene expression alterations related to the BDNF signaling in potentially impulsive behavior comes together with gene expression alterations of the WNT pathway, known to play a role in the action of psychoactive drugs.
Keywords: suicide, impulsivity, BDNF, Wnt signal, schizophrenia.
Disclosures: G. Punzi, Nothing to Disclose; G. Ursini, Nothing to Disclose; K. Maynard, Nothing to Disclose; J. Shin, Nothing to Disclose; B. Xie, Nothing to Disclose; Y. Gao, Nothing to Disclose; J. Kleinman, Nothing to Disclose; T. Hyde, Nothing to Disclose; K. Martinowich, Nothing to Disclose; D. Weinberger, Nothing to Disclose.

W100. Potential Methylation in the 5HT System: Analysis in Suicidal Behavior

Vincenzo de Luca*, Ali Bani-Fatemi, Jiali Song, Aaron Howe, Nuwan Hettige, Ahmed Hassan

CAMH, Toronto, Canada

Background: Studies of various genes have shown that SNPs are associated with suicidal behavior rather than

with primary psychiatric diagnosis. Given the above rationale, the primary aims of the current study are: (1) To identify SNPs in the 5HT system that are associated with suicide attempt in schizophrenia; (2) Create a SNP map in our sample, by selecting SNPs that affect the potential methylation within the 5HT system.
Methods: We have collected detailed clinical information and DNA samples from 221 schizophrenia patients, allowing us to perform genetic association analyses in suicide attempters and non-attempters. Using the the Columbia Suicide Severity Rating Scale, we determined the presence of suicide attempts lifetime that can be tested using DNA variants in the serotonin (5HT) system to detect the genetic markers associated with suicide risk in schizophrenia. This cross-sectional DNA sample included subjects with a diagnosis of schizophrenia ascertained by structured interview, all carefully re-assessed for lifetime suicide attempt by the means of Beck Scale for Suicide Ideation (BSS). In the initial step, we will apply a conventional genetic association strategy in order to find any SNP associated with suicide attempt in schizophrenia. The association study was performed using the duration of illness as main covariate incorporated in an additive model. A novel mapping analysis will be conducted using a specific bioinformatic tool we have developed, which analyzes only the polymorphic CpG sites in the 5HT system. This analysis will looks at the presence or absence of methylation sites affected by the SNP allele. Using this analysis, each subject can have zero, one or two methylation sites for each SNP locus, which in turn can be translated in a methylation level of 0, 50 or 100%. This bioinformatic tool can detect the SNPs that are affecting the polymorphic CpG sites across the genome. In this analysis, the SNPs will be studied under a different perspective considering their direct contribution to the availability of methylation sites within the gene of interest. Furthermore the total number of potential methylation sites at gene level and in the overall 5HT system was calculated.
Results: Among the candidate 5HT genes we considered none of them was significantly associated with suicide attempt. There were approximately 33% of CpG SNPs in our sample that was investigated using a total of 611 SNPs in the 5HT system. The overall potential methylation was 56.1% in the attempters and 56.3% in the non-attempters. This difference was not significant ($p = 0.865$).
Conclusions: The overall results show no association between CpG SNPs in the 5HT system and suicide attempt however the information of the SNP CpG potential methylation can be used as covariate in future methylation analysis.
Keywords: schizophrenia, epigenetics, 5ht, suicide, SNPs.
Disclosures: v. de luca, Nothing to Disclose; A. Bani-Fatemi, Nothing to Disclose; j. song, Nothing to Disclose; a. howe, Nothing to Disclose; n. hettige, Nothing to Disclose; a. hassan, Nothing to Disclose.

W101. Discovery and Validation of Blood Biomarkers for Suicidality

Alexander B Niculescu*, Helen Le-Niculescu, Daniel Levey, Mikias Ayalew, Nitika Jain, Evan Winiger, Ganesh Shankar, Mark Radel, Elizabeth Belanger, Hilary Duckworth, Robert Schweitzer, Michael Yard, George Sandusky, Anantha Shekhar, Nicholas Schork, Daniel Salomon

Indiana University School of Medicine, Indianapolis, Indiana

Background: Suicides are a leading cause of death in psychiatric patients, and in society at large. Developing more quantitative and objective ways (biomarkers) for predicting and tracking suicidal states would have immediate practical applications, and positive societal implications. We undertook such an endeavor.
Methods: First, building on our previous blood biomarker work in mood disorders and psychosis, we decided to identify blood gene expression biomarkers for suicidality, looking at differential expression of genes in the blood of subjects with a major mood disorder (bipolar disorder), a high risk population prone to suicidality1. We compared no suicidal ideation states and high suicidal ideation states using a powerful intra-subject design, as well as an inter-subject case-case design, to generate a list of differentially expressed genes. Second, we used a comprehensive Convergent Functional Genomics (CFG) approach to identify and prioritize from the list of differentially expressed genes biomarkers of relevance to suicidality. CFG integrates multiple independent lines of evidence- genetic and functional genomic data, as a Bayesian strategy for identifying and prioritizing findings, reducing the false-positives and false-negatives inherent in each individual approach. Third, we examined whether expression levels of the blood biomarkers identified by us in the live bipolar subject cohort are actually altered in blood in an age-matched cohort of suicide completers collected from the coroner's office. Fourth, we look if the markers can predict future, as well as past hospitalizations for suicidality. Fifth, we combined clinical and biomarker data to develop multimodal predictors.
Results: We report that 13 out of the 41 top CFG scoring biomarkers (32%) show step-wise significant change from no suicidal ideation to high suicidal ideation and then to the suicide completers group. 6 out of them (15%) remained significant after strict Bonferroni correction for multiple comparisons. We also show that blood levels of SAT1, the top biomarker identified by us, at the time of testing for this study, differentiated future as well as past hospitalizations with suicidality, in a live cohort of bipolar disorder subjects, and exhibited a similar but weaker pattern in a live cohort of psychosis (schizophrenia/schizoaffective disorder) subjects. Three other (PTEN, MARCKS and MAP3K3) of the six biomarkers that survived Bonferroni correction showed similar but weaker effects. Taken together, the prospective and retrospective hospitalization data suggests SAT1, PTEN, MARCKS and MAP3K3 might be not only state biomarkers but trait biomarkers as well. We then show how a multidimensional approach using SAT1 blood expression levels and two simple visual-analog scales for anxiety and mood

enhances predictions of future hospitalizations for suicidality in the bipolar cohort (ROC curve with AUC of 0.813). Of note, this simple approach does not directly ask about suicidal ideation, which some individuals may deny or choose not to share with clinicians. Lastly, we conducted bioinformatic analyses to identify biological pathways, mechanisms, and medication targets. Overall, suicidality may be underlined, at least in part, by biological mechanisms related to stress, inflammation and apoptosis.
Conclusions: Taken together, our results have implications for the understanding of suicide, as well as for the development of objective laboratory tests and tools to track suicidal risk and response to treatment.
Keywords: convergent functional genomics; suicide; bipolar disorder; biomarkers; blood.
Disclosures: A. Niculescu, **Part 1:** Speaker's Bureau, Sunovion; H. Le-Niculescu, Nothing to Disclose; D. Levey, Nothing to Disclose; M. Ayalew, Nothing to Disclose; N. Jain, Nothing to Disclose; E. Winiger, Nothing to Disclose; G. Shankar, Nothing to Disclose; M. Radel, Nothing to Disclose; E. Belanger, Nothing to Disclose; H. Duckworth, Nothing to Disclose; R. Schweitzer, Nothing to Disclose; M. Yard, Nothing to Disclose; G. Sandusky, **Part 2:** Lilly stock; A. Shekhar, Nothing to Disclose; N. Schork, Nothing to Disclose; D. Salomon, Nothing to Disclose.

W102. Growth Factors as Biomarkers of Major Depressive Disorder and Potential Predictors of Antidepressant Drug Response

Angelos Halaris*, Anne Clark-Raymond, Edwin Meresh, Aparna Sharma, Robin Kang, Brandon Hage, Kathryn Morrissey, Jawed Fareed, Ghanshyam Pandey

Loyola University Stritch School of Medicine, Maywood, Illinois

Background: Major Depressive Disorder (MDD) is a highly prevalent mood disorder worldwide, carries significant morbidity and mortality risk, and high co-morbidly with a host of other disease entities. In spite of numerous scientific breakthroughs, many questions remain about the pathophysiology of the disorder. Similarly, the precise mechanisms of antidepressant drug action and the reasons why only a minority of patients remit after initial treatment are unclear. Recent targets of investigation in an effort to address these questions have included Growth Factors (GFs), notably, Vascular Endothelial Growth Factor (VEGF) and Brain Derived Neurotrophic Factor (BDNF). VEGF, while extensively characterized as an angiogenic mitogen, is widely expressed in the body, and extensive research has identified its role in the brain. VEGF is involved in hippocampal neurogenesis and response to stress, and it exerts a neuroprotective effect. BDNF is densely distributed throughout the brain and a variety of peripheral tissues. In addition to its role in early development and neuronal survival, BDNF also plays a critical role regulating activity-dependent plasticity mechanisms. Based on these findings, the neurotrophic model of depression has been proposed. It postulates that a decline in neurotrophins may potentially cause atrophy of limbic structures that control mood, resulting in symptoms of depression. Antidepressant treatment may reverse this atrophy and restore levels of neurotrophins, such as VEGF and BDNF. The objective of this study was to investigate the potential utility of serum VEGF and BDNF as biomarkers for depression and as predictors of response to treatment. We measured VEGF and BDNF levels in serum of MDD patients and compared it to age/sex-matched healthy controls. Additionally, we assessed whether baseline VEGF and BDNF levels correlate with the response to treatment either with the SSRI, escitalopram (ESC), or the atypical antipsychotic, quetiapine (QTP), used as monotherapeutic agents.
Methods: MDD patients ($N = 54$) were evaluated with a battery of standard depression and anxiety scales, pertinent family history, blood chemistries, urinalysis, and toxicology screens. Healthy controls (HC) ($N = 25$) were evaluated using the same rating instruments. Once enrolled, baseline blood was analyzed for GFs and other biomarkers. Patients received ESC or QTP starting immediately after the baseline blood drawing. Dosing for ESC began at 10 mg/day and was maintained in the range of 10–30 mg/day. Dosing for QTP was started at 25 mg/day and was gradually increased up to 300 mg/day at the discretion of the physician. Patients returned for follow-up assessments and blood measurements at weeks 1, 2, 4, 8, and 12. During the study, the patients did not receive any other form of therapy. Correlations were sought between the pre- and post-treatment biomarkers and various clinical and psychological rating scales to gain more insight into the role of GFs in depression.
Results:

- A statistically significant elevation in serum VEGF at baseline was found in the MDD patients *vs* the healthy controls (10.5 *vs* 5.9 pg/ml, $p = 0.001$) and in responders to treatment *vs* non-responders (11.8 *vs* 5.5 pg/ml, $p < 0.001$), but no such differences were found for BDNF
- Baseline serum BDNF levels were lowest in patients with no history of prior treatment with antidepressant drugs, but no such trend was seen for VEGF
- Patients treated with a SSRI within the past 30 days had significantly higher GF levels
- Patients with depressive episodes lasting greater than 6 months had significantly higher BDNF levels ($p = 0.04$), but no such trend was seen for VEGF
- No correlation was found between baseline VEGF or BDNF and age, gender, or ethnicity, BMI, or depression severity
- Although symptoms of depression dissipated, there was no change in GF levels

A $\chi 2$ test comparing GF levels to treatment response showed only patients with high GF levels were treatment responders.
Conclusions: We conclude that serum BDNF and VEGF may help stratify subgroups of MDD patients and may also be useful markers in predicting response to antidepressant drug therapy. Furthermore, by elucidating the role of GFs in depression, we can better understand the pathophysiology of MDD and the mechanism of action of its treatment modalities. To our knowledge, this is the largest study to analyze serum VEGF in depressed *vs* healthy subjects and the first to be done in North America. Additionally, to our knowledge, this is the first study to demonstrate the

potential of these GFs as clinically useful biomarkers in the diagnosis and treatment of MDD.
Keywords: growth factors, VEGF, BDNF, depression, antidepressants.
Disclosures: A. Halaris, **Part 4:** I received an Investigator-initiated grant from AstraZeneca that supported the study with Quetiapine; A. Clark-Raymond, Nothing to Disclose; E. Meresh, Nothing to Disclose; A. Sharma, Nothing to Disclose; R. Kang, Nothing to Disclose; B. Hage, Nothing to Disclose; K. Morrissey, Nothing to Disclose; J. Fareed, Nothing to Disclose; G. Pandey, Nothing to Disclose.

W103. Task-related Brain Activation in Subjects with Chronic, Stable Schizophrenia and the Effects of a Single-dose α-7 Nicotinic Acetylcholine Receptor Agonist (AQW051): A Placebo-controlled, Double-blind, Randomized Study

Deanna M Barch*, Stephen R Marder, Michael P Harms, Lars F Jarskog, Will Cronenwett, Li-Shiun Chen, Markus Weiss, Ralph P Maguire, Nicole Pezous, Dominik Feuerbach, Cristina Lopez-Lopez, Rhett B Behrje, Baltazar Gomez-Mancilla, Robert W Buchanan

Washington University, Saint Louis, Missouri

Background: There is evidence that differential expression of nicotinic acetylcholine receptor (nAChR) is associated with cognitive impairment in schizophrenia. Furthermore, studies have shown that administration of AQW051, an α-7 nAChR partial agonist, can improve cognitive functioning in rodent models of learning and memory. The primary goal of the current study in chronic, stable outpatients with schizophrenia was to evaluate task-related activation in key regions of interest (ROI) in the brain during performance of a working memory task (WMT) and an episodic memory task (EMT), with both encoding and retrieval components, and to examine the effect of AQW051 on task-related functional brain activation.
Methods: Subjects included males and females aged 18–60 years with a diagnosis of schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders IV. The study was conducted at seven centers in the USA and was a double-blind, randomized, placebo-controlled, stratified trial, employing a single-dose, two-period, cross-over design. Subjects were randomized (1 : 1 : 1) to receive an active single dose of 7.5, 50 or 100 mg AQW051. The primary outcome of the study was the task-related activation as measured by fMRI during performance of working and episodic memory tasks in specific pre-defined ROI (WMT: dorsolateral pre-frontal cortex, inferior pre-frontal cortex, dorsal parietal cortex; EMT: anterior and posterior hippocampus, anterior and posterior parahippocampal gyrus) under both placebo and AQW051 conditions. Effect sizes of AQW051 administration *vs* placebo were calculated by dividing mean change in least square means by the pooled total standard deviation of the blood-oxygen-level-dependent response. A moderate effect of the drug was defined as an effect size $\geqslant 0.4$ occurring in two or more ROI during a task, and a strong effect was defined as an effect size $\geqslant 0.7$ occurring in one or more ROI. Safety evaluations included the recording of all adverse events (AEs).
Results: Of 68 subjects enrolled, 60 subjects completed the study. Baseline demographics were comparable between dose groups. Significant and predicted changes in fMRI signal (activation) were detected in the placebo conditions during performance of WMT and EMT. No effect on task-related brain activation was detected in response to AQW051 *vs* placebo during WMT. During the EMT-encoding phase, a strong effect on task-related brain activation was detected in response to 7.5 mg AQW051 *vs* placebo (anterior hippocampus, mean effect size [95% CI]: 0.795 [0.228, 1.362]). No clinically relevant increase in brain activation was detected during the EMT retrieval phase at ROI in response to AQW051 administration. Reported incidences of AEs during the study were numerically similar for all three dose cohorts (7.5 mg AQW051 cohort; drug: 36.4%, placebo: 47.4%. 50 mg AQW051 cohort; drug: 43.5%, placebo: 34.8%. 100 mg AQW051 cohort; drug: 45.0%, placebo: 50.0%).
Conclusions: AQW051 administered in a single dose of 7.5, 50 or 100 mg was generally well tolerated. Under placebo conditions, fMRI responses were robustly detected at multiple target ROI during performance of WMT and EMT, illustrating the feasibility of implementing a multi-site fMRI study to examine treatment effects on cognition in psychiatric disorders. Brain activation in response to AQW051 administration only deviated from placebo during EMT at the lowest dose, suggesting some sensitivity of this approach to measuring treatment efficacy in individuals with schizophrenia. Expanding the lower dose range and testing longer treatment exposures may be required to understand the potential cognitive benefits of AQW051.
Keywords: AQW051, schizophrenia, fMRI.
Disclosures: D. Barch, **Part 1:** Research grants from Novartis (though longer than 2 years), Allon (longer than 2 years), and Dainippon Sumitomo Pharma, Consulting for Pfizer and Amgen, **Part 4:** Research grants from Novartis (though longer than 2 years), Allon (longer than 2 years), and Dainippon Sumitomo Pharma; S. Marder, **Part 1:** Advisory Boards: Genentech, Roche, Brsitol Meyers Squibb, Otsuka, Abbott, Targacept, EnVivo, Boeringer Ingelheim, Lundbeck, Pfizer, Shire, Reckser, Stockholder: MedAvante, **Part 4:** Sunovion, Amgen, Novartis; M. Harms, **Part 4:** Research support from Novartis Pharmaceuticals for a study involving a nicotinic acetylcholine receptor agonist.; L. Jarskog, **Part 1:** DSMB membership for Janssen, **Part 4:** Research grant funding from Sunovion, Amgen, Genentech, Merck; W. Cronenwett, **Part 1:** Will Cronenwett has received research funding from Novartis Inc., Sunovion Pharmaceuticals Inc., Otsuka Pharmaceuticals Inc., and Naurex Inc., **Part 4:** Will Cronenwett has received research funding from Novartis Inc., Sunovion Pharmaceuticals Inc., Otsuka Pharmaceuticals Inc., and Naurex Inc.; L. Chen, Nothing to Disclose; M. Weiss, **Part 5:** Novartis Pharma; R. Maguire, **Part 5:** Novartis Pharma AG; N. Pezous, **Part 5:** Employee of Novartis Pharma AG; D. Feuerbach, **Part 1:** I am an employee of Novartis Pharma AG., **Part 2:** I am an employee of Novartis Pharma AG., **Part 3:** I am an employee of Novartis Pharma AG., **Part 5:** I am an employee of Novartis Pharma AG.; C. Lopez-Lopez, **Part 1:** Permanent full Novartis Employee., I am holding Novartis Shares/Stocks, **Part 5:** Novartis; R. Behrje, **Part 5:** Novartis Pharma AG; B. Gomez-Mancilla, **Part 1:** Novartis Employee, **Part 5:**

Novartis Institutes for Biomedical Research; R. Buchanan, **Part 1:** Advisory Boards: Abbott; Amgen; Janssen Pharmaceutical, Inc.; NuPathe; Pfizer; Roche; Takeda, Consultant: Abbott; Amgen; Bristol-Myers Squibb; EnVivo; Omeros; Pfizer, DSMB: Otsuka; Pfizer.

W104. Opioid Antagonism Decreases Hedonic Responses to Social Stimuli in Healthy Adults

Margaret C Wardle*, Anya K Bershad, Matt Pulaski, Harriet de Wit

Univeristy of Chicago, Chicago, Illinois

Background: Studies in laboratory animals suggest the opioid system is critical to social functioning, and particularly social reward. In laboratory animals, opioid agonists enhance the rewarding effects of social contact, and may even partially substitute for social reward, reducing motivation for social interaction. In contrast, opioid antagonists increase motivation for social interaction, while simultaneously reducing the pleasure derived from social contact. However, few studies have examined the role of opioids in responses to social stimuli in humans. Here we examine the effect of naltrexone (NTX), a μ-, δ-, and κ-opioid antagonist used to treat opioid and alcohol dependence, on subjective and psychophysiological responses to emotional pictures containing social and non-social content. Pictures with social content effectively activate the neural 'social network', including areas implicated in μ-opioid regulation of social reward. In this study, emotional reactions were assessed using subjective and psychophysiological methods. We hypothesized that NTX would preferentially decrease hedonic positive responses to pictures with positive social content.
Methods: Healthy normal adults ($N = 26$) participated in a three-session, within-participant, double-blind study in which they received oral NTX 25, 50 mg or placebo. At each session they self-reported subjective drug effects, mood, and physical symptoms, and viewed standardized and matched positive, negative and neutral pictures with and without social content. Pictures with social content contained two or more people, while pictures without social content contained no people. Participants rated the pictures for subjective positivity immediately after each picture. Physiological responses to the pictures were quantified using activity in the corrugator (frown) muscle, which deactivates during positive emotion, and activity in the zygomatic (smile) muscle, which activates during positive emotion, collected during each picture.
Results: NTX increased self-reported fatigue and nausea, but did not affect overall positive mood. NTX reduced zygomatic responses to all types of social pictures (positive, neutral and negative), without affecting responses to non-social pictures, suggesting reduced hedonic responses to social stimuli. This effect was significant even after controlling for self-reports of fatigue and nausea. NTX did not alter subjective positive responses to the pictures, or corrugator responses.
Conclusions: This study is the first demonstration that a non-specific opioid antagonist reduces hedonic responses to social stimuli in humans. In contrast to our hypothesis, this effect was not specific to positive social pictures, but was evident regardless of picture valence (positive, neutral and negative). These findings are consistent with non-human studies suggesting reduced reward from social stimuli during opioid blockade. This has implications for the use of NTX as a treatment for opioid and alcohol dependence, as the drug may reduce the positive impact of social support, a critical factor in successful treatment of these addictions.
Keywords: opioids, human psychopharmacology, naltrexone, social cognition, psychophysiology.
Disclosures: M. Wardle, Nothing to Disclose; A. Bershad, Nothing to Disclose; M. Pulaski, Nothing to Disclose; H. de Wit, Nothing to Disclose.

W105. Inpatient Resource Utilization and Cost-related Benefits of Long-acting Injectable Antipsychotics Across Different Age Groups of Medicaid-insured Schizophrenia Patients

Craig Karson*, Steve Offord, Ross A Baker, Anna Eramo, Jay Lin, Siddhesh Kamat

CNK Consulting Group LLC, Delray Beach, Florida

Background: Schizophrenia is associated with a substantial clinical and economic burden for patients and the healthcare system. An estimated 1.1% of adults (2.6 million) in the US have schizophrenia (1-year prevalence rate; based on 2010 census data) with onset in early adulthood. Among patients with schizophrenia, relapse rates are as high as 40–50% at 1 year and 80% at 5 years. Although preventing future relapses and worsening of symptoms is the main goal of antipsychotic therapy, up to 74% of patients are non-adherent to oral antipsychotics, which is associated with increased incidence of relapse and hospitalization. Relapse prevention may help to preserve function, especially in younger patients with schizophrenia. Long-acting injectable (LAI) formulations of antipsychotic agents were developed with the primary aim of improving treatment adherence, and thus, reducing the risk of future relapses and hospitalizations. Although LAIs have been shown to significantly prevent relapses among patients with schizophrenia, they are used less often among younger patients, and are usually used among older patients who have failed multiple oral treatments. The objective of this study is to assess the effectiveness of LAIs among different age categories of patients in terms of inpatient resource utilization and costs.
Methods: Adult patients ($\geqslant 18$ years) with at least 1 inpatient or 2 outpatient healthcare claims on separate dates with a primary or secondary diagnosis of schizophrenia (ICD-9-CM codes 295.0X, 295.1X, 295.2X, 295.3X, 295.5X, 295.6X, 295.8X, 295.9X) before initiating treatment with LAI antipsychotics were identified from the Thomson Reuters MarketScan® Research Medicaid database (1/1/2006–12/31/2010). Patients were required to have 12 months of continuous medical and pharmacy health plan enrollment before (baseline period) and after (follow-up period) LAI initiation (index event). Adherence to oral medications was evaluated during the baseline period using the Medication Possession Ratio (MPR = sum of days' supply of oral antipsychotic therapy during the baseline 12-month period/12 months; MPR of 1 indicates full adherence and 0 indicates poor adherence) for four age categories: 18–30,

31–40, 41–50, and 51–60 years. Inpatient resource utilization and associated paid costs were evaluated and compared with the baseline and follow-up periods for the four age categories described.

Results: Of 3094 patients identified with schizophrenia and initiated on LAIs, 983 (31.8%) were 18–30 years, 617 (19.9%) were 31–40 years, 877 (28.3%) were 41–50 years, and 617 (19.9%) were 51–60 years. Adherence to oral medications during baseline period was significantly lower among younger patients compared with other age categories: mean MPR was 0.47 for patients 18–30 years, 0.49 for 31–40 years, 0.54 for 41–50 years, and 0.53 for 51–60 years ($p = 0.0014$). Descriptive analysis indicated that before initiating LAIs, all-cause inpatient resource utilization (mean number of days spent in the hospital) ranged from 13.1 days in the 18–30 years age group to 10.3 days among patients 31–40 and 41–50 years, and associated baseline inpatient paid costs from $18 406 (51–60 years) to $12 700 (31–40 years). All age groups experienced significant ($p < 0.0001$) reduction in the mean number of days spent in the hospital during the 12-month follow-up compared with baseline. In terms of inpatient paid costs associated with inpatient resource utilization, mean reduction in costs were $4369 (18–30, $p < 0.0001$), $3681 (31–40, $p < 0.0001$), $2051 (41–50, $p = 0.1332$), and $4492 (51–60, $p = 0.0107$) during the 12-month follow-up compared with baseline.

Conclusions: For Medicaid-insured patients, these data indicate that LAIs are as effective in younger patient groups as they are for older patients. Based on the suggestion of lower compliance during the baseline period for younger patients, and because the usage of LAIs appears to have the same favorable impact across all age groups on inpatient resource utilization and cost, these data provide real-world evidence suggesting that prescribers should consider earlier use of LAIs in younger patients with schizophrenia.

Keywords: schizophrenia, adherence, relapse prevention, long-acting injectable, resource utilization and cost.

Disclosures: C. Karson, **Part 1:** Otsuka America Pharmaceutical, Inc., **Part 2:** Otsuka America Pharmaceutical, Inc.; S. Offord, **Part 1:** Employee of Otsuka Pharmaceuticals, **Part 2:** Otsuka, Sanofi Stock, **Part 3:** Otsuka, **Part 5:** Otsuka America Pharmaceuticals, Inc.; R. Baker, **Part 1:** Employee of Otsuka Pharmaceuticals, **Part 5:** Otsuka; A. Eramo, **Part 5:** Lundbeck; J. Lin, **Part 1:** Otsuka—consultant, **Part 2:** Otsuka—consultant, **Part 3:** Otsuka—consultant; S. Kamat, **Part 1:** Employee of Otsuka America Pharmaceutical, Inc., **Part 3:** Otsuka America Pharmaceutical, Inc., **Part 4:** Indirectly through grants to previous employers AstraZeneca, Bristol-Myers Squibb, Pfizer, **Part 5:** Otsuka America Pharmaceutical, Inc.

W106. A Trial of D-Cycloserine to Treat the Social Deficit in Older Adolescents and Young Adults with Autism Spectrum Disorders

Maria R Urbano*, Leonore Okwara, Paul Manser, Kathrin Hartmann, Stephen Deutsch

Eastern Virginia Medical School, Norfolk, Virginia

Background: Social communication deficits, manifested by poor knowledge of social skills or the inappropriate use of these skills, are a core symptom domain of ASDs; there are few effective treatments that target this domain especially in older adolescents and young adults (OAYA). A role for glutamatergic dysfunction in the pathophysiology of ASDs has been shown. GlycineB site agonists work cooperatively with glutamate to promote channel opening and Ca2 + conductance. Importantly, D-cycloserine (DCS), which is a partial glycineB agonist, improved sociability in several mouse models of ASDs. The sensitivity of the obligatory glycineB co-agonist binding site may change with daily administration of DCS as a result of 'agonist-induced desensitization'. We compared the efficacy of a 'pulsed' once weekly administration *vs* 'daily' administration of DCS in order to address the important issue of 'tachyphylaxis' or receptor desensitization.

Methods: The study was a double-blind, randomized 10 week trial, consisting of 8 weeks of active drug with a 2 week follow-up visit. Two dosing strategies, 50 mg weekly (placebo taken for the remaining 6 days of the week) and 50 mg daily, were compared. Subjects were required to attend 4 visits: baseline, midpoint of active drug administration at 4 weeks, end of active drug administration at 8 weeks, and follow-up at 10 weeks. The study measures were completed at each visit unless otherwise indicated. Males and females, ages 14–25, with a documented diagnosis of ASD and whose symptoms met a cutoff on a checklist completed by the parent were enrolled. All subjects were required to provide documentation of a normal physical examination, laboratory screen (Comprehensive Metabolic Panel-CMP and Complete Blood Count-CBC), EKG, and IQ test (IQ > 70) within a year prior to starting the study. Medication and therapy regimens were required to be stable for 4 weeks prior to beginning the study. The Aberrant Behavior Checklist (ABC){15–18}and the Social Responsiveness Scale (SRS), were administered at every visit. The Social Perception (SP) test of the Advanced Solutions cognitive test battery were administered at every visit: (1) Affect Naming (AN)—Subjects shown pictures of adults experiencing various emotions are tasked with pointing to which of several affective terms best describe the emotions and (2) Prosody-Face Matching (PFM)—Subjects listening to audiotapes of people speaking are tasked with pointing to which of several faces best represents the feeling expressed on the audiotape. Summary statistics included mean and standard deviation for continuous variables as well as frequencies for categorical variables. Linear mixed effects models were used to assess changes in clinical outcomes over time according to group assignment. *T*-tests were used to test for differences in baseline demographics between the two groups. Fisher's exact test was used to test for differences in gender and number of clinical responders between the two groups. Principal component analysis was used to produce an exploratory bi-plot of patient improvement from baseline to week 8. A significance level of alpha = 0.05 was used for all analyses. *P*-values were adjusted for multiple comparisons using the Bonferroni correction. SAS version 9.2 was used for all analyses.

Results: A total of 21 subjects were enrolled in the study and randomized to either daily or weekly dosage of DCS using a block randomization procedure. One subject discontinued DCS after the baseline visit due to minor depression, resulting in 20 total subjects in trial. Our primary outcome measure of efficacy was the SRS. Both daily and weekly dosing strategies showed a significant downward trend

when modeled separately (P-value = 0.004, 0.001 respectively). However, we were unable to detect a significant difference in linear trend between the two groups (P-value = 0.335). If we instead define a threshold for clinical response equal to change greater than one standard deviation (16 points), we again see no difference in the proportion of clinical responders in each group (3 of 6 in daily, 4 of 7 in weekly) as assessed by Fisher's Exact test (P-value = 0.370). All other measures similarly showed statistically significant trends over time that was not different between the two groups. No differentiation in response was found between subscales of the SRS. DCS was very well tolerated.

Conclusions: Treatment of the core social deficit in ASDs has been elusive. The current study provides proof of concept/proof of principle that targeting the NMDA receptor in the context of a comprehensive, individualized interdisciplinary treatment plan holds promise for addressing this core symptom domain. Positive therapeutic effects of DCS were detected with parent rating scales (ie, ABC and SRS), as well as objective tests completed by the subjects (ie, SP-AN). The results suggest that a once weekly 'pulsed' dosing strategy can be adopted in future clinical trials, which will enhance compliance, minimize the potential for side effects and reduce cost. DCS was safe and well-tolerated in this study sample. Importantly, medication trials that include the OAYA population with ASDs are rare. The current investigation supports a double-blind, placebo-controlled trial of 'pulsed' weekly DCS administration in a larger sample of OAYA with ASDs.

Keywords: autism, D-cycloserine, social deficits, NMDA receptor.

Disclosures: M. Urbano, Nothing to Disclose; L. Okwara, Nothing to Disclose; P. Manser, Nothing to Disclose; K. Hartmann, Nothing to Disclose; S. Deutsch, Nothing to Disclose.

W107. Selective Effects of the 5-HT2C Receptor Agonist Meta-chlorophenylpiperazine (mCPP) on Intake of a Palatable Snack Food in Healthy Female Volunteers: Correlation with Regional Brain Activations Measured by BOPLD fMRI

Colin T Dourish*, Jason M Thomas, Suzanne Higgs

P1vital, Wallingford, United Kingdom

Background: The 5-HT2C receptoragonist meta-chlorophenylpiperazine (mCPP) has been reported to decrease food intake in lean and obese volunteers although the behavioural selectivity of these effects and the brain mechanisms involved are unclear. In a recent study using a universal eating monitor to measure meal microstructure we showed that mCPP caused a dose related decrease in appetite ratings and enhanced the satiation quotient (change in hunger ratings divided by caloric intake) in lean females. However, despite these changes in rating derived measures the effects of mCPP on total food intake were not statistically significant. Therefore, in the present study, we investigated for the first time the potential influence of palatability on the response to mCPP by comparing the effects of the drug on the consumption of a pasta meal and a palatable snack in lean females. In addition, to investigate the brain mechanisms involved we used functional magnetic resonance imaging (fMRI) to determine the effects of a dose of mCPP that decreases appetite on blood oxygen level dependent (BOLD) signals.

Methods: The study used a within-subject double blind placebo controlled design and 24 healthy female volunteers received placebo and 30 mg mCPP in a counterbalanced order one week apart. Participants were scanned using BOLD fMRI pre and post oral dosing and after the second scan were provided with a pasta meal and allowed to eat to satiety. Food intake and meal microstructure were recorded using a Sussex Ingestion Pattern Monitor (SIPM). The SIPM comprises a concealed weighing system and computer software to enable detailed collection and analysis of human eating behaviour and continuously monitors food intake in parallel with measures of appetite and satiety. When participants had finished their pasta meal they were offered a palatable snack (chocolate chip cookies) and were again allowed to eat to satiety. Cookie intake and snack microstructure were recorded using the SIPM.

Results: mCPP significantly reduced hunger and the desire to eat but did not reduce the amount of pasta consumed during the meal. However, the drug significantly reduced pasta eating rate and increased pause length between mouthfuls of pasta. In contrast, mCPP significantly reduced the amount of cookies consumed. In addition, the drug reduced the size of cookie mouthfuls, the total number of cookie portions eaten and the cookie eating rate and increased the pause duration between mouthfuls of cookies. mCPP also decreased pleasantness ratings of both the pasta meal and the cookie snack but the temporal patterns of these responses were significantly different. Thus, the effect of mCPP on pleasantness ratings of pasta had a slow onset and increased throughout the meal whereas the effect of the drug on pleasantness ratings of cookies was immediately apparent and was maintained at the same level throughout the snack intake. Analysis of the BOLD fMRI results showed that mCPP attenuated activity in the hypothalamus, insula, brainstem, anterior cingulate cortex and dorsolateral prefrontal cortex. In addition, correlational analyses revealed that mCPP-induced BOLD changes in the hippocampus, anterior cingulate cortex, midbrain and orbitofrontal cortex were significantly and negatively correlated with cookie eating rate. These correlations were not apparent with pasta eating rate.

Conclusions: These results show for the first time that mCPP decreases the consumption of a highly palatable snack food in humans. Furthermore, this was a selective effect as the drug had no effect on the consumption of a pasta meal suggesting that the effects of mCPP on eating may be determined by the hedonic properties of food. This interpretation is consistent with the contrasting time courses of the effects of mCPP on pleasantness ratings of the pasta and cookies during the meal. The fMRI results show that mCPP attenuated BOLD signals in key areas involved in the processing of appetitive, rewarding and motivational stimuli. Furthermore, changes in hippocampus, anterior cingulate cortex, midbrain and orbitofrontal cortex were negatively correlated with cookie eating rate but not pasta eating rate suggesting that these brain regions which are known to be involved in reward and memory

processing could mediate the selective hedonic effects observed. Finally, as a selective 5-HT2C receptor agonist has recently been approved by the FDA for the treatment of obesity these findings could have important implications for drug therapy in obese patients where the over consumption of highly palatable foods may be an important contributory factor to the development and maintenance of the disease.

Keywords: eating, fMRI, mCPP, reward, obesity.

Disclosures: C. Dourish, **Part 1:** Employee, Director and shareholder of P1vital, **Part 2:** Employee, Director and shareholder of P1vital, **Part 3:** Employee, Director and shareholder of P1vital, **Part 5:** P1vital; J. Thomas, **Part 1:** PhD studentship part funded by P1vital, **Part 3:** PhD studentship part funded by P1vital, **Part 4:** PhD studentship part funded by P1vital; S. Higgs, **Part 1:** Academic Supervisor of PhD studentship part funded by P1vital, **Part 4:** Academic Supervisor of PhD studentship part funded by P1vital.

W108. Measurement of Immune Activation via Blood Gene Expression Early and Accross Treatment of Major Depressive Disorder

Marisa Toups*, Thomas Carmody, Cobi Heijnen, Robert Dantzer, Madhukar Trivedi

UT Southwestern Medical Center, Dallas, Texas

Background: Increased activation of the immune system is found in patients with Major Depressive Disorder (MDD) and may be related to its pathophysiology and treatment outcomes. Most studies have focused on peripheral cytokines, and show a decrease in some cytokines with Selective Serotonin Reuptake Inhibitors (SSRI) treatment. Early indications show similar changes in the expression of genes for inflammatory cytokines in whole blood. Changes over a whole course of treatment (generally 8–12 weeks) demonstrate that antidepressants are anti-inflammatory but it is not known if these changes represent a mediator effect because change in gene expression has not usually been measured early in the course of treatment before clinical improvement can be detected.

Methods: In this pilot study, twenty-one adults, age 21–55 with untreated MDD provided baseline whole blood mRNA samples and were treated with an SSRI for 12 weeks. Sixteen subjects had sufficient outcome data to analyze. Additional samples were collected at week 1 and week 12. Samples were analyzed using RT-PCR for expression of candidate genes previously found to relate to immune activation in depressed patients: *COX2*, *CREB1*, *FKBP5*, *IL-1β* (interleukin 1-*β*), *IL-6*, *IDO1* (indolamine 2.3-deoxygenase), *KMO* (kyneurenine 3-monooxygenase), *NR3C1* (glucocorticoid receptor), and *S100A10* (p11). Gene expression levels were compared at the three time points using the Wilcoxon Signed Rank test of the median expression level. Spearman's correlations compared gene expression change with outcome status adjusted for age and type of SSRI. A mixed effects model examined the effect of baseline gene expression on change over time of depression symptom severity on the Inventory of Depression Symptomatology—Clinician rated (IDS-C), Concise Associated Symptom Tracking (CAST) scale, and the Concise Health Risk Tracking (CHRT) scale. Alpha of 0.05 was considered significant, and good correlations are considered to be rho $>$ 0.6.

Results: *IL-6* levels dropped significantly between baseline and week 1, and levels of *KMO* between baseline and week 12. Baseline *FKBP5* level was significantly correlated with outcome on the IDS-C; baseline *CREB1* was significantly inversely correlated with CAST score. Week 1 drop in IL-6 was significantly correlated with improvement of symptoms on the IDS-C and suicidality on the CHRT over the 12 week study period. Good correlation (after adjustment) between week one change and outcome on the CAST was seen for *NR3C1* and *S100A10*; on the CHRT for *KMO* and *FKBP5*. When comparing gene expression change over the entire 12 week treatment period with symptom score change, we found strong correlations between CAST score and several genes (*CREB1*, *IDO1*, *IL-6*, *KMO*, *NR3C1*, and *S100A10*) with significance for *CREB1*, *IL-6* and *S100A10* after adjustment; change in *COX2* correlated well with CHRT scores. Good correlations between adjusted IDS-C score and *COX2*, *CREB1*, *IDO1*, *IL-6*, and *NR3C1* were found although none reached significance.

Conclusions: In this small pilot study we found evidence of early gene expression change in subjects undergoing SSRI treatment of MDD. Gene expression change over the first week of treatment produced good correlation with eventual change in general symptom scores as measured on the IDS-C. We also found 12 week change in several genes correlated with activation symptoms as measured on the CAST, suggesting that these symptoms may be especially related to inflammatory activation. This is consistent with the theory that inflammation is associated with neurovegetative symptoms in depressed patients. This small study provides support for future randomized trials examining early changes in immune function as mediators of antidepressant outcome.

Keywords: depression, inflammation, gene expression, SSRIs, biomarkers.

Disclosures: M. Toups, Nothing to Disclose; T. Carmody, Nothing to Disclose; C. Heijnen, Nothing to Disclose; R. Dantzer, Nothing to Disclose; M. Trivedi, Nothing to Disclose.

W109. Pregnenolone for Depression in Outpatients with Bipolar Disorder

E Sherwood Brown*, John Park, Christine E Marx, Linda Hynan, Domingo Davila, Alyson Nakamura, Prabha Sunderajan, Alexander Lo, Traci Holmes

UT Southwestern Medical Center, Dallas, Texas

Background: Pregnenolone is a neurosteroid from which many other steroids are synthesized. Low cerebrospinal fluid (CSF) levels of pregnenolone are reported in depression and bipolar disorder. Many psychiatric medications alter neurosteroid levels. Depression in bipolar disorder is challenging to treat. We previously a reported reduction, as compared to placebo, in depressive symptom severity in patients with unipolar or bipolar depression given pregnenolone (100 mg/day). We now report findings from a larger

trial of pregnenolone as an add-on therapy for bipolar depression that used a higher dose.
Methods: A total of 80 outpatients with bipolar depression were enrolled in a 12 week randomized, double-blind, placebo-controlled trial of pregnenolone. Included were men and women, age 18–75 years, bipolar I, II, or NOS disorders currently meeting criteria for a major depressive episode confirmed by a structured clinical interview. Excluded were those with active suicidal ideation with plan and intent, vulnerable populations or medically unstable, and those with psychiatric medication changes in the past 10 days. Outcome measures included the Hamilton Rating Scales for Depression (HAM-D) and anxiety (HAM-A), Inventory of Depressive Symptomatology-Self Report (IDS-SR) and Young Mania Rating Scale (YMRS). Neurosteroid levels were obtained at baseline and week 12 and analyzed using gas chromatography/mass spectrometry. Randomization was accomplished using a random number sequence. All staff members with participant contact were blinded to treatment assignment. Pregnenolone or placebo was initiated as add-on therapy at two capsules/day (50 mg twice daily if active medication, 100 mg/d), titrated to 100 mg in the morning and 200 mg in the evening (300 mg/d) at week 2 and 200 mg in the morning and 300 mg in the evening (500 mg/d) at week 4 allowing slower upward titration or decreased dose, based on clinician judgment, if side effects reported. Assessments were performed every two weeks. Data were analyzed using a mixed model ANCOVA with a between factor of treatment assignment, a within factor (repeated) of visit, and the baseline measure as a covariate. The best fitting covariance model for these data was a first order autoregressive.
Results: Demographic characteristics of those with at least one post-baseline visit ($n=38$ pregnenolone, $n=35$ placebo) were similar in the pregnenolone and placebo groups (age 43 ± 8 *vs* 44 ± 10 years, bipolar II disorder 55 *vs* 48%, education 13 ± 2 *vs* 13 ± 2 years) with the exception of gender (42 *vs* 69%) which was controlled for in the analysis. A significant group*visit effect for the HRSD ($DF=288$, $F=2.61$, $p=0.025$) was observed. Remission rates on the IDS-SR (exit IDS-SR $\leqslant 12$) were greater with pregnenolone than placebo (61 *vs* 37% placebo ($\chi 2(1)=3.99$, $p=0.046$) with no significant between-group differences in remission on the HRSD. No differences in overall side effects burden was observed between groups. The study completion rate was 71% with pregnenolone and 83% with placebo ($p=$ NS). Some significant relationships between neurosteroid levels were observed. Baseline pregnenolone levels correlated positively with YMRS scores ($r=0.31$, $p=0.028$). Participants randomized to pregnenolone (but not participants randomized to placebo) demonstrated significant increases in pregnenolone post-treatment at week 12 compared to baseline, as anticipated (mean pregnenolone change 1216 ± 2358 pg/ml in the pregnenolone group *vs* -38 ± 148 pg/ml in the placebo group). In addition, participants randomized to pregnenolone showed significant increases in downstream GABAergic neurosteroids with anxiolytic properties, including allopregnanolone (mean allopregnanolone increase 305 ± 521 in the pregnenolone group *vs* -4 ± 125 pg/ml in the placebo group) and pregnanolone (mean increase of 694 ± 2148 in the pregnenolone group *vs* 7 ± 114 pg/ml in the placebo group). A modest decrease in androsterone was seen in the pregnenolone group (-27 ± 42 *vs* -7 ± 37 pg/ml). In the pregnenolone group, changes in the HRSA correlated with changes in allopregnanolone ($r=-0.43$, $p=0.036$) and pregnanolone ($r=-0.48$, $p=0.019$) levels.
Conclusions: The findings suggest that pregnenolone at 500 mg/day is well tolerated in patients with bipolar depression and may be associated with an improvement in depressive symptoms.
Keywords: neurosteroid, pregnenolone, bipolar disorder, depression, clinical trial.
Disclosures: E. Brown, **Part 1:** Research support from Forest Laboratories, Inc. and Sunovion Pharmaceuticals. **Part 4:** Research support from Forest Laboratories, Inc. and Sunovion Pharmaceuticals.; J. Park, Nothing to Disclose; C. Marx, **Part 3:** Pending patents on the use of neurosteroids and derivatives in CNS disorders and for lowering cholesterol (no patents issued, no licensing in place). Unpaid scientific advisor, Sage Theraepeutics.; L. Hynan, Nothing to Disclose; D. Davila, Nothing to Disclose; A. Nakamura, Nothing to Disclose; P. Sunderajan, Nothing to Disclose; A. Lo, Nothing to Disclose; T. Holmes, Nothing to Disclose.

W110. Marijuana Withdrawal and Relapse in the Human Laboratory: Effect of Zolpidem Alone and in Combination with Nabilone

Margaret Haney*, Ziva Cooper, Gillinder Bedi, Stephanie Collins Reed, Divya Ramesh, Richard W Foltin

Columbia University, New York, New York

Background: Disrupted sleep is a robust marijuana withdrawal symptom and is one of the few factors predicting the severity of marijuana relapse, defined in our laboratory model as a resumption of marijuana self-administration after a period of abstinence (Haney *et al*, 2013a). These observations suggest that alleviating withdrawal-related sleep disruption might decrease the likelihood that abstinent marijuana smokers will return to active marijuana use. Zolpidem, a widely used hypnotic with low abuse liability, has been shown to improve sleep during marijuana withdrawal (Vandrey *et al*, 2011), yet the effects of zolpidem on models of marijuana relapse are not known. The cannabinoid agonist, nabilone (4 mg BID, 6 mg once/day) robustly decreased a range of marijuana withdrawal symptoms (negative mood, marijuana craving, disrupted sleep) and decreased marijuana relapse in our laboratory model relative to placebo (Haney *et al*, 2013b), but can also produce slight decrements in cognitive task performance. The objective of this study was to determine if zolpidem alone and in combination with a lower acute dose of nabilone than previously tested, would attenuate marijuana withdrawal and relapse compared to placebo.
Methods: Nontreatment-seeking marijuana smokers completed three 8-day inpatient phases. Each phase tested a different dose condition in counter-balanced order: placebo (0.0 mg zolpidem, 0.0 mg nabilone BID), zolpidem (12.5 mg once/day, 0.0 mg nabilone BID), and zolpidem (12.5 mg once/day) combined with nabilone (3.0 mg BID). On the

first inpatient day of each phase, participants repeatedly smoked active marijuana (5.6% THC) under controlled conditions to standardize recent marijuana exposure. For the following 3 days (*withdrawal*), they had the option to self-administer inactive marijuana (0.0% THC). On the subsequent 4 days (*relapse*), active marijuana (5.6% THC) was available for self-administration. Participants had to pay for self-administered marijuana using study earnings. Ratings of mood, drug-related effects, sleep, food intake and cognitive task performance were assessed repeatedly throughout each day.
Results: Male, daily, nontreatment-seeking marijuana smokers (10 + 5 marijuana cigarettes/day) completed the study ($n = 11$). *Marijuana withdrawal:* Relative to placebo, zolpidem alone and in combination with nabilone decreased objective measures of sleep latency and increased subjective ratings of 'Fell Asleep Easily.' Zolpidem plus nabilone also significantly improved objective measures of sleep efficiency and ratings of 'Slept Well,' while decreasing ratings of 'Woke often.' ($p < 0.05$). Zolpidem alone had no effect on mood symptoms during marijuana withdrawal, replicating earlier findings (Vandrey *et al*, 2011), but zolpidem plus nabilone significantly reduced withdrawal-related increases in ratings of 'Miserable,' 'Anxious,' and 'Irritable.' Neither medication condition altered cognitive task performance or ratings of capsule 'liking' or desire to take the capsule again, but zolpidem plus nabilone increased ratings of capsule strength relative to placebo. *Marijuana relapse:* Neither medication condition altered the amount of active marijuana self-administered the first day it became available after 3 days of abstinence. However, after this initial relapse day, the zolpidem plus nabilone condition significantly decreased marijuana self-administration ($p < 0.05$), while zolpidem alone had no effect relative to placebo.
Conclusions: These results suggest that although zolpidem alone decreased the amount of time it took to fall asleep during marijuana withdrawal, it did not reduce the mood symptoms of withdrawal and did not reduce marijuana self-administration. Of note, quetiapine (Cooper *et al*, 2012) and mirtazapine (Haney *et al*, 2010) similarly improved sleep during marijuana withdrawal but had few other promising effects. However, the combination of zolpidem and nabilone produced a robust attenuation of marijuana withdrawal including normalizing sleep disruption and reduced marijuana self-administration in daily marijuana smokers, similar to our earlier findings with nabilone alone, while producing no decrements in cognitive performance as seen with the larger dose of nabilone. We conclude that although improving sleep may be an important characteristic of an effective treatment for marijuana use disorder, improving sleep alone does not appear to be sufficient to impact marijuana use. In light of our earlier findings of nabilone's effects when given alone (Haney *et al*, 2013b), these findings support future clinical testing with nabilone.
Keywords: cannabis, cannabinoid, self-administration, hypnotic, agonist therapy.
Disclosures: M. Haney, Nothing to Disclose; Z. Cooper, Nothing to Disclose; G. Bedi, Nothing to Disclose; S. Collins Reed, Nothing to Disclose; D. Ramesh, Nothing to Disclose; R. Foltin, Nothing to Disclose.

W111. Relationship Between Tobacco Consumption and Lifetime Cannabis Use Status in Outpatients with Schizophrenia

Tony P George*, Rachel A Rabin

Centre for Addiction and Mental Health, Toronto, Ontario, Canada

Background: While high prevalence of tobacco and cannabis use is well established in people with schizophrenia, reports on their co-morbid use are limited. Therefore, we explored cigarette smoking behaviors as a function of cannabis use patterns in outpatients with schizophrenia.
Methods: Using a cross-sectional design, cigarette smoking behaviours were assessed in schizophrenia outpatients ($N = 60$) with current cannabis dependence (CD; $n = 24$), former cannabis dependence (in remission > 6 months) (FD; $n = 24$), and those with no lifetime cannabis dependnece (ND; $n = 12$).
Results: We found significant differences in cigarettes per day (CPD) across the three groups: $F\ (2, 57) = 15.77$, $p < 0.01$. *Post hoc* tests revealed that CD patients ($M = 11.6$, $SD = 6.3$) smoked significantly less CPD than ND ($M = 26.8$, $SD = 12.8$; $p < 0.01$) and FD patients ($M = 20.1$, $SD = 6.3$; $p < 0.01$). There was a trend for FD patients to smoke less CPD than ND ($p < 0.07$). Nicotine dependence scores assessed using the FTND also differed with respect to cannabis use status, with CD patients being the least dependent and ND patients demonstrating the highest level of nicotine dependence. Expired carbon monoxide (CO) levels were similar across groups.
Conclusions: Our preliminary findings support an effect of cannabis use status on tobacco consumption in outpatients with schizophrenia. In the presence of cannabis, patients may decrease cigarette smoking, suggesting state-dependent effects of cannabis on tobacco. Our on-going studies utilize a prospective design using a 30-day cannabis abstinence model to further explore this relationship between tobacco and cannabis use in schizophrenia patients *vs* non-psychiatric controls.
Keywords: schizophrenia, tobacco, cannabis, co-morbidity, outpatients.
Disclosures: T. George, **Part 1:** Member of a Data Monitoring Committee for Novartis, **Part 4:** Investigator-initiated and industry-sponsored grants from Pfizer; R. Rabin, Nothing to Disclose.

W112. Intravenous Methamphetamine Self-administration by Humans in a Modified Progressive-ratio Paradigm

Rajkumar J Sevak*, Carmen Freire-Cobo, Eric Wagreich, Edythe D London

University of California, Los Angeles, California

Background: Methamphetamine (MA) abuse and dependence are major public health concerns; however, a widely accepted pharmacotherapy has not yet been identified. Efforts in this regard have been limited, in part, by the inadequate sensitivity of human laboratory methods for

measuring abuse-related behavioral effects (eg, reinforcing effects) of MA. Human laboratory procedures with enhanced sensitivity to reveal reinforcing effects could facilitate the testing of pharmacological agents that can modify these effects. Only a handful of studies have measured the reinforcing effects of MA in the human laboratory, and the procedures used (eg, choice between drug and monetary rewards) have yielded effects that were moderate at best. The magnitude of reinforcing effects of MA found with these procedures limited the extent of potential pharmacological modification. Alternatively, the progressive-ratio procedure is an efficient method for assessing the reinforcing effects of abused drugs. In this procedure, the response requirement (ie, ratio) to obtain reinforcement progressively increases until the participant stops responding. The final ratio, ie the 'break point', reflects the maximum effort expended to receive the reinforcer. Progressive-ratio procedures have not yet been used in studies of human MA self-administration although d-amphetamine self-administration has been examined using this procedure. Progressive-ratio studies with d-amphetamine, however, showed only modest effect, most likely due to certain experimental design parameters. In these studies, response requirements ranged from either 50–6400 or 25–3200 responses (ie, mouse clicks) to earn capsules. This contributed to high responses for placebo and low responses for d-amphetamine, resulting in small to moderate magnitudes of the measured reinforcement. Here, wee used different response requirements (ie, 600–850 clicks) under a progressive-ratio schedule, reasoning that the higher 'minimal' and lower 'maximal' ratios would decrease responding for placebo and increase responding for MA, with an enhanced magnitude of the reinforcing effects of MA.

Methods: Eight MA-dependent individuals completed a total of 10 sessions (two practice sessions, four sampling sessions and four self-administration sessions) during their inpatient stay at the Ronald Reagan UCLA hospital. At each sampling session, participants sampled one of the i.v. doses of MA (0, 6, 12, and 24 mg), and the next day during the self-administration session, they received opportunities to work for the sampled dose on a progressive-ratio procedure. Subjective self-report and cardiovascular measures were obtained before and at several time points after i.v. administration of MA. The order of MA dose administration was random, except that, for safety purposes, subjects never received the highest dose (24 mg) before 6- or 12-mg doses. The area under the timecourse curve (AUC) data were analyzed using repeated-measures ANOVA with MA dose (0 [PL], 6, 12, 24 mg) as the within-subjects factor. When significant effects were observed in the ANOVA, Fishers LSD *post hoc* tests were conducted to compare effects of 6, 12, or 24 mg doses with those of 0 mg dose (placebo).

Results: Placebo maintained low levels of responding, and all three active doses of MA increased the number of injections earned under the progressive-ratio procedure significantly above placebo levels. MA produced prototypical subjective effects (eg, Good Effects, Like Drug) that varied with dose. MA produced a greater increase in heart rate and blood pressure compared to placebo. However, the increases were not clinically significant, and there were no major adverse events.

Conclusions: The progressive-ratio procedure employed yielded low levels of placebo taking and a large difference between the number of placebo and MA injections earned, revealing a large magnitude of reinforcing effect of MA. This enhanced magnitude of reinforcing effects could facilitate the detection of shift in the MA dose–response curve with a pharmacological treatment. Thus, this research provides a sensitive laboratory method for assessing reinforcing effects of MA that could be well-suited for evaluating pharmacological modification of the reinforcing effects of MA. These findings, therefore, may facilitate the human laboratory screening of putative pharmacotherapies for managing MA dependence.

Keywords: methamphetamine, progressive-ratio, human laboratory, reinforcing effects.

Disclosures: R. Sevak, Nothing to Disclose; C. Freire-Cobo, Nothing to Disclose; E. Wagreich, Nothing to Disclose; E. London, Nothing to Disclose.

W113. Topiramate Treatment of Heavy Drinkers: Moderation by a GRIK1 Polymorphism

Henry Kranzler*, Jonathan Covault, Richard Feinn, Stephen Armeli, Howard Tennen, Albert Arias, Joel Gelernter, Timothy Pond, Cheryl Oncken, Kyle Kampman

University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania

Background: Topiramate has been shown to reduce the frequency and intensity of drinking in alcohol-dependent individuals whose goal was to *stop* drinking. The present study evaluated (1) the efficacy and tolerability of topiramate in heavy drinkers whose treatment goal was to *reduce* drinking to safe levels and (2) the moderating effect of a single nucleotide polymorphism (rs2832407) in *GRIK1*, which encodes the kainate GluR5 subunit. We focused on *GRIK1* because topiramate's effects on AMPA/kainate receptors are most potent and selective for those containing the GluR5 subunit. We tested only rs2832407 as a moderator based on prior evidence of association of the C-allele at that locus with alcohol dependence.

Methods: We randomly assigned 138 individuals (62.3% male) to receive 12 weeks of treatment with topiramate ($N = 67$), at a maximal daily dosage of 200 mg, or matching placebo ($N = 71$). Both groups received brief counseling that focused on adherence to the medication regimen, reducing heavy drinking, and increasing abstinent days.

Results: The rate of treatment completion was high (84.9%) and did not differ by treatment group. Topiramate treatment significantly reduced heavy drinking days ($p < 0.001$) more than placebo. In the last week of treatment, the odds of experiencing a heavy drinking day in the placebo group was 5.33 (95%CI = 1.68–7.28) times that of the topiramate group. There were twice the number of treatment responders (ie, no heavy drinking days in the last 4 weeks of treatment) in the topiramate group ($N = 24$, 35.8%) as in the placebo group ($N = 12$, 16.9%) (OR = 2.75, 95%CI = 1.24–6.10). Topiramate also increased days abstinent more than placebo ($p = 0.032$) and by the last week of treatment, the odds of having an abstinent day in the

topiramate group was 2.57 (95%CI = 1.13–5.84) times that of the placebo group. There were similar differences favoring topiramate in the concentration of GGTP ($p = 0.06$ at 6 weeks and $p = 0.013$ at 12 weeks) and a measure of alcohol-related problems (SIP score; $p = 0.001$ at 12 weeks). In the European-American subsample ($N = 122$), the effects of topiramate on heavy drinking days ($p = 0.004$) was significantly greater than placebo only in rs2832407 C-allele homozygotes. In follow-up comparisons in patients with the CC genotype, topiramate reduced heavy drinking significantly more than placebo ($p < 0.001$), whereas in A-allele carriers, the difference between topiramate and placebo was not significant ($p = 0.37$). The interaction of medication group by the genotype group on abstinent days was not statistically significant ($p = 0.26$).

Conclusions: These findings support the use of topiramate 200 mg/day to reduce the frequency of heavy drinking and increase the number of abstinent days in heavy drinkers. There was a significant effect of rs2832407 in *GRIK1* as a moderator of topiramate's effects. If validated, these findings would facilitate the identification of heavy drinkers who are likely to respond well to topiramate treatment and provide an important personalized treatment option for this undertreated patient population.

Keywords: topiramate, genetic moderation, pharmacogenetics, GRIK1.

Disclosures: H. Kranzler, **Part 1:** Consulting or advisory board membership: Alkermes, Lundbeck, Lilly, Pfizer, Roche. Membership in the American Society of Clinical Psychopharmacology's Alcohol Clinical Trials Initiative, supported by Lilly, Lundbeck, Abbott, and Pfizer. **Part 2:** Lundbeck, **Part 4:** Adial; J. Covault, Nothing to Disclose; R. Feinn, Nothing to Disclose; S. Armeli, Nothing to Disclose; H. Tennen, Nothing to Disclose; A. Arias, Nothing to Disclose; J. Gelernter, Nothing to Disclose; T. Pond, Nothing to Disclose; C. Oncken, **Part 4:** Pfizer provided active medication and placebo for a smoking cessation trial.; K. Kampman, Nothing to Disclose.

W114. Onset of Efficacy of Long-acting Injectable Paliperidone Palmitate for Negative Symptoms and Anxiety/Depression in Subjects With Schizophrenia

Dong-Jing Fu*, Cynthia A Bossie, Jennifer Kern Sliwa, Yi-Wen Ma, Larry Alphs

CNS Clinical Development, Titusville, New Jersey

Background: Negative symptoms (eg, those affecting motivation, social interactions, and emotional expression and responsiveness; apathy; slowed movements) are a core feature of schizophrenia. They are significant contributors to long-term functional disability, cognitive dysfunction, and poor prognosis. Negative symptoms are sometimes difficult todifferentiate from depressive symptoms, which also are common in schizophrenia, complicating diagnosis and effective treatment. Paliperidone palmitate (PP), a once-monthly injectable antipsychotic for schizophrenia, has been shown to be efficacious for the multiple symptom domains of schizophrenia. This analysis examined the onset of efficacy of PP specifically in the treatment of patients with negative and depressive symptoms.

Methods: A *post hoc* analysis of a 13-week, randomized, double-blind study of fixed doses of PP *vs* placebo in 636 subjects with schizophrenia (Pandina *et al*, *J Clin Psychopharmacol.* 2010;30:235–244 [NCT00590577]) examined effects on PANSS negative symptom and anxiety/depression factors and items. PP was administered at 234 mg on day 1, followed by 39, 156, and 234 mg on days 8, 36, and 64, respectively, with no oral antipsychotic supplementation. PANSS scores were collected at baseline and on days 4, 8, 22, 36, 64, and 92/endpoint. Data for PP arms were pooled for day 4 and day 8 measures (all received 234 mg on day 1). 'Symptom reduction' was assessed as within-group mean change from baseline using paired *t*-tests. 'Treatment *vs* placebo effect' was assessed as between-group mean changeusing ANCOVA models and LOCF methodology without adjustment for multiplicity.

Results: *PANSS negative symptom factors and items:* At baseline, the mean negative symptom factor score was 33% of maximum. Symptom reduction within group: Mean changes were significant by day 4 for all PP treatment groups ($P < 0.05$) and only at day 22 for the placebo group ($P = 0.01$). Treatment *vs* placebo: A significant effect was observed for the total PP group *vs* placebo by day 8 ($P = 0.023$; all received 234 mg onday 1). For the 234- and 156-mg groups, a significant treatment *vs* placebo effect was observed from day 36 to endpoint (all $P < 0.05$). For the 39-mg group, a significant difference was observed only at endpoint ($P = 0.032$). For all individual items (blunted affect, emotional withdrawal, poor rapport, passive social withdrawal, lack of spontaneity, motor retardation, active social avoidance), significant improvement varied by item and by dose. *PANSS anxiety/depression factors and items:* At baseline, the mean anxiety/depression factor score was 28% of maximum. Symptom reduction within group: Mean changes were significant by day 4 for all PP treatment groups and for the placebo group ($P < 0.05$). Treatment *vs* placebo: A significant effect was observed for the PP 234-mg group *vs* placebo up to day 36 ($P = 0.048$) and at endpoint ($P = 0.010$), and for the 156-mg group by day 22 through endpoint (all $P < 0.05$). No significant differences were observed for the 39-mg PP group *vs* placebo at any time point. For all individual items (anxiety, guilt, tension, depression), significant improvement varied by item and by dose.

Conclusions: Mean negative symptom and anxiety/depression factor scores improved relative to baseline by day 4 (within-groupchange) with PP and no oral antipsychotic supplementation. Onset of symptom reduction with PP compared with placebo varied by individual symptoms and by PP dose. A differential placebo response was noted among factors and items. Findings illustrate the complexity of studying response to treatment for these related symptoms.

Support: Janssen Scientific Affairs, LLC. $< / p < 3.8 \times 10$.

Keywords: paliperidone palmitate, long-acting injectable antipsychotic, negative symptoms, anxiety/depressive symptoms, schizophrenia.

Disclosures: D. Fu, **Part 2:** Johnson & Johnsomn Shareholder, **Part 5:** Janssen Scientific Affairs, LLC; C. Bossie, **Part 1:** I have been a full time employee of Janssen Scientific Affairs, LLC during this time period, and a Johnson & Johnson stockholder, **Part 2:** I have been a full time employee of Janssen Scientific Affairs, LLC during this time

period, and a Johnson & Johnson stockholder, **Part 3:** I have been a full time employee of Janssen Scientific Affairs, LLC during this time period, and a Johnson & Johnson stockholder, **Part 5:** I am full time employee of Janssen Scientific Affairs, LLC; J. Sliwa, **Part 1:** Employee of Janssen Scientific Affairs, LLC (Johnson & Johnson); Johnson & Johnson stock-holder, **Part 2:** Employee of Janssen Scientific Affairs, LLC (Johnson & Johnson); Johnson & Johnson stock-holder, **Part 3:** Employee of Janssen Scientific Affairs, LLC (Johnson & Johnson); Johnson & Johnson stock-holder, **Part 5:** Employee of Janssen Scientific Affairs, LLC (Johnson & Johnson); Johnson & Johnson stock-holder; Y. Ma, **Part 5:** Johnson & Johnson, Jassen Pharmaceutical Companies; L. Alphs, **Part 2:** During this period I have been an employee of Janssen Scientific Affairs, a division of Johnson & Johnson. I hold stock in Johnson & Johnson, **Part 5:** Janssen Scientific Affairs, LLC.

W115. Effects of Varenicline on Neural Correlates of Motivation for Alcohol in Heavy Drinkers: The Alcohol-food Incentive Delay Task

Vatsalya Vatsalya, Reza Momenan, Melanie L Schwandt, Marion Coe, Selena Bartlett, Daniel W Hommer, Markus Heilig, Vijay A Ramchandani*

NIAAA Bethesda, Maryland

Background: There is considerable evidence for a role of the nicotinic system in the rewarding effects of alcohol and alcohol consumption. Varenicline is a nicotinic partial agonist currently approved for smoking cessation, and pre-clinical and clinical studies suggest that varenicline may attenuate the motivation for as well as the rewarding effects of alcohol. The brain reward system, particularly the striatum, has demonstrated activation during anticipation of working for reward as well as following reward notification. The Monetary Incentive Delay (MID) task has been used extensively to study reward processing in pharmacological and neuroeconomic imaging research, and the BOLD signal change in the striatum during anticipation for monetary reward is thought to measure the incentive salience of reward cues (Knutson, 2001, Bjork, 2004). The objective of this study was to examine the effect of varenicline on striatal activation and the incentive salience of cues associated with alcohol administration during reward processing, using a variation of the MID task, in non-treatment-seeking heavy drinkers.
Methods: In this double-blind randomized study, 36 male and female heavy drinkers (20 smokers), aged 21–58 years, were randomized to receive varenicline (2 mg/day) or placebo for 3 weeks. Following 2 weeks of treatment, participants underwent an fMRI scan while performing a variation of the MID task designed to evaluate the incentive salience of alcohol cues. In this version called the Alcohol-Food Incentive Delay (AFID) task, instead of monetary rewards, participants were presented with visual cues signaling alcohol (intravenous alcohol infusion), food (highly palatable snacks) or no rewards. Participants were instructed to respond to targets to earn credits associated with their selection. BOLD responses to the presentation of cue, target and feedback were measured. Group data was evaluated using Mixed Effect Meta-Analysis (Chen, *et al*, 2012) to examine effects of cue-type (alcohol *vs* food *vs* neutral) and treatment (varenicline or placebo). Linear contrasts were computed using general linear models in AFNI (Cox, 1996), separately for the varenicline and placebo groups, as well as separately for responses for alcohol, food and neutral cues by performing voxel wise *t*-tests between event-related β coefficients of each response type. Clusters larger than 10 voxels at an individual voxel threshold level of $p<0.05$ (corrected) were considered significant.
Results: Results indicated that participants in the placebo group ($n=17$) showed significantly higher BOLD activation in striatal regions during anticipation of working for alcohol reward (alcohol cue) than the varenicline group ($n=19$). The placebo group showed robust striatal activation to alcohol cue that was attenuated in the varenicline group. A direct contrast of treatment groups showed significantly lower activation in the caudate for varenicline compared to placebo group (TLRC -1, 8, 3, $p<0.05$ corrected). There were no significant differences in response to food cues between treatment groups. The placebo group also showed striatal activation in response to notification of alcohol reward (alcohol hits), and this response was also attenuated in the varenicline group. A direct contrast of treatment groups showed significantly lower activation in varenicline compared to placebo group (TLRC -6, 11, -5, $p<0.05$ corrected). Participants in the varenicline group also reported significantly lower feelings of happiness and excitement on subjective mood scales in response to alcohol cues compared to the placebo group.
Conclusions: These results indicate significant activation of striatal regions to alcohol cues and notification of alcohol reward in the placebo but not in the varenicline group. This suggests that varenicline may exert its effects by modulating the neural substrates underlying motivation for and incentive salience of alcohol reward in heavy drinkers. Consistent with these neural changes, subjective responses to alcohol cues were also attenuated in response to alcohol cues in the varenicline compared to the placebo group. These results also suggest that the AFID task may have utility as a brain biomarker of motivation and incentive salience for alcohol and other rewards, as well as of the effect of medications on the neural correlates of these reward processes.
Keywords: varenicline, alcohol, fMRI, motivation, incentive salience.
Disclosures: V. Vatsalya, Nothing to Disclose; R. Momenan, Nothing to Disclose; M. Schwandt, Nothing to Disclose; M. Coe, Nothing to Disclose; S. Bartlett, D. Hommer, Nothing to Disclose; M. Heilig, Nothing to Disclose; V. Ramchandani, Nothing to Disclose.

W116. Treatment with Paroxetine Increases Levels of Nociceptine in Cerebrospinal Fluid in Females with Fibromyalgia

Lars H Tanum*, Morten Vinje, Gunnar Ordeberg, Fred Nyberg

Akershus University Hopsital, Oslo, Norway

Background: Fibromyalgia syndrome is a chronic musculoskeletal disorder characterized by pain and tenderness,

and is often accompanied by fatigue, sleep disturbance, and morning stiffness. The syndrome has been attributed to changes in a number of neurochemical biomarkers including lower CSF levels of nociceptin. Nociceptin exerts a potent anti-nociceptive action through binding to opioide-like receptor complexes both in the brain and the spinal cord, and been postulated to take part in a broad range of physiological and behavioural responses. A lowered level of nociceptin may contribute to induce an increased sensitivity for pain involving development of hyperalgesia and allodynia generally observed in fibromyalgia females. However, it is still unclear to what extend CSF levels of nociception may be altered by treatment with an anti-depressant. The aim of the present study was to investigate to which extend pharmacological treatment would could alter the level of CSF nociceptin.

Methods: Thirty-six females with a diagnosis of fibromyalgia syndrome according to the ACR 1990 criteria (American College of Rheumatology) but with no other axis-1 disorder according to the DSM-IV were included in the study. All patients underwent a 16 weeks randomized controlled treatment with paroxetine ($n = 19$) or placebo ($n = 17$) as part of a larger treatment study. CSF was collected at baseline and after 16 weeks on fasting basis between eight and nine in the morning and immediately frozen. Pain was assessed by daily VAS diary and bi-weekly Clinical Global improvement Scale and McGill Pain Inventory. CSF was also collected from 12 healthy female volunteers under equal conditions. High Pressure Liquid Chromatography (HPLC) was used to detect nociceptin in cerebrospinal fluid. All analyses were performed at the Department of Pharmaceutical Biosciences, University of Uppsala, Sweden.

Results: Patients receiving paroxetin for 16 weeks showed a significant increase in nociceptin levels in contrast to placebo treated patients who showed only a minor increase ($p = 0.024$). Mean delta value of nociceptin after treatment was 8.5 and 2.9 femtomol/ml for paroxetin and placebo, respectively. The mean nociceptin level in the paroxetin group after treatment was still significantly lower than in healthy controls (84.8 *vs* 98.0 femtomol/ml; $p = 0.015$). The patients age correlated inversely with change in nociceptin (RR -0.48; $p = 0.036$). However, reported clinical improvement on any pain scale or even major relief of symptoms did not correspond with changes in individual nociceptin levels.

Conclusions: Our study showed that treatment with paroxetine but not placebo modulated nociceptin levels in a number of females with fibromyalgia. We assume that this effect is not a specific for paroxetin but for SSRI as a group, known to modulate genetic expressions for number of neuropeptids involved in pain and depression. A short-time limited increase of nociceptin may not be sufficient to change its postulated role in pain modulation. This corresponds with previous inverse findings that short-time pain conditions do not modulate nociceptin CSF levels.

Keywords: Fibromyalgia syndrome, chronic pain nociceptine, paroxetine, cerebrospinal fluid.

Disclosures: L. Tanum, Nothing to Disclose; M. Vinje, Nothing to Disclose; G. Ordeberg, Nothing to Disclose; F. Nyberg, Nothing to Disclose.

W117. Efficacy of Lurasidone in the Treatment of Schizophrenia with Prominent Negative Symptoms: A *Post-hoc* Analysis of Short-term Trials

Nina R Schooler*, Andrei Pikalov, Jay Hsu, Josephine Cucchiaro, Robert Goldman, Antony Loebel

SUNY Downstate Medical Center, Brooklyn, New York

Background: Negative symptoms in schizophrenia are associated with impairment in quality of life and functioning. Reviews indicate that negative symptoms are more difficult to treat than positive symptoms (1). The aim of this *post-hoc* analysis was to evaluate the efficacy of lurasidone in treating patients with prominent negative symptoms hospitalized for an acute exacerbation of schizophrenia.

Methods: This *post-hoc* analysis utilized pooled data from three large 6-week, double-blind, placebo-controlled multi-regional trials (2, 3) of patients ($N = 1206$) with an acute exacerbation of schizophrenia who were randomized to fixed, once-daily, 40–160 mg oral doses of lurasidone. Efficacy assessments included PANSS total, PANSS positive and negative subscale scores, and the CGI-severity score. Patients with prominent negative symptoms at baseline were identified based on the following criteria: a PANSS negative subscale score $\geqslant 25$ (median score); and a PANSS positive subscale score < 26 (median score). MMRM analyses were performed for change in PANSS total, negative subscale and CGI-S scores. Treatment responder status at endpoint was evaluated for both the PANSS-total and PANSS-negative subscale scores, defined as reduction from baseline of $\geqslant 20$, $\geqslant 30$, or $\geqslant 40\%$ (week 6 LOCF-endpoint).

Results: A total of 247/1206 (20.5%) patients met criteria for prominent negative symptoms. For the prominent *vs* less-prominent negative symptom subgroups, mean baseline scores were higher for the PANSS negative subscore (27.4 *vs* 23.2), lower for the PANSS positive subscore (22.6 *vs* 26.8), and similar for the PANSS total (96.0 *vs* 96.9) and CGI-S scores (4.9 *vs* 5.0). Treatment of the prominent negative symptom subgroup with lurasidone (*vs* placebo) was associated with significantly greater improvement at week 6 in the PANSS total score (-23.1 *vs* -16.2; $p < 0.01$), PANSS-negative subscale score (-6.7 *vs* -4.5; $p < 0.01$), and CGI-S (-1.4 *vs* -1.0; $p < 0.01$); but not the PANSS-positive subscale score (-5.4 *vs* -3.9; $p = 0.13$). Treatment of the prominent negative symptom subgroup with lurasidone (*vs* placebo) was associated with significantly greater endpoint response using the PANSS total 20% improvement criterion (71.3 *vs* 52.5%; $p < 0.01$), 30% criterion (55.1 *vs* 37.5%; $p < 0.01$), and 40% criterion (42.5 *vs* 28.7%; $p < 0.05$). Treatment of the prominent negative symptom subgroup with lurasidone (*vs* placebo) was associated with significantly greater endpoint PANSS negative symptom responder status using both the 20% improvement criterion ($p < 0.001$), 30% criterion ($p < 0.001$), and 40% criterion ($p < 0.05$). In the subgroup with less-prominent negative symptoms, treatment with lurasidone (*vs* placebo) was also associated with significantly greater endpoint responder rates using the PANSS 20% improvement criterion (55.0 *vs* 40.0%; $p < 0.001$), and 30% criterion (40.7 *vs* 28.1%; $p < 0.001$), but not using 40% criterion (28.1 *vs* 22.7%; $p = 0.08$). Study completion rates were non-significantly

higher for lurasidone (*vs* placebo) in both the subgroup with prominent negative symptoms (75.4 *vs* 70.0%) and the subgroup with less-prominent negative symptoms (66.7 *vs* 58.3%). Discontinuation due to adverse events was low in both the prominent negative symptom subgroup (5.4 *vs* 1.2%) and the less-prominent negative symptom subgroup (5.9 *vs* 4.7%) for lurasidone *vs* placebo, respectively. In the prominent negative symptom subgroup, the 3 most common adverse events reported for lurasidone (and greater than placebo) were somnolence (22.2 *vs* 2.5%), akathisia (15.0 *vs* 3.8%), and parkinsonism (11.4 *vs* 5.0%). The incidence of AEs on lurasidone in the less-prominent negative symptom subgroup was similar to the incidence in the prominent subgroup.

Conclusions: In patients with an acute exacerbation of schizophrenia, those who presented with prominent negative symptoms responded to treatment with lurasidone with significantly improved PANSS total and negative subscale scores comparable to what has previously been reported for the full patient sample. Treatment with lurasidone was well-tolerated in the prominent negative symptom subgroup with similar adverse event rates when compared with the subgroup with less prominent negative symptoms, and a comparable rate of discontinuation due to adverse events.

Keywords: schizophrenia, negative symptoms, clinical trials, lurasidone, acute treatment.

Disclosures: N. Schooler, **Part 1:** Johnson and Johnson, NUPATHE, Abbott Laboratories, Eli Lilly, Shire, Janssen Psychiatry, AMGEN, Lundbeck, Roche, Otsuka, **Part 4:** Astra Zeneca, Bristol Meyers Squibb, Eli Lilly and Company, OrthoMcNeil Janssen, Neurocrine, Otsuka, Pfizer Inc; A. Pikalov, **Part 5:** Sunovion Pharmaceuticals, Inc.; J. Hsu, **Part 5:** Sunovion Pharmaceuticals, Inc.; J. Cucchiaro, **Part 5:** Sunovion Pharmaceuticals, Inc.; R. Goldman, **Part 5:** Sunovion Pharmaceuticals, Inc.; A. Loebel, **Part 5:** Sunovion Pharmaceuticals, Inc.

W118. Critical Testing of the Alcohol Incentive-sensitization Model in Young Heavy Binge Drinkers Developing Symptoms of Alcohol Use Disorder

Andrea King*, Patrick McNamara, Dingcai Cao

University of Chicago, Chicago, Illinois

Background: Sensitization to the motivational rewarding properties of alcohol (wanting) but not the hedonic rewarding effects (liking) has been posited by Robinson & Berridge for over two decades. The theory is formed largely based on animal studies and *ad-hoc* patient reports. However, the theory has remained largely theoretical since critical tests of the model using human laboratory and longitudinal methods have been lacking. The present study represents significant critical testing of the incentive-sensitization model in young adult (Mean = 25 yrs) non-alcoholic drinkers. A group of heavy drinkers engaging in weekly bingeing and a control group of light drinkers were tested for response to alcohol (*vs* placebo beverage) at enrollment, followed-up for five years on their drinking behaviors and symptoms of alcohol use disorder, and then re-tested on alcohol and placebo beverage response five years after baseline testing. If the incentive sensitization model is correct, then alcohol liking and wanting would be expected to be higher in heavy compared to light drinkers and this effect should be reproducible in an independent sample. Further, in heavy drinkers, alcohol reward would be expected to predict the development of alcohol problems over time. At re-testing, alcohol wanting, but not liking, would be hypothesized to increase in heavy drinkers relative to baseline testing.

Methods: This double-blinded, within-subjects study examined visual analogue (0–100) ratings for *like* and *want more* from the Drug Effects Questionnaire (DEQ). In individual testing sessions 4–5 h in length, the DEQ was administered at several intervals after consumption of 0.8 g/kg alcohol or a placebo beverage. For alcohol responses, change scores (alcohol—placebo) were calculated at peak BrAC (60 min after beverage consumption). Participants were 208 male and female heavy drinkers ($n = 104$ in two cohorts) and 86 light drinkers. Follow-up retention in the first cohort was 98.2% through five years. Re-examination testing of alcohol and placebo response was conducted on 156 (88%) of the 178 participants deemed eligible, ie, current drinkers who were not pregnant, lactating, or psychiatrically impaired. Participants were given travel and expenses allowances with 37% returning to Chicago from another state or country outside the U.S.

Results: Heavy drinkers, compared with light drinkers, exhibited greater alcohol liking (15.3 *vs* 1.2, respectively) and wanting (22.4 *vs* 6.1) ($ps < 0.001$). These effects were replicated in a second cohort of heavy drinkers, with increases in liking (19.0) and wanting (21.2) for alcohol relative to placebo similar to that observed in the first cohort. Through follow-up, higher alcohol liking and wanting predicted greater number of symptoms of alcohol use disorder (GEE models: $\beta > 0.156$, $se > 0.61$, $ps = 0.01$). Binge drinking remained frequent for most heavy drinkers, ranging from twice monthly to 4–5 times weekly, and 0.9–6.6 out of 11 DSM-IV alcohol use disorder symptoms met in the heaviest drinking follow-up. Problem drinking was not evident in the light drinkers. At re-testing, in heavy drinkers the difference between wanting and liking increased compared to baseline testing responses ($\beta = 0.101$, $se = 0.04$, $p = 0.02$); but this was not the case in light drinkers whose liking and wanting scores remained low at both phases with the difference between them similar at re-testing and baseline testing ($\beta = 0.005$, $se = 0.05$, $p = 0.92$).

Conclusions: The results provide the first direct empirical support in humans for the incentive sensitization model of the development of alcohol addiction. At-risk binge drinkers in their mid-20s showed greater alcohol reward (liking and wanting) than light drinker controls. With repeated binge drinking and alcohol problems over time, as they approached their third decade of life, they exhibited increases in alcohol wanting relative to liking in the well-controlled laboratory challenge paradigm. The alcohol response phenotype in heavy drinkers is robust, as effects were reproduced in a second independent cohort. The findings in the first human longitudinal test-retest study of placebo-controlled alcohol challenge responses support incentive sensitization of motivational reward.

Keywords: alcohol response, incentive-sensitization, reward sensitivity, wanting, binge drinker.

Disclosures: A. King, Nothing to Disclose; P. McNamara, Nothing to Disclose; D. Cao, Nothing to Disclose.

W119. Is Quality of Life Related to Cognitive Performance or Negative Symptoms in Patients with Schizophrenia? Results from a Double-blind, Active-controlled, Lurasidone Extension Study

Philip D Harvey*, Antony Loebel, Josephine Cucchiaro, Debra Phillips, Cynthia Siu

University of Miami Miller School of Medicine, Miami, Florida

Background: Everyday functioning and quality of life are markedly impaired in schizophrenia. These impairments are related to both negative symptoms and cognitive deficits. Treatment of these symptoms would seem to have the potential to improve these critical real-world outcomes. The objective of this *post-hoc* analysis was to examine cross-sectional and longitudinal relationships between quality of life and both negative symptoms and cognitive performance in patients with schizophrenia treated with lurasidone or quetiapine XR over a 6-month assessment period.
Methods: This double-blind, extension study included subjects with schizophrenia who had completed an initial randomized, double-blind, placebo-controlled, 6-week treatment trial. Subjects received continued treatment with flexible once-daily doses of lurasidone (40–160 mg; $n=151$, LUR-LUR) or quetiapine XR (200–800 mg; $n=85$, QXR-QXR) over a 12-month treatment period; results through the 6-month cognitive assessment period are presented here. Subjects initially treated with PBO were started on flexible once daily doses of lurasidone (40–160 mg; $N=56$) (PBO-LUR). Negative symptoms were assessed with the PANSS negative subscale. Cognitive performance and functional capacity were assessed by the CogState computerized cognitive battery and the UPSA-B at baseline and 6 weeks, and at 3 and 6 months in the extension phase. Quality of life was measured using the Quality of Well-Being (QWB-SA) scale, which is a preference-weighted questionnaire with a combined score ranging from 0 to 1.0 (death to optimal functioning).
Results: At the core phase baseline, the QWB-SA total score was similar for the LUR-LUR (0.57, SE 0.02) and QXR-QXR (0.57, SE 0.02) groups. Significant improvement in QWB-SA total score from core baseline at months 3 and 6 [0.20 (SE 0.01) and 0.22 (SE 0.01)], respectively, were found in the LUR-LUR group and the QXR-QXR group (0.20, SE 0.02 for both Months 3 and 6) ($p>0.05$, LUR-LUR *vs* QXR-QXR). Improvement of the PANSS negative symptom subscale from baseline was significantly greater at the 6-month extension endpoint for LUR-LUR (−7.0, SE 0.36) *vs* QXR-QXR (−5.7, SE 0.50) ($p=0.037$); a trend favoring LUR-LUR (−6.7, SE 0.3) *vs* QXR-QXR (−5.8, SE 0.5) was observed at Month 3 ($p=0.117$). Improvement in cognitive performance was also significantly better for LUR-LUR compared to QXR-QXR at both Months 3 ($d=0.32$, $p=0.05$) and 6 ($d=0.49$, $p<0.01$). Improved QWB-SA score was longitudinally associated with reductions in negative symptoms ($p<0.01$, in both the core and extension phases) and improvement in cognitive performance ($p<0.05$, in the extension phase only) Early improvement of cognitive performance at Week 6 was a significant predictor of quality of life outcome in the 6-month continuation study. In contrast, baseline cross-sectional analysis showed a higher QWB-SA score (better quality of life) was associated with lower cognitive performance ($p<0.05$), more severe impairment in awareness of illness (higher PANSS G12 score), more severe negative symptoms ($p<0.05$) and a trend towards lower functioning capacity ($p=0.097$).
Conclusions: In this active-controlled, double-blind extensionstudy in patients with schizophrenia, improvement in quality of life wasdetected in a long term antipsychotic treatment, with improvements in cognitionand negative symptomsbeing the significant predictors of improvement. Consistent with previous research, QWB washigher at baseline in patients with more severe deficits, reflecting probabledeficits in self assessment. These findingsunderscore the importance of improving cognitive impairments and negativesymptoms in patients with schizophrenia in order to improve both objective andsubjective quality of life.
Keywords: quality of life; antipsychotic treatment; cognition; negative symptoms.
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W120. Abuse Potential of Intranasal Buprenorphine *vs* Buprenorphine/Naloxone in Buprenorphine-maintained Heroin Users

Jermaine D Jones*, Maria A Sullivan, Jeanne M Manubay, Shanthi Mogali, Verena Metz, Sandra D Comer

Columbia University College of Physicians and Surgeons, New York, New York

Background: In spite of the clinical utility of buprenorphine, diversion to parenteral abuse of this medication has been noted in several laboratory investigations and in the community. Studies have demonstrated the potential of the buprenorphine/naloxone combination to deter illicit use under various parametric conditions. However, clinical research has not yet examined the utility of the combined formulation to deter intranasal use in a buprenorphine-dependent population. The goal of the present study was to examine this question, as well as identify other factors that may minimize the abuse potential of buprenorphine alone and in combination with naloxone.
Methods: Intranasal and intravenous heroin users ($n=12$) lived in the hospital for 8–9 weeks and were maintained on each of three sublingual buprenorphine doses (2, 8, 24 mg/day). Under each maintenance dose, participants completed

laboratory sessions during which the reinforcing and subjective effects of intranasal doses of buprenorphine (8, 16 mg), buprenorphine/naloxone (8/2, 8/8, 8/16, 16/4 mg) and controls (placebo, heroin 100 mg, naloxone 4 mg) were measured.
Results: Intranasal buprenorphine alone typically produced increases in positive subjective effects and the 8-mg dose was self-administered above the level of placebo. Like the 8-mg dose, the 16-mg buprenorphine dose produced significant positive subjective. However, significant aversive effects were also reported following administration of the 16-mg dose and participants did not self-administer it above the level of placebo. The addition of naloxone to buprenorphine dose-dependently reduced positive subjective effects and increased aversive effects. None of the buprenorphine/naloxone dose combinations (8/2, 8/8, 8/16, 16/4 mg) were self-administered. The data also revealed several conditions under which larger sublingual buprenorphine maintenance doses reduced the positive subjective and reinforcing effects of intranasal buprenorphine and heroin. However, there were also circumstances where increasing the sublingual dose blunted the aversive effects produced by intranasal buprenorphine + naloxone.
Conclusions: These data suggest that within a buprenorphine-dependent population, intranasal buprenorphine/naloxone has significantly reduced abuse potential in comparison to buprenorphine alone. In comparison to buprenorphine alone, these data strongly argue in favor of buprenorphine/naloxone as the more effective option for managing the risk of buprenorphine misuse.
Keywords: intranasal, self-administration, buprenorphine, opioids, abuse liability.
Disclosures: J. Jones, Nothing to Disclose; M. Sullivan, Nothing to Disclose; J. Manubay, Nothing to Disclose; S. Mogali, Nothing to Disclose; V. Metz, Nothing to Disclose; S. Comer, **Part 4:** Investigator-Initiated Grant from Reckitt Benkinser Pharmacueticals.

W121. Positive Symptoms Respond to Add-on Aspirin in Schizophrenia Patients with High Sera CRP Levels: A *Post-hoc* Analysis of an RCT

Mark Weiser, Shimon Burshtein, Liana Fodoreanu, Roxana Chiriã, Ghiorghe Talãu, Diana Cirjaliu, Naama Fund, Robert Yolken, John M Davis, MD, Michael Davidson*

Tel Aviv University, Sackler School of Medicine, Ramat Gan, Israel

Background: This is a *post hoc* analysis of data from a previously performed RCT which administered add-on aspirin or placebo to patients with schizophrenia receiving anti-psychotics. We hypothesized that patients with high levels of CRP, perhaps reflecting high levels of inflammation, would have a better response to aspirin compared to patients with lower levels of CRP.
Methods: The original study was a multi-center, $N = 400$ trial was designed with one placebo arm to be employed as a comparator for 3 active arms. Inclusion criteria were 4 (moderate) or above on CGI-S and ł 4 (moderate) score on two of the following four PANSS items: delusions, hallucinatory behaviors, conceptual disorganization or suspiciousness/persecution, and/or a total PANSS negative symptoms score above 18. Before entering the trial and throughout the trial all subjects received anti-psychotics at doses within PORT recommendations. Upon entering the trial they were randomized to aspirin 1000 mg/d + pantoprazole 40 mg/d, minocycline 200 mg/d, pramipexole 1.5 mg, or placebo. Duration of the study was 16 weeks. Primary outcome measure was changes in total PANSS scores, secondary outcome measures included PANSS subscales.
Results: Mean age of patients was 42, 50% were females, mean duration of illness was 13 years, mean PANSS total score at baseline was 92. The ANOVA for overall change for all comparison of 3 drugs and placebo for the primary outcome of the total PANSS scores was significant, $p = 0.03$. Individual comparisons between each drug and placebo showed trends for significance (Effect size, ES = 0.28, $p = 0.056$) for aspirin, and were non-significant for minocycline (ES = 0.14, $p = 0.33$) and for pramipexole (ES = 0.01, $p = 0.95$). For positive symptoms the overall ANOVA was not significant, $p = 0.084$. Individual comparisons between each drug and placebo showed a trend for significance for aspirin (ES = 0.24, $p = 0.08$), and were non-significant for minocycline (ES = 0.04, $p = 0.77$) and pramipexole (ES = 0.11, $p = 0.45$). The sample was then divided into thirds according to CRP level at baseline. Patients with high (CRP > 3850 ng/ml) were significantly more likely to have improvements in their mean PANSS positive scores (ES = 0.61, $p = 0.03$), whereas patients with intermediate CRP scores (1300 < = "" ppp = "">.
Conclusions: The results of this *post-hoc* analysis might cautiously be interpreted as indicating that a subgroup of patients with relatively high levels of CRP, a non-specific marker of inflammation, have significant improvements in positive symptoms upon inhibition of COX-1 or COX-2, or other biological effects, both inflammatory and non-inflammatory of aspirin. The effect of aspirin on this small subgroup of responders might be the reason that previous studies found a small, consistently replicated over-all effect of aspirin in schizophrenia which was too small to be of clinical significance. This issue should be further tested by (1) performing similar *post-hoc* analyses on previous RCTs which administered aspirin or other anti-inflammatory agents in schizophrenia. Future studies might screen patients for CRP and randomize those with high CRP levels to add-on treatment with aspirin or placebo.
Keywords: aspirin, schizophrenia, CRP levels, *post-hoc* analysis.
Disclosures: M. Weiser, Nothing to Disclose; S. Burshtein, Nothing to Disclose; L. Fodoreanu, Nothing to Disclose; R. Chiriã, Nothing to Disclose; G. Talãu, Nothing to Disclose; D. Cirjaliu, Nothing to Disclose; N. Fund, Nothing to Disclose; R. Yolken, Nothing to Disclose; J. Davis, M.D, Nothing to Disclose; M. Davidson, Nothing to Disclose.

W122. Pharmacogenetics of CYP2C19 and Response to Escitalopram in Autism Spectrum Disorders (ASD)

Jeffrey R Bishop*, Fedra Najjar, Thomas Owley, Guter Stephen, Edwin H Cook

University of Illinois at Chicago College of Pharmacy, Chicago, Illinois

Background: Autism spectrum disorders (ASD) are characterized by impairments insocial interaction, communication,

irritability, as well as restricted andrepetitive behaviors. Selective serotonin reuptake inhibitors (SSRIs) arecommonly used to treat ASD. However, there is substantial heterogeneity intreatment response as well as a subset of patients who will become activated onthese medications. The CYP2C19 gene encodes the metabolic enzyme primarilyresponsible for the metabolism of escitalopram to the minimally activemetabolite desmethylcitalopram. Genetic polymorphisms in this gene may resultin increased or reduced metabolism of the SSRI escitalopram (ESC). Weinvestigated whether these variants were related to response to ESC treatmentin patients with ASD.

Methods: Study samples from two ESC treatment studies using similar enrollment, assessment, and treatment strategies were combined for this pharmacogeneticanalysis. Participants ($n = 97$, 5–50 years of age, $n = 78$ males) with a confirmeddiagnosis of an ASD (DSM-IV, ADI-R, ADOS) were enrolled. The ABC-CV wasadministered weekly with the irritability subscale measure predefined as aprimary outcome measure and other subscale measures as secondary end points. These open label studies used a forced titration schedule of weekly increasingdoses unless specific criteria for intolerable side effects were met. Theduration of treatment for Study 1 ($n = 58$) was 10 weeks, and the duration ofStudy 2 ($n = 39$) was 6 weeks. Changes in ratings from weeks 6–10 in Study 2 werenot significant, so end of study ABC-CV scores were used to assess improvementand were merged across studies. Participants were genotyped for twonon-functional (*2, *3) and one increased function (*17) variants of CYP2C19with ultrarapid/increased (UM), extensive/normal (EM), and reduced/poor (PM)metabolism groups defined using standard procedures. Analyses examiningtreatment response controlled for age, weight, final dose, sex, and pubertalstatus.

Results: Overall participants' scores on the ABC-CV Irritability subscaleimproved over the course of treatment ($F = 8.919$, $p = 0.004$). Changes from baselineto endpoint on the ABC-CV and subscales did not significantly differ acrossCYP2C19 metabolism groups.

Conclusions: In these open label studies, symptoms as measured by the ABC-CV improvedover the course of treatment. Genotypesrelated to altered metabolism capacity of CYP2C19 were not significantlyassociated with changes in ASD symptoms in these study samples. Prior studieshave identified relationships between CYP2C19 and citalopram response as wellas escitalopram plasma concentrations in adult studies of depression. Howeverthis is the first study to examine these relationships in ASD. *In vitro* studies indicate that CYP3A4 isalso involved with ESC metabolism. Thus reduced metabolic capacity may becompensated for by CYP3A4 and the extent of this relationship may be differentin younger patient populations. Future analyses will examine pharmacokinetic xpharmacodynamic relationships in the context of gene variants influencing thesevariables.

Keywords: escitalopram, autism, SSRI, pharmacogenetics, CYP2C19.

Disclosures: J. Bishop, **Part 1:** Physician's Choice Laboratory Services, **Part 4:** Ortho-McNeil Janssen, NIMH, NARSAD; F. Najjar, Nothing to Disclose; T. Owley, Nothing to Disclose; G. Stephen, Nothing to Disclose; E. Cook, **Part 1:** Seaside Therapeutics—Consultant and site investigator for clinical trial (with support to conduct the trial), **Part 4:** Seaside Therapeutics—sponsored clinical trial mentioned in 2 above.

W123. Implementation of Metabolic Monitoring Guidelines for Patients Receiving Antipsychotic Medications in a Large Outpatient Psychiatry Clinic: Interventions and Outcomes

Jayesh Kamath*, Rana Singh, Xuesong Chen, Helen Wu

University of Connecticut Health Center, Farmington, Connecticut

Background: Use of antipsychotic (AP) medications has been on the rise secondary to broader FDA approved indications as well as their off label use. Use of APs, however, has also been associated with higher risk of weight gain and metabolic syndrome with potentially serious medical consequences. A recent study suggested that AP use is independently associated with cardiometabolic risks, even after adjusting for patient's lifestyles characteristics. Other factors such as race, ethnicity, socioeconomic status, comorbidities, and non-compliance with treatment/monitoring recommendations might play a role in increasing cardiometabolic risks. In 2004, the American Psychiatric Association (APA) and the American Diabetes Association (ADA), in a joint statement, recommended specific guidelines for monitoring of metabolic syndrome associated with AP use. A survey of major public and private mental health systems in 2010 found that adherence to APA/ADA monitoring guidelines remains limited. In 2011, a two phase project was initiated in the outpatient psychiatry clinic at the University of Connecticut Health Center (UCHC) to investigate and improve cardiometabolic monitoring of patients receiving APs. The first phase conducted in 2011–2012 identified significant gaps in cardiometabolic monitoring (presented at the ACNP annual meeting, 2012). The present report describes second phase of the project which included specific evidence-based clinician-directed interventions to improve cardiometabolic monitoring and impact of these interventions on clinician's adherence to APA/ADA guidelines.

Methods: The project was approved by the UCHC Institutional Review Board. Review of implementation/dissemination scientific literature identified specific, evidence-based interventions for translation of evidence in clinical practice. These interventions were rigorously implemented over a 6 month period. Impact of these interventions was assessed using the same chart review form used for Phase I. The form gathered following information: patient demographic factors (age, gender, education, race and ethnicity), APs and indications for their use, comorbidities including cardiovascular issues and diabetes, familial risk factors, current monitoring of cardiometabolic health, specifically weight/height, waist circumference, vitals and metabolic monitoring (as recommended by the APA/ADA consensus statement, 2004). As in phase I, a chart review was conducted using this form. Patients were randomly selected from the clinic database. The data gathered was compared with the data from phase I.

Results: Interventions conducted to improve clinician adherence to APA/ADA guidelines included systemic (eg reminders on charts and throughout the clinic) and focused interventions (eg group/individual counseling sessions). The details of conducted interventions will be presented at the meetings. The chart review was conducted after completion of the 6 month intervention period. A total of 95 charts (50 males and 45 females) were reviewed. Patients were primarily white ($n=81$). The AP indications included primary psychotic disorders ($n=50$), and non-psychotic disorders ($n=45$) such as bipolar disorder and off-label indications such as impulse control disorder. A total of 9 patients were receiving at least 2 APs simultaneously. Approximately two third of patients carried a secondary psychiatric diagnosis. Approximately one third of patients ($n=37$) had known cardiovascular comorbidities and similar number of patients ($n=28$) were diagnosed with diabetes. Preliminary analyses showed improvement in metabolic monitoring compared to similar data from phase I. Statistically significant improvement was seen with weight and laboratory monitoring compared to phase I ($p<0.001$) with approximately 80% of patients receiving recommended laboratory monitoring. However, the review showed inadequate adherence with certain aspects of monitoring eg blood pressure and heart rate. Notably, the gaps in monitoring were primarily seen in patients receiving APs for non-psychotic indications.
Conclusions: The two phase project conducted in our large outpatient psychiatry clinic showed that evidence-based interventions can improve clinician adherence to metabolic monitoring guidelines for patients receiving AP medications. Specifically, improved monitoring was noted in patients receiving AP medications for primary psychotic disorders. Patients receiving AP medications for non-psychotic indications continue to receive inadequate monitoring. Further interventions are underway to address the gaps in monitoring and for complete adherence to the APA/ADA guidelines. Efforts are underway to improve coordination of care between psychiatric and medical providers to address medical comorbidities (eg hypercholesterolemia) identified with improved metabolic monitoring. Efforts are also underway to improve rigorous assessment of risk-benefit ratio when prescribing APs, especially for non-psychotic indications.
Keywords: antipsychotic medications, metabolic syndrome, cardiometabolic monitoring.
Disclosures: J. Kamath, Nothing to Disclose; R. Singh, Nothing to Disclose; X. Chen, Nothing to Disclose; H. Wu, Nothing to Disclose.

W124. Which Schizophrenia Subjects Relapse Despite Adherence to Long-acting Antipsychotic Therapy?

Henry Nasrallah*, Ibrahim Turkoz, Cynthia A Bossie, Dong-Jing Fu, Srihari Gopal, Larry Alphs, David Hough

University of Cincinnati, Cincinnati, Ohio

Background: Schizophrenia is a chronic illness in which most patients have periods of symptom exacerbation and relapse often attributed to nonadherence. However, studies show that a subgroup of patients will relapse despite uninterrupted antipsychotic (AP) long-acting therapy (LAT). An exploratory analysis examined variables associated with relapse despite ensured adherence with LAT.
Methods: Study inclusion criteria consisted of schizophrenia with duration $\geqslant 1$ year, long-acting injectable antipsychotic treatment, a relapse endpoint, and available patient-level data. Two studies were identified. The initial analysis was performed on Study 1 because it had the greater number of patients receiving LAT ($n=323$; 1-year study of risperidone long-acting injection in stable schizophrenia subjects; NCT00297388). Study 2 was used for confirmatory analyses (LAT $n=177$; 2-year study of risperidone long-acting injection in schizophrenia subjects; NCT00299702). Stepwise Cox proportional hazards regression models identified variables (demographic/clinical) associated with time to relapse. Stepwise selection alternates between forward and backward, bringing in and removing variables that met the criteria for entry or removal, were performed until a stable set of variables was attained. No adjustment was made for multiplicity.
Results: In Study 1, 59/323 (18.3%) subjects relapsed over 12 months despite continuous AP treatment. Variables associated with relapse include duration of illness (>10 years *vs* $\leqslant 5$ years associated with a 4.4-fold increase; $P=0.018$) and country (Canada *vs* US associated with a 4.6-fold increase; $P=0.0008$) based on entry and stay criteria of 0.1. In Study 2, 81/177 (45.8%) subjects relapsed over 24 months despite continuous AP treatment. No variables emerged at the significance level of 0.1 for Study 1. When less stringent significance levels were applied with an entry significance level of 0.3 and a stay significance level of 0.2 (excluding age and age at diagnosis), sex was identified as the most significant variable associated with relapse in this dataset (female *vs* male associated with a 68.0% decrease in risk of relapse; $P=0.0013$). Duration of illness (>10 years *vs* $\leqslant 5$ years associated with a 2.2-fold increase; $P=0.0425$) persisted as an important variable in this dataset, and race emerged (Asian/other *vs* Caucasian associated with a 2.3-fold increase; $P=0.0229$) as another important variable.
Conclusions: Findings suggest that patients with a longer duration of illness may have a higher risk of relapse despite assured adherence to treatment, supporting the need for early continuous treatment in schizophrenia. Other variables that emerged as relevant in only one of the datasets include sex, country, and race. These hypothesis-generating results have led to several important questions: Do repeated relapses eventually result in treatment resistance to dopamine antagonists? Does continuous effective AP treatment delay relapse indefinitely if given at the first episode? Is the first episode a distinct 'treatment-responsive stage' of the illness, which moves to a different stage after subsequent episode(s)? Why do females tend to have better response and outcome with AP treatment? These findings and their implications should be the focus of future research.
Keywords: antipsychotics, injectables, schizophrenia, adherence, relapse.
Disclosures: H. Nasrallah, **Part 1:** Gruenthal, Boehringer-Ingelheim, Genentech, Janssen, Merck, Lundbeck, Novartis, Otsuka, Roche, Shire, Sunovion, **Part 2:** Janssen, Merck, Novartis, Sunovion, **Part 3:** Janssen, Merck, Novartis, Sunovion, **Part 4:** Lilly, Shire, Otsuka, Roche (All provided

research grants through my University); I. Turkoz, **Part 1:** Full-time employee of JNJ, **Part 2:** Full-time employee of JNJ, **Part 5:** Janssen & Janssen; C. Bossie, **Part 1:** Full-time employee of JNJ, **Part 2:** Full-time employee of JNJ, **Part 5:** JNJ; D. Fu, **Part 1:** Full-time employee of JNJ, **Part 2:** Full-time employee of JNJ; S. Gopal, **Part 1:** Full-time employee of JNJ, **Part 2:** Full-time employee of JNJ; L. Alphs, **Part 1:** Full-time employee of JNJ, **Part 2:** Full-time employee of JNJ; D. Hough, **Part 1:** Full-time employee of JNJ, **Part 2:** Full-time employee of JNJ.

W125. Plasma Oxytocin Concentrations Following MDMA or Intranasal Oxytocin in Humans

Matthew G Kirkpatrick*, Sunday M Francis, Suma Jacob, Royce J Lee, Harriet de Wit

University of Chicago, Chicago, Illinois

Background: MDMA (±3,4-methylenedioxymethamphetamine, 'ecstasy') is reportedly used recreationally because it increases feelings of sociability and interpersonal closeness. Prior work suggests that the pro-social effects of MDMA may be mediated by release of oxytocin. However, the effects of MDMA and intranasal oxytocin on plasma oxytocin have not been compared directly. In this study healthy volunteers received acute doses of MDMA and intranasal oxytocin using controlled laboratory procedures.
Methods: Fourteen MDMA users participated in a 4-session, double-blind between- and within-subjects study. Each participant received two doses of oral MDMA (0.75, 1.5 mg/kg), one dose of intranasal oxytocin (20 IU or 40 IU), and placebo. Plasma oxytocin concentrations were assessed before and at several time points after drug administration. Cardiovascular and subjective effects were also assessed.
Results: MDMA dose-dependently increased heart rate and blood pressure, feelings of euphoria (eg, 'High' and 'Like Drug'), and feelings of sociability ($p<0.001$ for all comparisons), whereas oxytocin had no cardiovascular or subjective effects. Relative to placebo, MDMA (1.5 mg/kg only) increased plasma oxytocin levels to a mean peak of 83.7 pg/ml ($p<0.001$), peaking at 90–120 min. Intranasal oxytocin (40 IU, but not 20 IU) increased plasma oxytocin levels to a mean peak of 48.0 pg/ml, peaking 15 and 45 min after nasal spray ($p<0.05$ for both comparisons). None of the subjective or cardiovascular responses to MDMA were significantly correlated with plasma oxytocin levels, and there were no relationships between responses to MDMA and oxytocin.
Conclusions: Both oral MDMA and intranasal oxytocin increased plasma oxytocin levels, but MDMA produced levels higher than oxytocin. The effects of MDMA on oxytocin are consistent with previous animal and human findings. The finding that plasma oxytocin levels were unrelated to MDMA-related subjective effects suggests that oxytocin is not the primary mediator of the pro-social subjective effects of MDMA. Intranasal oxytocin only produced small and short-lived increases in plasma levels, even after a relatively large dose (ie, 40 IU). Future studies using MDMA to increase oxytocin levels may be useful to clarify the role of this pro-social peptide in other behaviors.

Keywords: MDMA, oxytocin, mood, plasma, human.
Disclosures: M. Kirkpatrick, Nothing to Disclose; S. Francis, Nothing to Disclose; S. Jacob, Nothing to Disclose; R. Lee, Nothing to Disclose; H. de Wit, Nothing to Disclose.

W126. Effects of MDMA and THC on Social Subjective States and Social Processing in Humans

Gillinder Bedi*, Daniel Burghart, Jenny Porter, Nicholas T Van Dam, Kevin N Ochsner, Margaret Haney

NYSPI, New York, New York

Background: Drug folklore suggests that a variety of abused drugs alter social experiences; these effects appear to motivate drug use. Consistent with this, emerging evidence indicates that several drugs modulate social feelings and behavior in ways likely to enhance the direct rewarding effects of the drugs themselves. For instance, ±3,4-methylenedioxymethamphetamine (MDMA; 'ecstasy'; 0.75 or 1.5 mg/kg): (1). Increased social approach-oriented feelings, such as friendliness, sociability, and loving feelings; (2). Decreased recognition of threat-oriented facial emotions (Bedi *et al*, 2010); and (3). Modulated neural response to socially threatening and rewarding stimuli in ways consistent with increased sociability (Bedi *et al*, 2009). Conversely, some drugs (eg marijuana) are reported to produce feelings of social anxiety or paranoia under certain situations (Luzi *et al*, 2008); few studies have investigated these negative social feelings after controlled drug administration. Moreover, few prior studies have systematically investigated the effects of more than one drug on a range of dimensions of social processing in the same individuals. Here, we aimed to characterize the effects of two abused drugs, MDMA and delta-9-tetrahydrocannabinol (THC; the main psychoactive constituent of marijuana) on: (1) positive and negative social subjective states; (2) social motivation; (3) empathic accuracy; and (4) neural correlates of social reward and social threat processing (measured with functional Magnetic Resonance Imaging; fMRI).
Methods: Healthy, non-treatment-seeking participants reporting prior ecstasy use (5–50 times) and current marijuana use (2–16 times/week) completed three, 7-h outpatient sessions in which they received oral MDMA (105 mg/70 kg), THC (10 mg/70 kg), or placebo (PBO). Drug administration was randomized, double-blind, and double-dummy. Socially positive (eg 'sociable'; 'talkative') and negative (eg 'socially uncomfortable'; 'self-conscious') subjective states were assessed repeatedly throughout the session, as were general drug effects (eg 'high', 'drug liking',). During the period of estimated peak drug effects, participants underwent a 90-min fMRI scan including standardized measures of social reward and social threat responding. They then completed a social motivation probe, making repeated choices between access to 'social time' (phone time and social internet sites) and money. One of these choices was randomly selected for implementation in the final hour of the session. Participants also underwent an empathic accuracy measure, which assessed on-line monitoring of others' emotional state from video clips.
Results: Data collection is ongoing. To date, 9 volunteers (6M; 3F) reporting ecstasy use on 18 (±11) occasions and

marijuana use 2 (±2) times per week have completed the protocol. Initial analyses of subjective data indicated that MDMA increased 'loving' and 'talkative' ratings relative to THC ($p<0.05$). Both MDMA and THC increased ratings of 'concerned about what others will think of me' and MDMA increased ratings of 'concerned about the way I present myself' compared to PBO ($p<0.05$). MDMA marginally increased ratings of 'self-conscious about the way I look' and THC marginally increased ratings of 'concerned that I will say or do the wrong thing', 'socially uncomfortable', and 'worried about the impression I am making on others' compared to PBO ($p<0.09$). Both MDMA and THC increased ratings of 'high' relative to PBO ($p<0.005$); MDMA increased 'drug liking' ratings compared to both THC and PBO ($p<0.05$). There was no effect of drug on choice for social time *vs* money in the social motivation task. Empathic accuracy and fMRI data are not yet available.
Conclusions: These findings indicate that, in addition to producing increases in prosocial feelings, MDMA and, more prominently, THC increase feelings associated with social discomfort when administered in the laboratory. The current procedures demonstrate the importance of using a range of measures to comprehensively assess drug effects on social processing. This ongoing research will further characterize the nuanced and multifaceted effects of MDMA and THC on different dimensions of social processing in humans.
Keywords: social processing, MDMA, ecstasy, THC, pharmacoMRI.
Disclosures: G. Bedi, Nothing to Disclose; D. Burghart, Nothing to Disclose; J. Porter, Nothing to Disclose; N. Van Dam, Nothing to Disclose; K. Ochsner, Nothing to Disclose; M. Haney, Nothing to Disclose.

W127. Pro-attentional Effects of Amphetamine in Healthy Adults are Predicted by Levels of Sensorimotor Gating

Neal R Swerdlow*, Savita G Bhakta, Jo A Talledo, Sarah N Lamb, Bryan Balvaneda, Justin Kei, Hsun-Hua Chou

UCSD School of Medicine, La Jolla, California

Background: The cost of identifying procognitive agents for schizophrenia (SZ) might be reduced if drugs could be screened based on cognitive effects in healthy subjects (HS). In a double-blind placebo-controlled cross-over study of 20 mg amphetamine (AMPH) in HS, we reported (Chou *et al*, 2013) procognitive effects on MATRICS Consensus Cognitive Battery (MCCB) performance among specific HS subgroups, and in specific cognitive domains. A challenge to detecting these drug effects is a substantial 'carry-over' or order effect of repeated testing on MCCB performance. Here, we applied a strategy to neutralize order effects and thereby better assess the behavioral and genetic correlates of pro-attentional AMPH sensitivity.
Methods: 79 HS (M:F = 37 : 23; age range 18–35; $n=60$ from Chou *et al*, 2013) were assessed via clinical instruments, and eyeblink EMG measures of prepulse inhibition (PPI) of acoustic startle. Effects of AMPH (placebo (PBO) *vs* 20 mg) on MCCB T-scores and PPI were tested in a double-blind, order-balanced, PBO-controlled crossover study. Single nucleotide polymorphisms (SNPs) associated with PPI, AMPH sensitivity or SZ were assayed. Mean order effects (MOE) on MCCB T-scores were calculated; for Attention/Vigilance (A/V), MOE was 3.9 units. For each subject, the arithmetic difference of this AMPH effect on A/V minus the MOE was the metric of 'A/V AMPH sensitivity,' and wasstudied for its clinical, PPI and genetic correlates.
Results: In 45 subjects (M;F = 27 : 18), A/V sensitivity exceeded the MOE ('A/V enhancers'), and for 34 subjects (M:F = 23 : 11), A/V sensitivity was less than the MOE ('A/V suppressers'). Mean A/V AMPH sensitivity was not significantly correlated with age, weight or education; A/V AMPH sensitivity was not associated with AMPH-induced subjective changes in alertness or happiness, or changes in autonomic measures. Screening PPI predicted A/V AMPH sensitivity ($p<0.02$); PPI was significantly lower among A/V enhancers *vs* A/V suppressers ($p<0.03$). In subsequent testing, AMPH significantly enhanced PPI among A/V enhancers ($p<0.05$), but not among A/V suppressers. All SNP effects and the relationships of AMPH sensitivities across MCCB domains and PPI will be reported.
Discussion: Among HS, AMPH sensitivity on MCCB performance can be quantified in a simple cross-over design; a 'test case' of A/V AMPH sensitivity demonstrated relationships with specific personality, psychophysiological and genetic markers that are consistent with our understanding of AMPH effects in HS. These findings support the feasibility of using drug-induced changes in MCCB performance in HS to screen for candidate procognitive drugs for SZ or other brain disorders.
Keywords: amphetamine, cognition, prepulse inhibition, schizophrenia, neurocognition.
Disclosures: N. Swerdlow, **Part 1:** Consultant for Neurocrine, **Part 2:** N/A, **Part 3:** N/A, **Part 4:** N/A, **Part 5:** N/A; S. Bhakta, Nothing to Disclose; J. Talledo, Nothing to Disclose; S. Lamb, Nothing to Disclose; B. Balvaneda, Nothing to Disclose; J. Kei, Nothing to Disclose; H. Chou, Nothing to Disclose.

W128. Cariprazine Demonstrates High Dopamine D3 and D2 Receptor Occupancy in Patients with Schizophrenia: A Clinical PET Study With [11C]-(+)-PHNO

Mark Slifstein*, Anissa Abi-Dargham, Deepak C D'Souza, Richard E Carson, István Laszlovszky, Suresh Durgam, Nika Adham, Béla Kiss, István Gyertyan, Margit Kapás, Yih Lee

Columbia University, New York, New York

Background: Blockade of dopamine D2 receptors is thought to be essential for antipsychotic efficacy. However, activity at other receptors may be beneficial in effectively managing the broad spectrum of symptoms associated with schizophrenia. The dopamine D3 receptor has recently emerged as a potential therapeutic target due to its preferential expression in areas of the brain thought to regulate emotional and cognitive processes. While several currently marketed antipsychotics have shown moderate *in vitro* affinity for the D3 receptor, some studies have suggested that these compounds do not demonstrate D3 receptor-

occupancy *in vivo* at therapeutic doses. Compounds displaying high occupancy at both D2 and D3 receptors may confer benefits for some of the more difficult-to-treat symptoms associated with schizophrenia, such as negative symptoms and cognitive impairment. Cariprazine is an orally active and potent dopamine D3 and D2 receptor partial agonist atypical antipsychotic with preferential binding to D3 receptors. *In vitro*, cariprazine displays an affinity for the D3 receptor that is almost one order of magnitude greater than for the D2 receptor and demonstrated high and balanced *in vivo* occupancy of D2 and D3 receptors in rats. The objective of the current study was to determine the occupancy of cariprazine at dopamine D3 and D2 receptors in patients with schizophrenia following acute and subchronic treatment.

Methods: This study was a Phase 1, open-label, multiple-dose, 3-cohort study in patients with a DSM-IV-TR primary diagnosis of schizophrenia. Initially, 2 patients (Cohort 1) received open-label cariprazine treatment for a total of 15 days; dosage was initiated at 1.5 mg on Day 1 and then increased to 3 mg on Day 2, 6 mg on Days 3–4, 9 mg on Days 5–6, and 12 mg on Days 7–15. Dosing strategies for subsequent cohorts were based on results from previous cohorts. Cohort 2 ($n = 3$) received cariprazine 0.5 mg on Day 1, 1 mg/d on Days 2–4, and 3 mg/d on Days 5–15. Cohort 3 ($n = 3$) received cariprazine 0.5 mg/d for Days 1–4 and 1 mg/d for the remaining 11 days. Dynamic Positron Emission Tomography (PET) scans using the D3-receptor preferring radiotracer [11C]-(+)-PHNO were performed at baseline and under 3 different time points representing different dosing conditions: acute (Day 1), Day 4/5, and subchronic (Day 15). Brain regions of interest included the caudate, putamen, ventral striatum, globus pallidus, thalamus, and a midbrain region encompassing the substantia nigra and ventral tegmental areas. PET data were analyzed by the simplified reference tissue method (SRTM) using cerebellum as a reference tissue to derive regional binding potentials (BPND). A non-linear regression model accounting for regional differences in relative D2 and D3 receptor availability was used to determine specific D2 and D3 occupancy measures.

Results: Cariprazine treatment resulted in high D2 and D3 receptor occupancy with all doses (range, 0.5–12 mg/d) and conditions (ie, acute, 4–5 day, or subchronic treatment). A clear dose-occupancy relationship existed for acute, 4–5/day, and subchronic treatment, with the highest occupancy for both D2 and D3 receptors observed with the highest dose (12 mg/d). Following subchronic treatment, mean D2 receptor occupancy was 95% ($n = 2$; range 94–96%) for the 12 mg/d dose, 79% ($n = 3$; range, 68–88%) for the 3 mg/d dose, and 45% for the 1 mg/d dose ($n = 3$; range, 14–64%). Mean D3 receptor occupancy following subchronic dosing was 99% ($n = 2$; range, 93–105%) with 12 mg/d, 92% ($n = 3$; 86–96%) for 3 mg/d, and 76% ($n = 3$; range, 58–89%) for 1 mg/d. ED50 values for D2 and D3 receptor occupancy with acute cariprazine treatment was 1.84 mg (95% CI, 1.41–2.40) and 1.52 mg (95% CI, 0.76–3.05), respectively, with a D3/D2 selectivity ratio of 1.21. Subchronic treatment yielded ED50 values of 1.03 mg (95% CI, 0.54–2.05) for the D2 receptor and 0.30 mg (95%CI, 0.15–0.59) for the D3 receptor, with a D3/D2 selectivity ratio of 3.43.

Conclusions: In this study, cariprazine showed high and dose-dependent *in vivo* occupancy of both dopamine D3 and D2 receptors in patients with schizophrenia. Following subchronic treatment, cariprazine showed 3-fold greater preference for D3 *vs* D2 receptors. The increased D3/D2 selectivity following subchronic dosing compared with acute dosing may be related to the pharmacological activity of cariprazine metabolites. To our knowledge, no currently marketed antipsychotic has demonstrated appreciable D3 receptor occupancy at clinically therapeutic doses; in this regard, the pharmacological profile of cariprazine is unique.

Keywords: cariprazine, dopamine receptor, PET, schizophrenia, antipsychotic.

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W129. Lack of Subjective Abuse-Related Effects of Intranasal Eluxadoline: A Novel Mu-delta Opiate Modulator for Oral Use in IBS-d

Naama Levy-Cooperman, Gail McIntyre, Laura Bonifacio, Mike Davenport, Paul Covington, Scott Dove, June Almenoff, Bijan Chakraborty, Kerri A Schoedel, Michael McDonnell, Edward M Sellers*

DL Global Partners Inc., Toronto, Ontario, Canada

Background: Eluxadoline (ELUX) is a mixed mu-opioid receptor (μOR) agonist and delta-opioid receptor (δOR) antagonist being developed for the treatment of diarrhea-predominant irritable bowel syndrome (IBS-d). When administered orally, its intended route of use, ELUX has low systemic bioavailability, negligible CNS side effects, and acts locally in the GI tract. ELUX inhibits gastrointestinal transit, consistent with its primary pharmacological profile as a μOR agonist, and its additional δOR antagonist activity may mitigate the constipating effect of unopposed peripherally acting μOR agonists (eg, loperamide). ELUX may have decreased abuse potential relative to pure μOR agonists because of its very low oral bioavailability, physicochemical properties which are not conducive to extraction or smoking, large tablet size (825 mg for 100 mg ELUX), and mixed μOR agonist/δOR antagonist profile. Because the intranasal bioavailability and pharmacodynamic effects of ELUX were not known, this study evaluated the intranasal abuse potential of ELUX compared to oxycodone and placebo (PBO) in recreational opioid users.

Methods: In this single dose, randomized, double-blind, PBO- controlled crossover study, 35 healthy recreational opioid users received single intranasal doses of ELUX (100 mg, and 200 mg), oxycodone (15 and 30 mg), and PBO. Because of large differences in weight between the ELUX and oxycodone tablets, in order to maintain subjects' blinding, 2 doses of PBO were administered that matched

the weight of oxycodone and ELUX (200 mg). Eligible subjects were required to first pass a qualification session to ensure they could reliably distinguish between and like the effects of 20 mg intranasal oxycodone compared to PBO. In the main study, subjective effects, including visual analog scales (VAS) eg, 'at the moment' Drug Liking (primary endpoint), global measures (eg, Overall Drug Liking, Take Drug Again) and Addiction Research Center Inventory (ARCI) Morphine-Benzedrine Group (MBG, euphoria), Pentobarbital Chlorpromazine Alcohol Group (PCAG, sedation) and Lysergic Acid Diethylamide (LSD, dysphoria) scales were evaluated at multiple time points up to 24 h post-dose. In addition, pharmacologic and local effects were assessed with objective measures, such as pupillometry and nasal-effects rating scales. Pharmacokinetic samples were also collected until 24 h post-dose.

Results: Peak Drug Liking was significantly higher for both doses of oxycodone compared to PBO (Median Difference of 36.0 for 15 mg and 47.0 for 30 mg, $p<0.0001$); confirming the validity of the study. For peak drug liking, the 2 doses of ELUX were not different from either one of the PBOs, and both ELUX doses showed significantly lower Drug Liking VAS peak values compared to both 15 mg (Median Difference -28.0 and -26.0, $p<0.0001$) and 30 mg oxycodone (Median Difference -45.0 and -42.0, $p<0.0001$). Both doses of ELUX were disliked relative to 15 mg and 30 mg oxycodone and both PBOs, indicated by Drug Liking VAS scores that were in the 'disliking' range of the bipolar scale (<50 points). Similarly, both doses of ELUX showed significantly lower effects compared to both oxycodone doses on most secondary subjective measures (eg, High and Good Effects VAS), with the exception of measures of sedation, where differences between oxycodone and ELUX were less pronounced. In general, both doses of ELUX were associated with significantly greater negative effects, including most nasal effects, in comparison to oxycodone and both PBOs, while PBO matched to ELUX 200 mg showed statistically greater bad effects compared to PBO matched to oxycodone ($p<0.05$). Pupil diameter was significantly smaller following oxycodone in comparison to PBO and both doses of ELUX; however, small but statistically significant decreases in pupil diameter were also observed following ELUX *vs* PBO. Clinically, the insufflation of ELUX was difficult for many subjects and on average, subjects were only able to insufflate approximately 50% of the drug, thereby confirming that maximally abusable doses were evaluated. Mean (% CV) peak ELUX plasma concentrations were 118.8 ng/ml (84.6%) and 191.4 ng/ml (87.4%) for 100 mg and 200 mg ELUX doses, respectively. Subjects difficulty in insufflation of both 100 mg and 200 mg ELUX doses is a contributor to the large variation observed in the PK parameters.

Conclusions: Eluxadoline effects were significantly lower than oxycodone on Drug Liking VAS and all positive effects measures. In general, ELUX was associated with greater negative effects, including nasal irritation. Intranasal ELUX was associated with some central 'opioid-like' effects at maximum insufflable doses; however, overall it was associated with significant disliking compared to both PBOs and to oxycodone. Moreover, subjects selected based on their recreational drug use experience and subjective responses to intranasal oxycodone, showed no willingness to take ELUX again. Subjective ratings of intranasal discomfort reported demonstrate that intranasal doses are associated with negative effects that would mitigate the risk of abuse. This study shows that ELUX is unlikely to be abused by recreational drug users since the abuse potential of intranasal ELUX is similar to or lower than PBO and much lower than oxycodone.

Keywords: human abuse liability, opioid, clinical research, subjective measures.

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W130. The Effects of 5HT-7 Antagonism on Sleep in Humans: A Placebo-controlled Cross-over Study of Lurasidone

Andrew Krystal*, Gary Zammit, Andrei Pikalov, Antony Loebel

Duke University School of Medicine, Durham, North Carolina

Background: 5HT-7 receptors are found in the hypothalamic suprachiasmatic nucleus and are thought to modulate sleep and circadian function. In preclinical work 5HT-7 antagonism delays sleep phase, though this effect varied among species. We sought to determine the sleep effects of 5HT-7 antagonism in humans by evaluating the effects of lurasidone, an atypical antipsychotic agent that is unique in being a potent 5HT-7 antagonist, which also has D2, 5HT-2A antagonist and 5HT-1A partial agonist effects but no appreciable histaminergic or cholinergic receptor affinity.

Methods: This was a two-site, 2-period, cross-over, polysomnographic study involving 54 non-sleep-deprived, healthy volunteers without sleep complaints. Each subject underwent two treatment periods (order randomized) each consisting of two nights in the laboratory. On night one they went to bed at their usual bedtime and received single-blind placebo. On night two they underwent a 4 h advance of sleep phase and received either lurasidone 40 mg or placebo 30 min prior to lights out. The next morning impairment testing was carried out (Sleepiness Visual Analogue Scale, Digit Symbol Substitution Test, Symbol Copying Test).

Results: Lurasidone increased total sleep time, the primary outcome measure, by an average of 28.4 min *vs* placebo ($p < 0.05$). Lurasidone also significantly decreased wake time after sleep onset (WASO) and wake time after the final awakening, and increased sleep efficiency, N2% (N2 was formerly referred to as Stage 2) and N2 sleep time compared with placebo. No effects were found with other sleep variables or any of measures of next-morning impairment.
Conclusions: Lurasidone had unique effects on sleep in this phase-advance paradigm including improving the ability to stay asleep, particularly at the end of the night, without shortening the time to sleep onset. The absence of an increase in slow-wave sleep suggests that 5HT-2A antagonism does not dominate lurasidone's sleep effects and suggests that the observed effects are likely due to 5HT-7 antagonism. The finding that wake time is decreased at the end of the night without shortening sleep onset is consistent with 5HT-7 antagonism being associated with a delay in sleep phase. This profile and the absence of next-day residual sleepiness or cognitive impairment suggests that lurasidone may be beneficial for the many patients with thought and mood disorders with disturbed sleep, particularly those with sleep maintenance problems/early morning awakening.
Keywords: lurasidone; 5HT-7; sleep.
Disclosures: A. Krystal, **Part 1:** Abbott, Astellas, AstraZeneca, BMS, Teva, Eisai, Eli Lilly, GlaxoSmithKline, Jazz, Johnson and Johnson, Merck, Neurocrine, Novartis, Ortho-McNeil-Janssen, Respironics, Roche, Sanofi-Aventis, Somnus, Sunovion, Somaxon, Takeda, Transcept, **Part 2:** Neurocrine Biosciences; Somaxon, AstraZeneca, **Part 4:** NIH, Teva, Pfizer, Sunovion, Transcept, Phillips-Respironics, Astellas, Abbott, Neosynch, Brainsway.; G. Zammit, **Part 1:** Grants/Research Support: Abbott, Apnex, AstraZeneca, Biomarin, BMS, Catalyst, CHDI, Eisai, Elminda, Genentech, Gilead, Glaxo Smith Kline, Gilead, H. Lundbeck A/S, Janssen, Johnson & Johnson, Lilly, Medtronic, Merck and Co., National Institute of Health (NIH), Neurocrine Biosciences, Naurex, Novo Nordisk, Otsuka, Pfizer, Respironics, Saladax, Shire, Takeda Pharmaceuticals North America, Teva, Thymon, Ultragenyx, Consultant: Acorda, Eisai, Purdue, **Part 2:** Clinilabs, Inc.; A. Pikalov, **Part 1:** employee of Sunovion Pharmaceuticals, Inc., **Part 2:** employee of Sunovion Pharmaceuticals, Inc., **Part 3:** employee of Sunovion Pharmaceuticals, Inc., **Part 5:** Sunovion Pharmaceuticals, Inc.; A. Loebel, **Part 5:** Sunovion Pharmaceuticals.

W131. Trajectories of Response to Repeat Dose of Intravenous Subanesthetic Ketamine in Treatment Resistant Depression

Paulo R Shiroma*, Brian Johns, Michael Kuskowski, Paul Thuras, Kelvin O Lim

University of Minnesota, MInneapolis, Minnesota

Background: Placebo-controlled studies of a single ketamine infusion in unipolar or bipolar depression support the rapid antidepressant effects of ketamine in mood disorders. Preliminary evidence suggest giving more than one ketamine infusion may result in better clinical outcomes for treatment resistant depression (TRD) than what might be achieved with a single infusion (Murrough *et al*, 2012; Rasmussen *et al*, 2013). Most studies of ketamine infusion wash subjects out of prior medication for at least two weeks, which may be impractical in a clinical setting. Administration of multiple infusions allows the trajectory of treatment response to be examined, especially important for those subjects that do not respond after a single infusion. In this pilot study, we sought to determine whether antidepressant response and remission can be increased by completing six ketamine infusions as compared to a single infusion while continuing with stable antidepressant dosages. The study also aimed to determine the trajectories of clinical response during infusions and to estimate time to relapse among responders after completion of the six infusions.
Methods: TRD subjects were defined as three or more adequate trials of an antidepressant as determined by the Antidepressant Treatment History Form criteria (score $>=3$). Subjects were required to have at least 2-month period of stable dose of antidepressants and other medications prior to entering the study. Subjects received six IV infusions of 0.5 mg/Kg ketamine over 40 min on a Monday-Wednesday-Friday schedule during a 12-day period. Those who meet response criterion after the last dose of ketamine were followed for 4 consecutive weeks or until relapse towards their baseline state was observed. Response was defined as $\geqslant$50% improvement in depressive symptoms as measured by the Montgomery–Åsberg Depression Rating Scale (MADRS). Remission was defined as a MADRS score $\leqslant 9$. Relapse was defined as $<$50% improvement in MADRS score at that visit compared with baseline. MADRS scores were ascertained at baseline (t0), at the end of infusion (t + 40 min), and again at t + 100 mins and t + 160 mins. Other outcome measures, all administered at the same time points as the MADRS, included self-rated Visual Analog Scales (VAS) for happiness, sadness, energy, tiredness, calmness, worry, worthless and self-value; four-item positive symptom subscale of the Brief Psychiatric Rating Scale (BPRS) and the Clinician-Administered Dissociative States Scale. Hemodynamic parameters were monitored throughout the infusion and two hours post infusion.
Results: Fourteen subjects were enrolled; one dropped out after the first infusion and the second after two infusions due to dissociative side effects. Out of twelve subjects who completed all six infusions, eleven (92%) achieved response criterion while eight (67%) met the remission criterion. After the first infusion, three subjects responded and one remitted. After three or more infusions, four responded and six remitted. Of the eleven patients who responded, seven relapsed within the four week followup period. The mean time to relapse after the last ketamine infusion was 16 days (range 7–28 days). For subjects that completed all infusions, no significant increases were observed on the positive symptom subscale of the BPRS or Clinician-Administered Dissociative States Scale.
Conclusions: Using a six infusion protocol, 92% of subjects responded and 67% remitted. This compares favorably with prior reports from single infusion studies in which between 56–71% of subjects responded. Within our own sample, adding five infusions increased response from 33 to 92%. We observed two patterns of response: a fast response where subjects responded after 1–3 infusions, and a slow

response where 4–6 infusions were needed to reach a clinical response. We were able to achieve response without a two week washout, making ketamine infusion for TRD easier to implement clinically. Future work should examine the baseline neural circuitry and changes in neural circuitry in responders *vs* non-responders and slow *vs* fast responders.

Keywords: treatment resistant depression; ketamine.

Disclosures: P. Shiroma, Nothing to Disclose; B. Johns, Nothing to Disclose; M. Kuskowski, Nothing to Disclose; P. Thuras, Nothing to Disclose; K. Lim, Nothing to Disclose.

W132. Cognitive Training with Pharmacological Enhancement in Schizophrenia

Ana D Stan*, Debra Bushong, Binu Thomas

UT Southwestern, Dallas, Texas

Background: Cognitive impairment is widely considered to be a distinct feature of schizophrenia and a critical target for new treatment development. Based on the considerable current knowledge about the mechanisms of cognition, we have chosen to combine two therapeutic directions, one pharmacologic and the other one behavioral to putatively improve cognition in schizophrenia.

Methods: 60 schizophrenia volunteers were randomized into four treatment groups: (1) atomoxetine plus cognitive remediation ($N=15$); (2) atomoxetine plus remediation control ($N=15$); (3) placebo plus cognitive remediation ($N=15$); and (4) placebo plus remediation control ($N=15$). Atomoxetine or matching placebo were administered at a dose of 40 mg bid or the placebo equivalent. The remediation sequence lasted for 90 min and was administered three times weekly; the remediation control was administered on the same schedule and for the same duration. Psychiatric rating scales were completed at baseline, 1, 2, 3, 4, 5, and 6 months, and a neuropsychological battery was completed at baseline and at 3 and 6 months. fMRI BOLD data was collected with the N-back task before and after the remediation. The primary data analysis was a 4-treatment group (atomoxetine plus remediation control, placebo plus remediation, placebo plus remediation control, atomoxetine plus remediation) X 3 time points (baseline, 3 months, 6 months) mixed-model ANOVA.

Results: Behavioral results show a significant group X time interaction in the Brief Visual Memory Test (BVMT) total recall t score ($p=0.039$). *Post –hoc* analyses revealed that within the groups that received Atomoxetine, there is a significant increase in the t scores at 24 weeks compared to baseline ($p=0.003$ for the atomoxetine plus cognitive remediation group; $p=0.008$ for the atomoxetine plus remediation control group). Imaging results were analyzed with SPM8 comparing two groups: subjects who underwent cognitive remediation *vs* subjects who underwent cognitive remediation control. Preliminary results from $N=12$ per group show an increased BOLD signal in the visual association areas of the occipital cortex and a decreased BOLD signal in the PFC, likely associated with increased processing efficiency.

Conclusions: Behavioral data show an atomoxetine effect on the visual learning memory, both alone and in combination with cognitive remediation. Preliminary functional change to cognitive training is increasedBOLD activation in primaryvisual cortex and a decreased BOLD activation in multimodal association areas.

Keywords: cognitive remediation; atomoxetine; schizophrenia.

Disclosures: A. Stan, Nothing to Disclose; D. Bushong, Nothing to Disclose; B. Thomas, Nothing to Disclose.

W133. Adverse Childhood Experiences Predict Heavier Drinking and Greater Alcohol Intake During Intravenous (IV) Alcohol Self-Administration in Non-Dependent Drinkers

Bethany L Stangl*, Melanie L Schwandt, Laura E Kwako, Jia Yan, Molly Zametkin, Vijay A Ramchandani

NIH, Bethesda, Maryland

Background: Adverse childhood experiences (ACE) have been shown to be associated with increased risk for alcohol use disorders. However, the relationship between ACEs and alcohol seeking behavior in non-dependent drinkers is less clear. The objective of this study was to examine the influence of ACE on drinking history and IV alcohol self-administration in non-dependent drinkers.

Methods: Data were obtained from healthy non-dependent drinkers ($N=212$). ACE was assessed using the Childhood Trauma Questionnaire (CTQ), a 28-item self-report measure that yields a score for overall trauma severity (CTQTotal), total number of traumatic events experienced (CTQNumCat), and severity scores for five subtypes of trauma; physical abuse, sexual abuse, emotional abuse, physical neglect, and emotional neglect. Recent drinking history was assessed using the Timeline Follow Back (TLFB) and the Alcohol Use Disorders Identification Test (AUDIT). Alcohol self-administration behavior was measured in a subset ($N=81$) using the Computer-Assisted Self-infusion of Ethanol (CASE) method that allows individuals to self-administer IV alcohol in a laboratory setting, while controlling the breath alcohol concentration (BrAC) using a physiologically-based pharmacokinetic model-based algorithm. The CASE session consisted of a 25-min priming phase where subjects were prompted to push a button to receive standardized alcohol infusions, followed by a 125-min open-bar phase during which they could push the button *ad lib* for additional infusions. Self-administration measures included number of button presses (NBP), peak (PEAK) and average (AVG) BrAC and total ethanol (EtOH). Subjective responses obtained during the session included the Drug Effects Questionnaire (DEQ) as well as the Alcohol Urge Questionnaire (AUQ). Personality measures included the NEO Five Factor Personality Assessment, the Alcohol Effects Questionnaire (AEFQ) which measures the participant's expectation for change in various facets after alcohol consumption, and the Sensitivity to Punishment and Reward Questionnaire (SPSRQ).

Results: There was a 39% prevalence of at least one form of childhood trauma in the sample. TLFB measures (total drinks, number of drinking days, drinks per drinking day, and heavy drinking days) and AUDIT total score were linearly related to CTQTotal (all $p<0.001$). CTQNumCat

was also significantly associated with TLFB measures and AUDIT total score (all $p<0.001$). Individuals that reported childhood emotional abuse, emotional neglect, physical abuse, physical neglect, and sexual abuse had heavier drinking histories ($p<0.02$). The same relationships were seen in the CASE subset. Within the CASE subset, CTQTotal was significantly associated with EtOH ($p<0.02$) and AVG ($p<0.05$) and trending for PEAK ($p<0.06$). CTQNumCat was significantly associated with EtOH ($p<0.01$), AVG ($p<0.02$) and PEAK ($p<0.03$). Physical neglect was associated with EtOH ($p<0.02$), AVG ($p<0.04$) and PEAK ($p<0.02$), and emotional neglect was associated with EtOH ($p<0.03$). DEQ subjective measures of 'like' and 'want' during self-administration were strongly associated with CTQTotal, CTQNumCat, emotional neglect, as well as physical abuse (all p values <0.02). AUQ total score was associated with CTQTotal, CTQNumCat, and emotional neglect (all p values <0.05). TLFB measures were significantly associated with AVG, PEAK, and EtOH (all $p<0.01$) in this group. NEO measures of Extraversion was negatively associated with CTQNumCat ($p<0.01$) while Openness was negatively associated with severity of emotional abuse ($p<0.01$). Facets of the AEFQ were also strongly associated with the CTQ. Specifically, global positive score was associated with emotional neglect and CTQTotal, social and physical pleasure score was associated with emotional neglect, relaxation and tension reduction scores were associated with emotional abuse, while expectation for cognitive and physical impairment was associated with physical neglect (all p values <0.01). Sensitivity to reward was associated with both emotional and physical neglect, as well as CTQTotal.

Conclusions: Childhood trauma had a significant effect on recent drinking history measures in non-dependent drinkers, as well as on the amount of ethanol self-administered during the CASE session, and subjective hedonic and craving responses during alcohol self-administration. There was also evidence of a strong relationship between childhood trauma and personality factors, specifically sensitivity to reward and expectations from alcohol consumption showing associations with various subtypes of trauma. These data add to the growing evidence of the impact of childhood trauma on alcohol intake behavior and related measures, including subjective effects, expectancies, and reward sensitivity in non-dependent drinkers.

Keywords: childhood trauma, CTQ, IV alcohol, CASE, self-administration.

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W134. Δ^9-THC Attenuates and d-amphetamine Potentiates Responses to a Psychosocial Stressor

Emma L Childs*, Harriet de Wit

University of Chicago, Chicago, Illinois

Background: There is growing evidence that drugs of abuse alter the processing of and reactivity to external emotional stimuli, and that these effects occur independently of direct effects of the drug on mood. Recently we reported that Δ^9-tetrahydrocannabinol (THC) reduces perception of facial threat and diminishes amygdalar activation in response to threat-related faces and that d-amphetamine (AMP) potentiates positive responses to both positive and negative emotional stimuli. These findings have implications for drug use and abuse; drugs which either make positive stimuli more attractive or negative stimuli less threatening may be particularly desirable to some individuals.

Methods: In the current study, we evaluated the effects of THC (0, 7.5, 12.5 mg; $N=35$) and AMP (0, 5, 10 mg; $N=50$) on responses to a negative event, the Trier Social Stress Test (TSST), in healthy young adults. Participants received the drug or placebo before undergoing the TSST and a non-stressful control task, in randomized order, under double blind conditions. We measured self-reported emotional states and drug effects, cardiovascular indices and salivary cortisol.

Results: Both THC and AMP produced mild mood effects before the tasks, consistent with these drugs' expected effects. The TSST increased feelings of tension, heart rate, blood pressure and salivary cortisol. In comparison to placebo, THC significantly attenuated negative emotional responses to the TSST ('I feel stressed', $p<0.05$) but it prolonged blood pressure responses to the task ($p<0.05$). AMP (10 mg) increased ratings of how threatening and challenging the task would be ($p<0.05$), and increased ratings of how stressful they found the task ($p<0.05$). AMP (10 mg) also prolonged heart rate and blood pressure responses to the task ($p<0.05$). The lower dose of AMP (5 mg) affected participants' ratings of the task; before completing the task they reported feeling more confident in their ability to perform the task and after the task, the drug increased their perceived ability to influence the task.

Conclusions: These findings add support to the idea that THC attenuates processing of and responses to negative external stimuli. AMP affected performance and ratings differently at low and high doses. Together, the results support the hypothesis that drugs affect responses to emotional stimuli in ways that may influence their use and abuse.

Keywords: THC, amphetamine, stress, emotion.

Disclosures: E. Childs, Nothing to Disclose; H. de Wit, Nothing to Disclose.

W135. Meta-analysis: Response Curve to SSRIs in OCD

Michael H Bloch*, Yasmin Issari, Ewgeni Jakubovski

Child Study Center, New Haven, Connecticut

Background: Selective-Serotonin Reuptake Inhibitors (SSRIs) are the first-line pharmacological treatment for Obsessive-Compulsive Disorder (OCD). Current clinical guidelines recommend the use of higher doses of SSRIs and for a longer period of time for OCD compared to Major Depression. Many treatment guidelines suggest that a clomipramine, the most serotonergically-selective tricyclic antidepressant is more effective than SSRIs for the treatment of OCD.

Methods: We conducted a meta-analysis of randomized, placebo-controlled trials of SSRIs and clomipramine in

OCD to describe the dose response curve of SRIs in OCD (and compare it to MDD) and examine the effect of dose, individual agent and year of publication on the response curve of SRIs in OCD.
Results: Meta-analysis included 18 trials of SSRIs and 5 trials of clomipramine in OCD including over 4000 subjects. A logarithmic response curve, indicating decreasing symptom improvement over time, best fit the response curve of SSRIs and clomipramine in OCD. A significantly greater response was associated with using higher doses of SSRIs. Clomipramine demonstrated a significantly greater treatment response compared to SSRIs. The benefit of clomipramine was robust to controlling for dosage or year of publication.
Conclusions: The average response curve in OCD to SSRIs is similar to that observed in similarly conducted meta-analyses in Major Depression. Higher doses of SSRIs appear more effective in OCD (in contrast to Major Depression). Evidence exists that clomipramine and fluoxetine may be particular effective in OCD treatment.
Keywords: obsessive-compulsive disorder, meta-analysis, serotonin reuptake inhibitors, clomipramine.
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W136. A Pilot Study of a Dopamine β-Hydroxylase Inhibitor, Nepicastat, in the Treatment of PTSD with Genotype Outcome Analysis

Lori Davis*, David P Graham, Hamner Mark, David Nielson, Thomas Kosten, Iouri Makotkine, Rachel Yehuda

Tuscaloosa VA Medical Center, Tuscaloosa, Alabama

Background: Despite the evidence that increased noradrenergic (NA) activity is associated with PTSD, studies have shown variance in NA levels among persons with PTSD. One reason for this heterogeneity may depend on an individual's genetics. The etiology of PTSD is multifactorial and can best be viewed as polygenetic. This study was conducted in advance of a larger, multicenter, randomized clinical trial investigating the dopamine β-hydroxylase (*DBH*) inhibitor nepicastat as a novel treatment for PTSD, specifically the hyper-arousal symptoms. Nepicastat is a potent, competitive, and selective DβH inhibitor which should reduce the abnormally high NA activity generally found in PTSD, and thereby, result in reduced PTSD hyper-arousal symptoms. The *DBH* gene has a functional polymorphism, with higher plasma DβH activity having been associated with the CC *DBH* genotype in combat Veterans without PTSD as compared to Veterans with PTSD, while the DβH activity was similar to those without PTSD in Veterans carrying the T allele, with the CC genotype having overall greater activity than the T carrier genotypes. This suggests that DβH activity is attenuated by PTSD in the higher functioning CC genotype, but not in the lower functioning T carrier genotypes and may represent a compensatory response to prolonged hyperarousal condition seen in PTSD. The DBH genotype may have relevance to an individual's treatment response to nepicastat.
Methods: This 6-week randomized, double-blind, placebo-controlled study examined the utility of using genetics and functional noradrenergic biology to predict response to nepicastat, a specific and targeted pharmacological treatment for PTSD hyper-arousal symptoms in Veterans ($n = 23$). Measures included the Clinician Administered PTSD Scale—D (hyperarousal) subscale (*CAPS-D*), dopamine β-hydroxyzine (*DBH*) genotype, and urinary 3-methoxy-4-hydroxyphenylglycol (MHPG).
Results: We found a primary effect for pre- to post-treatment change in *CAPS-D* score within the nepicastat group ($p = 0.006$), but no significant effect based solely on treatment ($p = 0.832$) or *DBH* genotype ($p = 0.983$). However, there was a significant treatment by genotype interaction regarding pre- to post-treatment change in *CAPS-D* score ($p = 0.013$). Changes in *CAPS-D* scores were uncorrelated with changes in MHPG (all $p > 0.05$).
Conclusions: This study suggests specific genetic polymorphisms may be more useful in predicting an individual's response to targeted intervention than directly measuring a designated functional biological measure.
Keywords: nepicastat, posttraumatic stress disorder, dopamine-beta-hydroxylase inhibitor, gene, polymorphism.
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W137. Preliminary Investigation of EEG Predictors in an Open-label, Flexible-dose, Repeated Infusions of Ketamine as Augmentation in Treatment Resistant Depression

Cristina Cusin*, Matthias Eikermann, Sebastian Zaremba, Kara Pavone, Kelley Durham, Trina Chang, Paolo Cassano, Christina Dording, David Soskin, David Mischoulon, Maurizio Fava

Massachusetts General Hospital, Boston, Massachusetts

Background: Several studies have shown that the N-methyl-d-aspartate (NMDA) antagonist ketamine at a dose of 0.5 mg/kg produces significant antidepressant effects in patients with treatment-resistant major depressive disorder (MDD). Identifying neural correlates of its acute antidepressant effect and psychotropic side effects would be an important advance for the development of rapid antidepressant agents and could facilitate the early identification of patients who may develop a sustained response to those agents and are most likely to benefit from treatment. We conducted a pilot study of open-label flexible-dose repeated infusions of ketamine as augmentation in a sample of treatment-resistant, chronically suicidal outpatients with MDD. We conducted preliminary analyses in a subsample to investigate possible EEG predictors of acute response. We also performed continuous monitoring of physiologic variables to assess the safety of ketamine as augmentation of their ongoing medications.
Methods: Inclusion criteria were primary diagnosis of MDD (DSM-IV), no history of psychotic features, age 18–65 years, Hamilton Depression Rating Scale 28-Items (HAM-D 28)

score $\geqslant 20$, history of 3 or more failed treatments during the current episode, current suicidal ideation (SI) for at least 3 months and on a stable antidepressant regimen stable for 4 weeks. Patients received 6 infusions of ketamine over 3 weeks, followed by 3-months of follow-up visits occurring every other week. The dose was 0.5 mg/kg, infused over 45 min. If the participant did not experience an improvement $\geqslant 30\%$ in HAM-D score after the 3rd infusion, the dose was increased to 0.75 mg/kg for infusions 4–6. Response was defined as 50% improvement on the HAM-D score from screen to the 6th infusion; remission as HAMD score $\leqslant 7$. The Clinician Administered Dissociative States Scale (CADSS) was administered at time points 0, 60, and 120 min of every infusion visit. Respiratory function, eye movements, and arousal state were recorded using frontal electroencephalography (EEG) recording (F3-A1 and F4-M1), submental electromyography (EMG), electrooculography (EOG), measurement of respiratory effort (impedance plethysmography), nasal respiratory flow, and capillary blood oxygen saturation (SpO2, Alice PdX monitor, Respironics).

Results: The study is ongoing and to date, 15 subjects have been screened and 12 have been enrolled in the study (10F/2M, mean age 51.1 ± 7.9). The mean HAM-D score at screening was 28.9 ± 4.7. Subjects were taking an average of 1.8 ± 0.8 antidepressants and 1.8 ± 1.4 other psychotropic medications (anxiolytics, mood stabilizers, atypical antipsychotics), and they had failed an average of 7.6 ± 5.1 antidepressant trials in the current depressive episode. After the 6th infusion, 4/12 (33%) patients met criteria for response and 2/12 (17%) met criteria for remission. After the last infusion 5 out of 12 (42%) patients reported not having any SI. The patients experienced minimal sedation and mild dissociative symptoms during the infusions, with an average score of 2.7 ± 1.8 on the CADSS at T60 and 0.2 ± 0.4 at T120. One participant discontinued the study after the second infusion, and five dropped out during follow-up *EEG*: Preliminary analyses in a subsample found that the patients who respond to ketamine treatment and sustained the improvement at 4 weeks showed an increase in EEG power in the gamma band, similar to the one observed in healthy volunteers during the onset of ketamine induced sedation (Lee U *et al* Anesthesiology 2013). In contrast, delta power was higher in the patients who did not show acute response to ketamine treatment. Given the small sample size, however, the differences are not significant. Change in gamma or delta power did not seem to correlate with acute dissociative side effects. Safety: Ketamine infusions at 0.5 and 0.75 mg/kg were well tolerated. Vital signs showed mild increase in systolic BP during the infusion, and no appearance of apneas or hypopneas during or after the infusions of ketamine 0.5 and 0.75 mg/kg, indicating upper airway stability and no impairment of respiratory drive.

Conclusions: Ketamine infusions in outpatients with TRD were feasible and well tolerated. The overall efficacy of ketamine in our sample seems to be lower compared to published samples, which could be due to interactions with concurrent medications. We are investigating possible EEG biomarkers as they may become useful predictors of long-term response to ketamine in patients with TRD and further analyses of larger samples are necessary.

Keywords: ketamine, treatment-resistant, depression, EEG, biomarker.

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(CPFQ), Sexual Functioning Inventory (SFI), Antidepressant Treatment Response Questionnaire (ATRQ), Discontinuation-Emergent Signs & Symptoms (DESS), and SAFER; Lippincott, Williams & Wilkins; Wolkers Kluwer; World Scientific Publishing Co. Pte.Ltd., **Part 2:** Belvoir Media Group for monthly newsletter editing, **Part 4:** Research Support: Abbot Laboratories; Alkermes, Inc.;Aspect Medical Systems; AstraZeneca; BioResearch; BrainCells Inc.; Bristol-Myers Squibb; CeNeRx BioPharma; Cephalon; Clintara, LLC; Covance; Covidien; Eli Lilly and Company; ElMindA, Ltd.; EnVivo Pharmaceuticals, Inc.; Euthymics Bioscience, Inc.; Forest Pharmaceuticals, Inc.; Ganeden Biotech, Inc.; GlaxoSmithKline; Harvard Clinical Research Institute; Icon Clinical Research; i3 Innovus/Ingenix; Janssen R&D, LLC; Jed Foundation; Johnson & Johnson Pharmaceutical Research & Development; Lichtwer Pharma GmbH; Lorex Pharmaceuticals; MedAvante; National Alliance for Research on Schizophrenia & Depression (NARSAD); National Center for Complementary and Alternative Medicine (NCCAM); National Institute of Drug Abuse (NIDA); National Institute of Mental Health (NIMH); Neuralstem, Inc.; Novartis AG; Organon Pharmaceuticals; PamLab, LLC.; Pfizer Inc.; Pharmaceutical Research Associates., Inc.; Pharmavite® LLC;PharmoRx Therapeutics; Photothera; Roche Pharmaceuticals; RCT Logic, LLC (formerly Clinical Trials Solutions, LLC); Sanofi-Aventis US LLC; Shire; Solvay Pharmaceuticals, Inc.; Synthelabo; Wyeth-Ayerst Laboratories.

W138. Intranasal Methamphetamine Self-administration in Humans During D-amphetamine Maintenance

Paul Glaser*, Erika Pike, Lon Hays, William W Stoops, Craig R Rush

University of Kentucky, Lexington, Kentucky

Background: Agonist replacement has been successfully implemented for opiate abuse but remains less proven for stimulant use disorders. This study sought to determine the effects of *d*-amphetamine maintenance on methamphetamine self-administration in human volunteers. We predicted *d*-amphetamine maintenance would reduce methamphetamine self-administration.
Methods: Eight volunteers were recruited meeting criteria for stimulant abuse but not currently stimulant dependent nor seeking treatment. Subjects were admitted to the hospital and started one of two *d*-amphetamine maintenance conditions in counter-balanced order (0 and 40 mg/day). Once in steady state for maintenance d-amphetamine or placebo, four experimental sessions were started sampling one of four doses of intranasal methamphetamine (0, 10, 20, or 30 mg). Volunteers then had the opportunity to respond on a computerized progressive ratio task to earn portions of the sampled methamphetamine dose. Subject-rated drug-effect and physiological measures were completed at regular intervals prior to and after sampling methamphetamine.
Results: Methamphetamine was self-administered as an orderly function of dose regardless of the maintenance condition. Methamphetamine produced prototypical subject-rated effects, some of which were attenuated by *d*-amphetamine maintenance. Methamphetamine was well tolerated during *d*-amphetamine maintenance and no adverse events occurred. Methamphetamine significantly increased systolic blood pressure by 10 mm Hg, but did not affect diastolic blood pressure and heart rate. Maintenance with *d*-amphetamine reduced the effect on systolic blood pressure.
Conclusions: Although *d*-amphetamine attenuated some subject-rated effects of methamphetamine, the self-administration results are concordant with those of a clinical trial showing that *d*-amphetamine did not reduce methamphetamine use. Human laboratory self-administration studies can be used to screen other putative agonist replacement pharmacotherapies prior to clinical trial testing.
Keywords: amphetamine abuse methamphetamine.
Disclosures: P. Glaser, Nothing to Disclose; E. Pike, Nothing to Disclose; L. Hays, Nothing to Disclose; W. Stoops, Nothing to Disclose; C. Rush, Nothing to Disclose.

W139. Lurasidone in the Treatment of Early-stage Schizophrenia: A *Post-hoc* Analysis of Three Pooled Acute Treatment Studies

Jeffrey Lieberman*, Andrei Pikalov, Jay Hsu, Josephine Cucchiaro, Fred Grossman, Antony Loebel

Columbia Univesity, New York, New York

Background: Lurasidone has demonstrated efficacy in the treatment of schizophrenia in patients who typically have a high degree of chronicity. Lurasidone is well-tolerated with a relatively favorable safety profile, low propensity for weight gain and metabolic abnormalities, making it a potentially useful agent to treat patients in the early stages of schizophrenia. In previous studies, the criteria for defining early-stage schizophrenia has ranged from 2 to 5 years since onset of illness (1–3). The aim of this *post-hoc* analysis was to evaluate the efficacy of lurasidone in patients with early-stage schizophrenia (ESS).
Methods: This was a pooled analysis of patients experiencing an acute exacerbation of schizophrenia who had participated in three 6-week, randomized, double-blind, placebo-controlled, phase 3 trials. ESS was defined using two early stage criteria: onset of illness within 3 years (ESS-3 yr), or onset within 5 years (ESS-5 yr), prior to study entry. Two chronic subgroups were defined based on duration of illness greater than 3 years (chronic-3 yr) or greater than 5 years (chronic-5 yr). Efficacy was evaluated using a mixed-model repeated-measures (MMRM) analysis of change from baseline to Week 6 in PANSS total and subscale scores. Effect sizes were calculated using LOCF-endpoint data.
Results: In this pooled analysis, a total of 857 patients were randomized to lurasidone and 366 patients were randomized to placebo. The proportion of patients meeting 3 year and 5 year ESS criteria was 12 and 23%, respectively. Using the 3 year onset criterion, mean age was lower in the ESS *vs* chronic subgroups (ESS-3 yr: 29 years *vs* chronic-3 yr: 39 years) and mean PANSS total score at baseline was similar (ESS-3 yr: 97 *vs* chronic-3 yr: 97). In the 3 year criterion ESS subgroup, treatment with lurasidone (*vs* placebo) was associated with significantly greater improvement at week

6 on the PANSS total score (−25.9 *vs* −17.3; $p<0.05$), the PANSS positive subscore (−9.6 *vs* −6.0; $p<0.01$), and the PANSS negative subscore (−5.6 *vs* −3.2; $p=0.014$). In the 3 year criterion chronic subgroup, treatment with lurasidone (*vs* placebo) was associated with significantly greater improvement at week 6 on the PANSS total score (−24.6 *vs* −17.9; $p<0.001$). For the 3 year criterion subgroup, the proportion of patients who discontinued due to an adverse event was similar in the ESS *vs* chronic subgroups (4 *vs* 2%). The 3 most frequent adverse events for lurasidone (*vs* placebo) were somnolence (ESS-3 yr: 22.5 *vs* 13.6%; chronic-yr: 21.4 *vs* 11.1%), Parkinsonism (ESS-3 yr: 21.6 *vs* 9.1%; chronic-3 yr: 3.1 *vs* 7.6%), and nausea (ESS-3 yr: 18.6 *vs* 18.2%; chronic-3 yr: 9.5 *vs* 7.6%). Comparable levels of improvement were found using 5 year *vs* 3 year ESS criteria; the effect size (lurasidone *vs* placebo) for change in PANSS total score was 0.39 for the ESS-5 yr subgroup and 0.42 for the ESS-3 yr subgroup. The incidence of adverse events was also similar when comparing 5 year and 3 year ESS subgroups.

Conclusions: In this pooled analysis, patients with early stage schizophrenia were, as expected, younger than chronic patients. In patients with early stage schizophrenia, treatment with lurasidone was associated with levels of improvement that were similar to levels observed in the chronic population. Discontinuation rates were low across all early stage and chronic subgroups. These results suggest that lurasidone is efficacious and well-tolerated in patients with early stage schizophrenia.

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Sanger *et al* (1999). *Am J Psychiatry* **156**: 79–873.
Girgis *et al* (2011). *J Psychiatric Res* **45**: 756–762.

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W140. Perceptions of Obsessive Compulsive Disorder and Potential Impact on Treatment Outcome

Michael Van Ameringen*, William Simpson, Beth Patterson, Jasmine Turna

McMaster University, Hamilton, Ontario, Canada

Background: The effect of an individual's perceptions of his or her illness on the outcome of the illness has primarily been examined in physical (as opposed to mental) illnesses. Understanding patients' perceptions of their physical illness or injury has been shown to predict important patient outcomes such as treatment adherence and return to work. There is a small literature examining how illness perceptions can be applied to mental health, however most of this work has been conducted in schizophrenia and psychotic disorders. In these populations, perceptions of mental illness have been found to be associated with anxiety and depressive symptoms. In relatives of patients with schizophrenia, illness perceptions are related to emotional andbehavioural responses to the individual with the illness and distress in the relatives who care for these patients (Petrie *et al*, 2007). Obsessive compulsive disorder (OCD) is a chronic, often disabling condition disorder affecting approximately 1.6% of the population of the United States at some point in their lifetime. Although not as prevalent as other anxiety disorders, OCD causes substantial impairments in a person's overall functioning and quality of life (QoL) (Moritz, 2008). Very little has been done to examine OCD patients' self-perceptions of their disorder and the impact of these perceptions on outcomes. As part of a naturalistic cross-sectional study of OCD, sponsored by the International College for Obsessive Compulsive Spectrum Disorders, we collected data concerning patient's views of their OCD.

Methods: Consecutive OCD patients ($n=504$), at various stages of treatment were evaluated in 9 international tertiary care anxiety disorders clinics. Patients completed a number of self-report measures, including the 36-item 'Views of OCD' questionnaire, the Sheehan Disability Scale (SDS) as well as a detailed clinical and structured interview, including the Yale-Brown Obsessive Compulsive Scale (YBOCS) and Clinical Global Impression-Severity Scale (CGI-S).

Results: The mean age of participants was 38.1 years ($\pm$ 12.9 years); 61.5% of the sample was female, and the mean Yale-Brown Obsessive Compulsive Scale score was 19.7 ($\pm$ 6.5), indicating a moderate level of severity. The majority of the sample ($\geqslant$73%) saw their OCD as permanent, having major consequences on their life, causing difficulties for those close to them and inducing fear and worry about the impact of this condition. However, 68% felt they had power to influence their OCD, 55% saw treatment as controlling their OCD, and 53% felt they had a good understanding of their condition. Negative perceptions were correlated with increased severity (YBOCS) and impairment (SDS), but were not related to treatment response. No associations were found for positive perceptions and degree of disability, symptom severity or treatment response. Although 45% believed that use of cognitive techniques would help, only 28% were using these techniques regularly ($\geqslant$3 times per week); 39% believed that regular use of exposure techniques would help, but only 18% were using them regularly. Similarly, 75% believed that medication was helpful, however only 63% reported taking their medication daily. Compared to those who did not take their medication daily, those who took it daily had higher SDS and CGI-S scale scores indicating higher functional impairment and global severity, but no significant difference in YBOCS scores (symptom severity).

Conclusions: The majority of the sample described their OCD as a serious and distressing, long-term, cyclical illness which they expect to have for the rest of their life. Most people indicated that their OCD has had major consequences on their lives and has caused difficulties for their loved ones. Interestingly, at least half of the sample believed that they had the ability to influence the course of their OCD, in terms of control over and management of their symptoms. Negative beliefs about an individual's OCD were significantly correlated with greater functional impairment and symptom severity, but not with treatment response. No

similar associations were found for positive perceptions. Surprisingly, a significant number of OCD patients are not using evidence-based treatments as prescribed.
Keywords: obsessive compulsive disorder perceptions qualtiy of life cross-sectional international college of obsessive compulsive spectrum disorders.
Disclosures: M. Van Ameringen, Nothing to Disclose; W. Simpson, Nothing to Disclose; B. Patterson, Nothing to Disclose; J. Turna, Nothing to Disclose.

W141. Do We Know Why There are Regional Differences in Signal Detection in Global Neuroscience Clinical Trials?

Amir Kalali*

Quintiles, San Diego, California

Background: The topic of regional differences in signal detection in neuroscience clinical trials has been the subject of intense discussion in the realm of drug development. There has been much speculation about the reasons for these differences and the possible implications for neuroscience drug development and regulatory approvals. One of the main proposed reasons is the subjective nature of many primary outcome measures in neuroscience clinical trials.
Methods: Data from several recent large global clinical development programs were examined with respect to regional difference in signal detection. These were compared to data from global programs outside of the area of neuroscience, including cardiovascular development programs.
Results: Regional differences in signal detection are seen in global drug development programs in neuroscience and in other therapeutic areas that do not utilize subjective outcome measures.
Conclusions: Data will be shown regarding regional differences in signal detection in neuroscience clinical trials as well as from programs in other therapeutic areas. As the regional differences exist across therapeutic areas, possible causes contributing to this phenomenon will be discussed as well as implications for global drug development and regulatory approval of future programs.
Keywords: signal detection clinical trials drug development.
Disclosures: A. Kalali, **Part 1:** Employee of Quintiles Inc., **Part 3:** Employee of Quintiles Inc., **Part 5:** Employee of Quintiles Inc.

W142. Trajectory of Neurocognition in First-episode Schizophrenia

Joey W Trampush*, Delbert G Robinson, Todd Lencz, John Kane, Anil Malhotra, Terry E Goldberg, Danielle Beech

Zucker Hillside Hospital, Glen Oaks, New York

Background: First-episode schizophrenia is associated with pronounced cognitive dysfunction across domains (Mesholam-Gately *et al*, 2009). However, less is known about the course of dysfunction following the initial presentation of psychosis in the neuropsychological domains most germane to schizophrenia (eg, general cognition, processing speed and verbal memory). As such, this longitudinal study utilized the MATRICS Consensus Cognitive Battery (MCCB) to evaluate the magnitude of impairment in first-episode schizophrenia at baseline, and then reevaluated patients after 12 and 52 weeks of antipsychotic treatment in order to track the stability neurocognitive dysfunction over time.
Methods: A total of 169 first-episode patients (mean age: 22.91 y; sd = 6.12 y; range = 15.25–45.15 y) contributed between 228 to 306 data points from the MCCB across baseline, 12-week follow-up and 52-week follow-up. Most patients were diagnosed with a DSM-IV-defined schizophrenia spectrum disorder (85.6%); the remaining patients received diagnoses of psychosis NOS. Patients were randomized to either aripiprazole or risperidone during the initial 12-week phase, and then maintained on antipsychotic treatment for the remainder of the year. Hierarchical linear modeling (HLM; Raudenbush and Bryk, 2002) was used to generate individual cognitive growth trajectories across the year. The Brief Psychiatric Rating Scale (BPRS) was used to dimensionalize psychosis severity at baseline and included as a mediating variable in secondary analyses.
Results: Adjusted for age and sex, the magnitude of impairment at baseline ranged from ~2 SD's below average for the Speed of Processing domain (T = 30.7) to ~1.25 SD's below average for the Social Cognition domain (T = 37.5; Table 1). The mean linear growth rate of increase in performance across time was significant for 6 out of 8 MCCB

Table 1 MCCB Performance at Baseline and Relative Change Over the Course of the year

Baseline status

MCCB domain	Coefficient	S.E.	t-ratio	d.f. (approx)	*p*-value
General cognition	33.70	0.69	49.09	166	<0.001
Speed	30.70	1.03	29.79	165	<0.001
Attention	32.09	0.95	33.69	124	<0.001
Working memory	34.08	1.02	33.53	162	<0.001
Verbal memory	36.07	0.65	55.86	166	<0.001
Visual memory	32.40	0.87	37.05	164	<0.001
Reasoning	37.27	0.79	47.44	161	<0.001
Social cognition	37.48	0.93	40.14	151	<0.001

Change over time

MCCB domain	Coefficient	S.E.	t-ratio	d.f. (approx)	*p*-value
General cognition	1.98	0.39	5.09	166	<0.001
Speed	1.62	0.71	2.28	165	0.024
Attention	1.30	0.65	1.99	124	0.049
Working memory	3.95	0.59	6.72	162	<0.001
Verbal memory	1.18	0.46	2.58	166	0.011
Visual memory	0.64	0.68	0.93	164	0.354
Reasoning	1.44	0.66	2.19	161	0.03
Social cognition	1.96	1.00	1.97	151	0.051

Note: adjusted for age, sex.

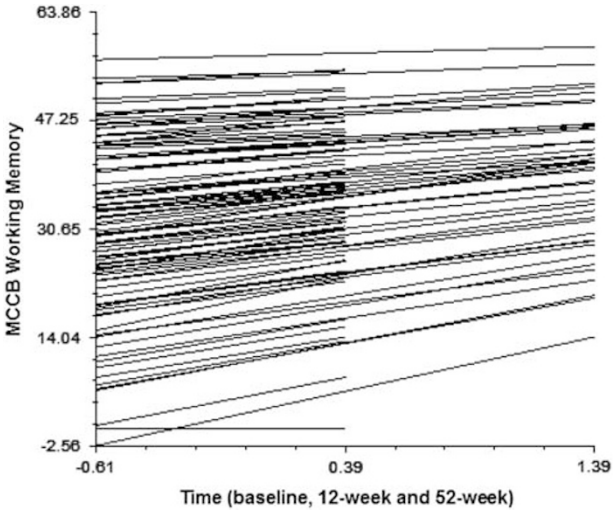

Figure 1 Individual linear growth rate for MCCB Working Memory domain.

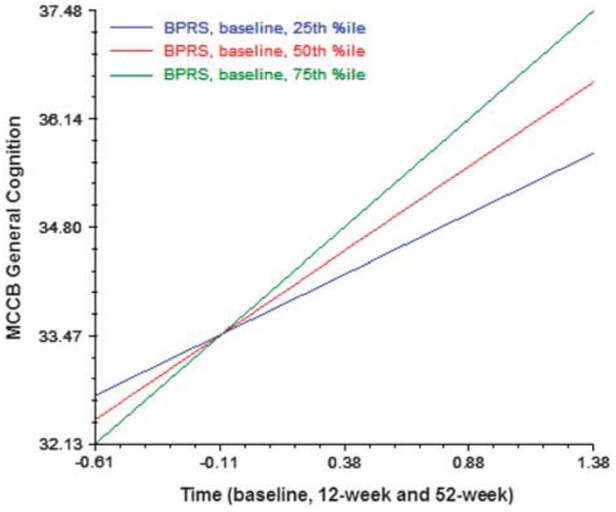

Figure 2 Model based plot of trajectory of general cognitive ability as a function of baseline psychosis severity rated with the BPRS.

domains and estimated as follows (in descending order of magnitude; see Table 1): Working Memory (3.95, $p<0.001$; Figure 1); General Cognition (1.98, $p<0.001$); Social Cognition (1.96, $p=0.051$); Speed of Processing (1.62, $p=0.024$); Reasoning and Problem Solving (1.44, $p=0.03$); Attention/Vigilance (1.30, $p=0.049$); Verbal Memory (1.18, $p=0.011$); and Visual Memory (0.64, $p=0.354$). Notably, the magnitude of change in General Cognition was significantly associated with BPRS severity at baseline such that the most severe patients at baseline improved the most over time (Figure 2).
Conclusions: Similar to prior reports (eg, Mesholam-Gately *et al*, 2009), our large sample of first-episode schizophrenia patients evidenced severe impairments across all cognitive domains at baseline as assessed with the MCCB. Across a year of antipsychotic treatment, positive though modest gains in performance were detected in most areas of cognitive functioning. However, whether these improvements can be traced back to antipsychotic treatment or whether the gains in performance were confounded by practice effects is unknown at this time. These data are preliminary; once the blind is broken on this double-blind study, we will examine this question in detail. Nonetheless, irrespective of whether improvements were due to practice, treatment or both, this is one of the largest studies to show that cognitive functioning does not appear to decline in the year following the first episode of psychosis if antipsychotic treatment is initiated.
Keywords: first-episode schizophrenia; neurocognition; growth trajectories; antipsychotic medication.
Disclosures: J. Trampush, Nothing to Disclose; D. Robinson, Nothing to Disclose; T. Lencz, Nothing to Disclose; J. Kane, Nothing to Disclose; A. Malhotra, Nothing to Disclose; T. Goldberg, Nothing to Disclose; D. Beech, Nothing to Disclose.

W144. Chronic High Dose Adjunctive Intranasal Oxytocin in Schizophrenia Patients

David Feifel*, Kai MacDonald, Cobb Patrice, Rebecca McKinney

University of California, San Diego, California

Background: Recently, several groups including ours have shown the therapeutic benefit of 20–40 IU BID intranasal (IN) oxytocin (OT) added to existing antipsychotic treatment in randomized, double blind, placebo-controlled studies. These finding have significant implications for OT as a novel antipsychotic therapy. However, each of these studies examined a single dose of OT (40 IU BID or 24 IU BID) for 2–8 weeks. As promising as these results are, the optimal dose of OT for schizophrenia remains unknown as does the effects of long term OT administration in this population. We present findings from a placebo controlled randomized study of 80 IU BID intranasal oxytocin and from a long term (6 month) open label study of flexibly dosed intranasal oxytocin (up to 168 IU).
Methods: To explore the efficacy and safety of higher OT doses than previously studied in schizophrenia patients we are conducting an open-label, 6-month evaluation of IN OT in antipsychotic-treated schizophrenia patients who have significant residual symptoms. IN OT was titrated up 168 IU BID on in a naturalistic fashion. We are also conducting a 3-week placebo crossover study of 80 IU BID OT—double the dose used our original study—added on to existing treatment in schizophrenia patients.
Results: Data from the first 11 patients who have received at least 12 weeks of open label OT revealed progressive reduction in PANSS scores ($P=0.035$ and $P=0.010$) over the 12-weeks of treatment. Treatments were well tolerated with no significant study related adverse effects noted. Preliminary analysis of the first 6 patients in the crossover study revealed that 3-weeks of 80 IU BID OT produced a greater reduction in PANNS total ($P=0.065$), PANSS positive subscale ($P=0.039$) and PANSS negative ($p=0.091$) scores compared to placebo. As in the open label study, OT was well tolerated with no evidence of adverse effects.
Conclusions: This is the first report of OT doses above 40 IU BID in schizophrenia patients and the first of daily OT treatment longer than 8 weeks. The results suggest that

higher doses of OT are both efficacious and well tolerated, providing further support that OT may be a safe and efficacious novel treatment for schizophrenia. Head to head studies of different doses of IN OT in schizophrenia patients are needed to establish the optimal dose.
Keywords: oxytocin, schizophrenia.
Disclosures: D. Feifel, Nothing to Disclose; K. MacDonald, Nothing to Disclose; C. Patrice, Nothing to Disclose; R. McKinney, Nothing to Disclose.

W145. The Impact of Cocaine Use Patterns, Demographic and Mood Variables, and Addiction Severity on Neurocognitive Functioning in Individuals with Cocaine Use Disorders

James Mahoney*, Ari Kalechstein, Christopher D Verrico, Tabish Iqbal, Thomas Newton, Richard De La Garza

Baylor College of Medicine, Houston, Texas

Background: One of the factors that impedes treatment success for individuals with cocaine use disorders is the presence of neurocognitive deficits produced or exacerbated by cocaine use. Thus, determining potential variables (ie demographic, drug use, and mood symptoms) that impact outcomes may be useful in the development of treatment plans. The goal of this study was to determine whether demographic (ie, ethnicity, gender, education, IQ), drug use (ie, years, recent, and daily cocaine use), and mood (ie BDI-II, LSC-R) variables, as well as addiction severity, affect neurocognitive functioning in participants with cocaine use disorders.
Methods: Participants with cocaine use disorders ($N = 125$) were administered the following neurocognitive tests: Continuous Performance Task (CPT; a measure of attention/information processing), N-Back (a measure of working memory), Hopkins Verbal Learning Task—Revised (HVLT-R; a measure of verbal memory), and the Wechsler Adult Intelligence Scale (a measure of IQ). The order of administration was the same and the assessments were identical with the exception of the HVLT-R where alternate forms were administered. Since the sample was comprised of mostly African American males, a subsample of those individuals were matched with Caucasian participants on the following variables: age, education, IQ, years, recent, and daily cocaine use (this same matching process was conducted for males *vs* females since there were significantly fewer females). For the non-categorical (continuous) variables, the sample was divided into tertiles to compare the highest 1/3 in each category to the lowest 1/3 in each category to determine differences between groups using one-way analysis of variance (ANOVA).
Results: Participants were primarily African-American males, ~45 years of age, reported using ~2 grams of cocaine/day, ~17 days out of the last 30 days, and an average of ~18 years of use. With respect to demographic comparisons, females outperformed males on the delayed recall component of the episodic memory task ($p = 0.039$), but there were no other gender differences nor were there any ethnic differences noted. When comparing those individuals with more education (14.8 ± 0.2 years; mean ± SEM) *vs* less education (10.1 ± 0.3 years), there were no differences on any of the domains assessed. When comparing individuals with higher IQ's (109.6 ± 0.8 years) to those with lower IQ's (85.1 ± 1.1), it was determined that those with higher IQ's performed significantly better on tasks of episodic ($p < 0.0001$) and working memory ($p < 0.0001$). Also, younger individuals (37.6 ± 0.8 years), were more impulsive and inattentive ($p = 0.02$) when compared to older individuals (51.5 ± 0.3 years); yet, younger participants demonstrated significantly better performance on a working memory task ($p = 0.006$). Of interest, individuals who reported more daily cocaine use (1.8 ± 0.0 *vs* 0.7 ± 0.0 grams) demonstrated better accuracy on the working memory task ($p = 0.04$). There were no differences in cognitive function between individuals who used for more years (25.2 ± 0.6 *vs* 10.1 ± 0.6 years) or more days in the last 30 (26.3 ± 0.5 *vs* 6.0 ± 0.6 days). Finally, individuals with higher depression, lifetime stress, and addiction severity did not differ on any of the neurocognitive domains assessed when compared to those endorsing fewer symptoms.
Conclusions: Overall, when compared to an age-matched normative sample, the current results indicate that cocaine users perform at a lower level on the neurocognitive tests administered. Of note, the lack of differences with respect to gender (with the exception of delayed episodic memory), ethnicity, and education demonstrated that these factors may not be as critical in cognitive functioning as reported in other populations. Moreover, the finding that drug use characteristics (including years and recent use) did not influence cognitive functioning may demonstrate that once an individual reaches a certain amount of cocaine usage, a 'floor' effect takes place and these deficits remain impaired, yet stable. In summary, our findings suggest that some demographic, drug use, and mood variables may (or, more accurately, may not) contribute to cognitive deficits in individuals with cocaine use disorders.
Keywords: cocaine, episodic memory, working memory, attention, drug use variables.
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W146. Efficacy and Safety of Treatment with Lurasidone Adjunctive to Lithium or Valproate in Bipolar I Depression: Results of Two 6-week Studies

Joseph R Calabrese*, Trisha Suppes, Kaushik Sarma, Robert Silva, Hans Kroger, Josephine Cucchiaro, Andrei Pikalov, Antony Loebel

University Hospitals Case Medical School, Cleveland, Ohio

Background: For the treatment of depressive episodes in bipolar I patients concurrently treated with mood stabilizers, expert clinical guidelines recommend adjunctive therapy, yet few studies have reported data supporting the efficacy of any adjunctive agent. The aim of the current analysis was to evaluate the efficacy and safety of

lurasidone adjunctive to lithium or valproate in bipolar I depression.

Methods: Data were pooled from two adjunctive therapy studies (D1050235 [Study 1]; D1050292 [Study 2]) with similar designs: patients meeting DSM-IV-TR criteria for bipolar I depression, with or without rapid cycling, with a Montgomery Åsberg Depression Rating Scale (MADRS) score $\geqslant 20$ and a Young Mania Rating Scale score $\leqslant 12$ were randomized to 6 weeks of once-daily, double-blind treatment with lurasidone 20–120 mg/day or placebo, adjunctive with either lithium (Li) or valproate (VPA). Therapeutic blood levels of Li or VPA were maintained for $\geqslant 28$ days prior to randomization in both studies; Study 1 allowed retrospective confirmation of treatment with Li or VPA prior to screening, while Study 2 allowed both retrospective and prospective treatment cohorts. Changes from baseline in MADRS (primary outcome) and Clinical Global Impression Bipolar Severity of Illness (CGI-BP-S; key secondary assessment) were analyzed using MMRM; secondary efficacy outcomes included the Quick Inventory of Depressive Symptomology—Self Report (QIDS-SR16), Hamilton Anxiety Rating Scale (HAM-A), the Quality of Life, Enjoyment and Satisfaction Questionnaire (Q-LES-Q-SF), and the Sheehan Disability Scale (SDS). These measures were evaluated using ANCOVA-LOCF, and logistic regression. Safety parameters were evaluated based on analysis of the LOCF-endpoint sample.

Results: A similar proportion of patients in the adjunctive lurasidone and placebo groups completed Study 1 ($N = 143$; 78% *vs* $N = 136$; 82%) and Study 2 ($N = 148$; 82% *vs* $N = 140$; 80%). Mean MADRS scores at baseline were similar (Study 1: 30.7; Study 2: 29.1). For the pooled analysis sample, treatment with lurasidone (*vs* placebo) was associated with significant week 6 improvement in the mean MADRS score (-14.4 *vs* -11.9; $p = 0.003$; effect size: 0.25). Treatment with lurasidone (*vs* placebo) was also associated with significant week 6 improvement in CGI-BP-S scores (-1.7 *vs* -1.3; $p = 0.001$), QIDS-SR16 scores (-7.4 *vs* -5.7; $p \leqslant 0.001$), HAM-A scores (-7.0 *vs* -5.0; $p \leqslant 0.001$ [LOCF]), and Q-LES-Q-SF scores ($+18.5$ *vs* $+13.2$; $p \leqslant 0.001$ [LOCF]), and non-significant improvement in SDS scores (-7.7 *vs* -6.4; $p = 0.067$ [LOCF]). Responder rates ($\geqslant 50\%$ reduction in MADRS at week 6) were significantly higher for the lurasidone group *vs* placebo (48 *vs* 37%; $p = 0.002$; LOCF-endpoint). Remission rates (defined as MADRS $\leqslant 10$) were significantly higher for the lurasidone group *vs* placebo (36 *vs* 27%; $p = 0.008$; LOCF-endpoint). In the pooled safety population, minimal LOCF-endpoint changes were observed for adjunctive lurasidone *vs* placebo in mean weight (0.1 *vs* 0.2 kg), median total cholesterol (-4.0 *vs* -1.0 mg/dl), LDL (-3.0 *vs* -1.0 mg/dl), triglycerides ($+4.0$ *vs* -2.0 mg/dl), and glucose (0.0 *vs* 0.0 mg/dl). Discontinuation rates due to treatment-emergent adverse events were similar (5.8 *vs* 4.8%); treatment-emergent adverse events with an incidence $\geqslant 5\%$ (and greater than placebo) were nausea (13.9 *vs* 10.2%), Parkinsonism (12.8 *vs* 8.1%), somnolence (11.4 *vs* 5.1%), and akathisia (10.8 *vs* 4.8%) in the combined adjunctive lurasidone and placebo groups, respectively. The incidence of protocol-defined treatment-emergent mania for the lurasidone *vs* placebo groups was similar (0.8 *vs* 1.5%).

Conclusions: The pooled results from two similarly designed, short-term placebo-controlled studies demonstrated that use of lurasidone as adjunctive therapy with lithium or valproate is an effective treatment for patients with bipolar I depression. Significant improvement was observed in depressive symptoms, and in patient-rated measures of function and quality of life. The low rate of discontinuation due to adverse events, and low incidence of adverse events, suggest that lurasidone was well-tolerated. Short-term treatment with lurasidone had minimal effect on weight or metabolic parameters.

Keywords: bipolar disorder, atypical antipsychotics, major depressive disorder.

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W147. Lurasidone Adjunctive Therapy with Lithium or Valproate for the Treatment of Bipolar I Depression: A Randomized, Double-blind, Placebo-controlled Study (PREVAIL 3)

Trisha Suppes*, Joseph R Calabrese, Robert Silva, Hans Kroger, Josephine Cucchiaro, Andrei Pikalov, Antony Loebel

VA Palo Alto Health Care System, Palo Alto, California

Background: Few studies have been reported that support the efficacy of adjunctive therapy for patients with bipolar I depression who have had an insufficient response to monotherapy with mood stabilizing agents. In a previous placebo-controlled trial (1; PREVAIL 1), treatment with lurasidone adjunctive with lithium or valproate significantly improved depressive symptoms and was generally well-tolerated. This was the second study to evaluate the short-term efficacy of lurasidone as adjunctive therapy with lithium (Li) or valproate (VPA) for the treatment of bipolar I depression.

Methods: In this multi-regional study, patients were required to meet DSM-IV-TR criteria for bipolar I depression, without psychotic features, with or without a rapid-cycling course of illness, and with a Montgomery-Åsberg Depression Rating Scale (MADRS) score $\geqslant 20$ and a Young-Mania Rating scale (YMRS) score $\leqslant 12$ at screening and baseline. Eligible patients were randomized to 6 weeks of double-blind treatment with either lurasidone 20–120 mg/day or placebo adjunctive with either Li or VPA. Treatment with Li or VPA could be ongoing at the time of screening (retrospective assessment cohort), or could be prospectively initiated by the investigator during a lead-in phase (prospective cohort). All patients were required to have a minimum of 28 days of treatment with Li (range, 0.6–1.2 mEq/l) or VPA (range, 50–125 μg/ml). Changes from double-blind baseline in MADRS (primary assessment) and Clinical Global Impression Bipolar Severity of Illness (CGI-BP-S; key secondary assessment) were analyzed using mixed model repeated measures (MMRM) analysis; other secondary outcomes were analyzed using ANCOVA (LOCF).
Results: A total of 180 patients were randomized to the adjunctive lurasidone treatment group and 176 were randomized to the adjunctive placebo group (treatment with Li/VPA was ongoing at the time of initial screening in 38.5% of patients, and prospectively initiated in 61.5% of patients); 82% in the lurasidone group and 80% in the placebo group completed the study. Mean MADRS scores at baseline were 29.1 in both treatment groups, indicative of moderate-to-severe depression. Treatment with lurasidone was associated with significant improvement compared with placebo at week 2 to week 5, but a significant difference was not observed at the primary (week 6) endpoint (-11.8 *vs* -10.4; $p = 0.18$; effect size, 0.16). A larger improvement for lurasidone *vs* placebo was observed in patients recruited into the study who were already being treated with Li/VPA (retrospective cohort; effect size: 0.31), compared to those treated prospectively with Li/VPA (effect size: 0.03). On the CGI-BP-S, endpoint improvement in the lurasidone group was not significantly different from the placebo group (-1.4 *vs* -1.1; $p = 0.095$; effect size, 0.20). On the Hamilton Anxiety Rating Scale (HAM-A) treatment with lurasidone was associated with significantly greater endpoint improvement compared with placebo (-5.7 *vs* -3.8; $p < 0.01$). Treatment with lurasidone was associated with significantly greater endpoint improvement on the Quality of Life, Enjoyment and Satisfaction Questionnaire (Q-LES-Q-SF; $+14.3$ *vs* $+10.2$; $p = 0.04$), but not on the Sheehan Disability scale or other secondary measures. Treatment with lurasidone *vs* placebo was associated with minimal endpoint changes in mean weight ($+0.2$ *vs* 0.0 kg), median total cholesterol (-4.0 *vs* -2.5 mg/dl), triglycerides ($+1.0$ *vs* -1.0 mg/dl), glucose (0.0 *vs* -0.5 mg/dl), and prolactin ($+1.8$ *vs* -0.1 ng/ml). Discontinuation rates due to treatment-emergent adverse events were 5.6% in the lurasidone group and 2.9% in the placebo group. The most frequently reported adverse events in the lurasidone *vs* placebo groups were akathisia (14.1 *vs* 5.3%), somnolence (11.9 *vs* 4.7%), and Parkinsonism (11.3 *vs* 7.6%).
Conclusions: In this study, which ascertained subjects utilizing both retrospective and prospective treatment cohorts (Li or VPA) in patients with bipolar I depression, adjunctive treatment with lurasidone did not result in significant improvement compared with placebo at study endpoint. Significant separation from placebo was observed starting at week 2 through week 5. Lurasidone was also significantly superior to placebo on the HAM-A and Q-LES-Q at endpoint. Lurasidone was generally well-tolerated in this study with a relatively low overall discontinuation rate, and rate of discontinuation due to adverse events. The lack efficacy in this study appeared in part related to inclusion of a cohort of patients treated prospectively with mood stabilizers prior to randomization. Use of a prospective treatment cohort during lead-in has previously been shown to present methodological challenges in augmentation studies of mood disorders (2). Minimal changes in weight, lipids and measures of glycemic control were observed.
Keywords: bipolar disorder, atypical antipsychotics, major depressive disorder.
Disclosures: T. Suppes, **Part 1:** Consultant or advisory capacity to Orexigen Therapeutics and Sunonvion Pharmaceuticals., Has received royalties or honorarium from Medscape, CNS Drug Supplement, and Jones and Bartlett (formerly Compact Clinicals)., **Part 4:** Research funding or medication support from Abbott Laboratories, AstraZeneca, JDS Pharmaceuticals, NIMH, Pfizer, the Stanley Medical Research Institute and Sunovion Pharmaceuticals.; J. Calabrese, **Part 1:** Consultant services to AstraZeneca, Biomedical Dev Corp, Convergent Health Solutions, Elan, Esai, Forest Labs, Health & Wellness, Hoffman LaRoche, Lundbeck, Merck, Otsuka, Pfizer, Scientia, Spirant Communications Private Limitrex, Sunovion, Takeda, and Teva., Payment for lectures from the American Foundation for Suicide Prevention, AstraZeneca, Benecke, Cortex Congress, GlaxoSmithKline, Lundbeck, Medwiz Healthcare, Merck, Pfizer, Promedica, Sunovion, Takeda, Ohio Psychiatry Association, Ohio State University, University of Cincinnati, and University of Toronto., **Part 4:** Cephalon, Elan, NARSAD, Sunovion, and Takeda; R. Silva, **Part 5:** Sunovion Pharmaceuticals, Inc.; H. Kroger, **Part 5:** Sunovion Pharmaceuticals, Inc.; J. Cucchiaro, **Part 5:** Sunovion Pharmaceuticals, Inc.; A. Pikalov, **Part 5:** Sunovion Pharmaceuticals, Inc.; A. Loebel, **Part 5:** Sunovion Pharmaceuticals, Inc.

W148. Lurasidone in Bipolar I Depression: A 24 Week, Open-label Extension Study

Terence Ketter*, Kaushik Sarma, Robert Silva, Jane Xu, Josephine Cucchiaro, Antony Loebel

Stanford School of Medicine, Stanford, California

Background: Lurasidone has recently been approved by the FDA for the treatment of acute bipolar I depression as monotherapy, and as adjunctive therapy with lithium or valproate. The aim of the current 6 month extension study was to evaluate the longer-term safety and tolerability of lurasidone (20–120 mg/day, flexibly dosed) in patients who recently completed acute treatment for bipolar I depression.
Methods: Patients completing 6 weeks of double-blind, placebo-controlled treatment with either lurasidone monotherapy (1 study) or lurasidone adjunctive therapy with lithium (Li) or valproate (VPA; 2 studies), were treated for 6 months with once-daily flexible doses of lurasidone,

20–120 mg/day in this open-label extension study. Patients were transitioned directly upon completion of the preceding 6 week study to receive 60 mg/day of lurasidone, with weekly dose adjustments to optimize efficacy and tolerability. In addition to mood stabilizers (Li, VPA), concomitant treatments with benzodiazepines or antidepressants were permitted. Safety/tolerability outcomes included the incidence of adverse events (AEs), discontinuations due to AEs, weight, and laboratory parameters, which were co-primary endpoints. Secondary efficacy endpoints included total scores for the Montgomery-Asberg Depression Rating Scale (MADRS), Clinical Global Impression for Bipolar Disorder, Overall Severity (CGI-BP-S), Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q), Sheehan Disability Scale (SDS), Hamilton Anxiety Scale (HAM-A), and Young Mania Rating Scale (YMRS). Safety endpoints were analyzed as change from double-blind baseline for study completers who had initially been randomized to lurasidone in the initial 6 week study (30 weeks of total exposure; monotherapy, $n = 154$; adjunctive, $n = 104$). Treatment-emergent mania was defined as a YMRS score $\geqslant 16$ on 2 consecutive visits, or an AE of mania or hypomania.

Results: Of the 817 patients entered into this open-label extension study, 316 (38.9%) were treated with lurasidone as monotherapy, and 497 (61.1%) were treated with lurasidone adjunctive with Li or VPA. For the total population, 47.5% were male, with a mean age of 42.7 years, and a mean MADRS score of 15.3 at baseline (ie at entry to this open extension study). A total of 68.4% of patients entering the extension study completed it. Treatment-emergent AEs with an incidence $\geqslant 5\%$ were akathisia (8.1%), headache (7.7%), nausea (7.6%), insomnia (6.4%), anxiety (5.8%), and Parkinsonism and EPS-related AEs (including akathisia, in aggregate 10.7%). Discontinuation due to an AE leading occurred in 7.0% of monotherapy patients and 8.7% of adjunctive therapy patients. Mean change in weight at month 6 was 0.45 kg in the monotherapy group and 0.90 kg in the adjunctive group. Clinically meaningful change in weight ($\geqslant 7\%$) for adjunctive therapy with lurasidone was: increase, 11.9%; decrease, 4.9%; and for monotherapy was: increase, 5.0%; decrease, 3.0%). The following median changes were observed at month 6 for total cholesterol (monotherapy, 0.0 mg/dl; adjunctive, −1.5 mg/dl); triglycerides (monotherapy, + 6.0 mg/dl; adjunctive, + 8.0 mg/dl); glucose (monotherapy, 0.0 mg/dl; adjunctive, + 1.0 mg/dl); and prolactin (monotherapy, + 1.3 ng/dl; adjunctive, + 1.3 ng/dl). The incidence of treatment-emergent mania was 1.3% in the monotherapy treatment subgroup and 3.8% in the adjunctive subgroup; the incidence of 'any suicidal ideation or behavior' on the Columbia Suicide Severity Rating Scale was 2.1%. The following mean changes were observed at month 6 (observed case) for the MADRS (monotherapy: −6.9; adjunctive: −6.5), CGI-BP-S (monotherapy: −0.87; adjunctive: −0.85), HAM-A (monotherapy: −2.8; adjunctive: −2.4), YRMS (monotherapy: −0.8; adjunctive: −0.5), SDS (monotherapy: −4.3; adjunctive: −3.3), and Q-LES-Q (monotherapy: + 9.6; adjunctive: + 7.5).

Conclusions: Long term treatment with lurasidone 20–120 mg/d for 6 months was safe and well tolerated with minimal effect on weight and metabolic parameters. There were minimal differences in tolerability or safety outcomes with lurasidone monotherapy or as adjunctive therapy with lithium or valproate. Treatment with lurasidone was associated with sustained improvement in both the MADRS, and in patient-rated measures of quality of life and function. *ClinicalTrials.gov Identifier: NCT00868959 Sponsored by Sunovion Pharmaceuticals Inc.*.

Keywords: bipolar disorder, atypical antipsychotics, major depressive disorder.

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W149. Short- and Longer-term Treatment with Lurasidone in Patients with Bipolar I Depression: Effect on Metabolic Syndrome

Susan McElroy*, Andrei Pikalov, Josephine Cucchiaro, Jay Hsu, Hans Kroger, Debra Phillips, Antony Loebel

Lindner Center of HOPE, Mason, Ohio

Background: Antipsychotics are commonly used in the management of bipolar disorder but may lead to adverse metabolic consequences, including weight gain and an increased risk for diabetes and cardiovascular disease. These adverse metabolic effects are in addition to effects associated with bipolar illness itself. This pooled analysis evaluated the effect of short- and longer-term treatment with lurasidone on the prevalence of metabolic syndrome in bipolar I depression.

Methods: The effects of lurasidone, in the dosing range of 20–120 mg/d, was evaluated on the prevalence of metabolic syndrome in three short-term and one longer-term extension study. In the short-term studies, patients meeting DSM-IV-TR criteria for bipolar I depression, with or without rapid cycling, with a Montgomery Åsberg Depression Rating Scale (MADRS) score $\geqslant 20$, were randomized to 6 weeks of once-daily, double-blind, placebo-controlled treatment with lurasidone, either as monotherapy (one study; total $N = 499$) or adjunctive to lithium (Li) or valproate (VPA; two studies; total $N = 694$). Patients completing the 6-week studies continued to receive 6 months of additional treatment with lurasidone 20–120 mg/d in an open-label extension study. NCEP criteria (1) for metabolic syndrome were used, with metabolic syndrome defined as meeting $\geqslant 3$ of the following: waist circumference (male, $\geqslant 102$ cm; female, $\geqslant 88$ cm), triglycerides ($\geqslant 150$ mg/dl), HDL-cholesterol (male, < 40 mg/dl; female, < 50 mg/dl), blood pressure ($\geqslant 130/85$ mmHg), or plasma glucose ($\geqslant 110$ mg/dl). Change at 6 months (for completers) was calculated from double-blind baseline of the acute study.

Results: At baseline, the prevalence of metabolic syndrome was similar for lurasidone and placebo, respectively, in the

adjunctive therapy studies (lurasidone, 14.8% and placebo, 13.5%) and in the monotherapy study (lurasidone, 14.3% and placebo, 15.5%). After 6 weeks of adjunctive therapy, the prevalence of metabolic syndrome in the lurasidone *vs* placebo groups was 17.0 *vs* 12.4% (LOCF); and after 6 weeks of monotherapy the prevalence was 15.8 *vs* 17.7% (LOCF). For patients treated with lurasidone in the double-blind study and who completed 6 months of extension phase treatment (30 weeks of total exposure), the prevalence of metabolic syndrome, among patients receiving monotherapy and adjunctive therapy, was 16.7 and 15.5%, respectively, at double-blind baseline, and 17.9 and 23.8% at month 6 of the extension. For the subgroup with metabolic syndrome at baseline in the monotherapy study, the following median changes were observed (after 30 weeks) in patients ($n = 30$) who completed 6 months of treatment with lurasidone in weight (−0.3 kg), cholesterol (−4.0 mg/dl), triglycerides (−22.0 mg/dl), and glucose (−2.0 mg/dl). For the subgroup of lurasidone patients with metabolic syndrome at double-blind baseline in the adjunctive therapy studies, the following median changes were observed in patients ($n = 31$) who completed 30 weeks of treatment with lurasidone in weight (0.0 kg), cholesterol (−6.0 mg/dl), triglycerides (+ 11.0 mg/dl), and glucose (+ 2.0 mg/dl).

Conclusions: In patients with acute bipolar I depression, up to 7 months of treatment with lurasidone, either as monotherapy, or as adjunctive therapy when added to Li or VPA, was associated with only minimal metabolic disturbance. In at-risk patients who met metabolic syndrome criteria at study entry, treatment with lurasidone was associated with no worsening of metabolic parameters.

Keywords: bipolar disorder, atypical antipsychotics, metabolic syndrome, lipids, glucose.

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W150. Early Improvement Predicts Endpoint Response to Lurasidone in Schizophrenia: Pooled Analysis of Five Double-blind Trials

Christoph U Correll*, Andrei Pikalov, Jay Hsu, Josephine Cucchiaro, Robert Goldman, Antony Loebel

Hofstra North Shore LIJ School of Medicine and Albert Einstein College of Medicine, Glen Oaks, New York

Background: Early improvement following initiation of antipsychotic medication (within 2–3 weeks of initiating treatment) is a potentially important predictor of subsequent treatment response that has clinical implications for the successful management of schizophrenia (1, 2). The goal of this pooled completer analysis was to evaluate the clinical value of early improvement in the PANSS total score and the CGI-Severity score as predictors of response to 6 weeks of treatment with lurasidone among patients with an acute exacerbation of schizophrenia.

Methods: Data were pooled from 5 similarly designed, six-week, double-blind, placebo-controlled trials of subjects hospitalized with an acute exacerbation of schizophrenia who were randomly assigned to fixed, once-daily doses of lurasidone 40–80 mg ($n = 404$) or 120–160 mg ($n = 264$), or placebo ($n = 280$), and who completed acute treatment. Endpoint responder rates were calculated using the PANSS criterion ($\geqslant$40% reduction from Baseline). Early improvement was separately assessed at weeks 1, 2, and 3 using two criteria (CGI-Severity $\geqslant$1-point improvement; PANSS improvement $\geqslant$20%). For each improvement criterion, calculations were made of sensitivity (the probability that a patient who achieved endpoint response was correctly identified based on early improvement) and specificity (the probability that a patient who did not achieve endpoint response was correctly identified based on early improvement). Receiver operating characteristic (ROC) curves were used to determine the optimal cut-scores for prediction of endpoint response, based on the highest area under the curve (AUC).

Results: Baseline demographic and clinical characteristics were similar in the group that achieved (*vs* did not achieve) early improvement, with the exception of duration of schizophrenia, which was longer in the group that did not achieve early improvement. In the combined lurasidone dosage groups, the proportion of subjects showing early improvement was similar for the PANSS$\geqslant$20% criterion and the CGI-Severity $\geqslant$1 criterion, respectively, at week 1 (32.5 and 36.1%), at week 2 (53.8 and 59.8%), and at week 3 (70.7 and 88.0%). Endpoint response (week 6) in the lurasidone group was 50.2% using PANSS$\geqslant$40% responder criteria. For the PANSS$\geqslant$40% responder criterion, at week 1 PANSS$\geqslant$20% improvement had a sensitivity = 46.6%, specificity = 81.6%, and AUCROC = 0.660; and CGI-S improvement $\geqslant$1 had a sensitivity = 46.2%, specificity = 74.0%, and AUCROC = 0.621. At week 2, PANSS$\geqslant$20% improvement had a sensitivity = 75.2%, specificity = 67.9%, and AUCROC = 0.733; and CGI-S improvement $\geqslant$1 had a sensitivity = 74.4%, specificity = 54.8%, and AUCROC = 0.650. At week 3, PANSS$\geqslant$20% improvement had a sensitivity = 91.9%, specificity = 50.5%, and AUCROC = 0.730; and CGI-S improvement $\geqslant$1 had a sensitivity = 87.3%, specificity = 43.7%, and AUCROC = 0.656. Among patients treated with lurasidone who met $\geqslant$40% responder criteria at week 3, 57/60 (95.0%) maintained their response at week 6. Among week 3 non-responders, 278/607 (45.8%) achieved a response (using the $\geqslant$40% PANSS criterion) by week 6.

Conclusions: The ROC area under the curve analysis found that the PANSS$\geqslant$20% improvement at week 2 provided adequate prediction of endpoint response. Change in CGI-Severity was less useful as a predictor. Early determination of the likelihood that a patient will have a favorable treatment response has important implications for the effective clinical management of schizophrenia. Further

research is needed to determine whether dose titration is an effective strategy for increasing responder rates on lurasidone among patients who do not respond early in the course of treatment.

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Keywords: schizophrenia, lurasidone, early response, prediction, PANSS.

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W151. Neurocognitive Impairments as Putative Predictors of Neuroleptic-induced Movement Disorders in People with Schizophrenia

Anthony Ahmed*

Medical College of Georgia, Augusta, Georgia

Background: Antipsychotic medication management is a standard intervention for schizophrenia symptoms but chronic or prolonged use of both first generation antipsychotics alone or in combination with atypical antipsychotics is associated with the emergence of tardive dyskinesia and other movement disorders. In the absence of effective treatments for neuroleptic-induced movement disorders, prevention through the identification of risk factors is a desirable proposition with potential to improve antipsychotic prescription practice. The current study examined neurocognitive phenotypes as putative predictors of tardive dyskinesia, Parkinsonism, and akathesia in schizophrenia patients.

Methods: We recruited 662 patients with a DSM diagnosis of schizophrenia, schizoaffective, psychotic disorder not otherwise specified, and mood disorder with psychotic features receiving antipsychotic treatments in outpatient and inpatient psychiatric settings. DSM diagnosis of a psychotic disorder was ascertained using a Structured Clinical Interview for DSM-IV Axis I (SCID-I). Participants were rated on the Maryland Psychiatric Research Center's (MPRC) Involuntary Movement Scale (MIMS), a 31-item measure of involuntary movements in several anatomic regions that include tongue; perioral region; eyes and periorbital region; face and jaw; fingers and wrists; elbows and arms; neck, shoulders, and head; thighs and knees; legs and feet; arm; pelvis; and diaphragm. We administered the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS); Wechsler Adult Intelligence Scale—Third Edition (WAIS-III); and Wide-Range Achievement Test (WRAT-3) to assess neurocognitive functions and intellectual functioning. We measure positive symptoms of schizophrenia using the Scale for the Assessment of Positive Symptoms, and negative symptoms using the Schedule for the Deficit Syndrome. We evaluated premorbid social and academic adjustment by administering the Premorbid Adjustment Scale (PAS). We measured psychosocial functioning with the Level of Functioning Scale, Global Assessment of Functioning (GAF) ratings, and the Quality of Life Inventory (QOLI). The demographic and clinical characteristics of study participants were obtained using a standard clinical interview. We used canonical correlational analysis (CCA) to examine the association between neurocognitive domain scores and subtypes of movement disorders. Given that distributions of study variables were skewed, we used non-parametric correlations to assess the association with clinical and demographic variables and anatomic regions.

Results: There were no statistically significant differences in global severity ratings of movement disorders across sex and ethnic groups. Age was however a statistically significant predictor of dyskinesia with older patients generally receiving higher severity ratings (rrho = 0.338, $p < 0.0001$). Level of education was a small but significant predictor of the severity of Parkinsonism (rrho = 0.148, $p < 0.001$). but not dyskinesia or akathesia. There was no association between the severity of positive symptoms and the severity of movement disorders. There was however an association between the severity of negative symptoms and Parkinsonism (rrho = 0.213, $p < 0.001$). With regard to psychosocial outcomes, the severity of dyskinesia (rrho = −0.320, $p < 0.001$) but not Parkinsonism or akathesia was significantly associated with GAF scores. In contrast, only Parkinsonism was significantly correlated with Level of Function total score (rrho = −0.169, $p < 0.001$). Dyskinesia, Parkinsonism, and Akathesia global ratings had significantly negative correlations with work status, hours worked per week, earning, and job satisfaction. The severity of dyskinesia had a small correlation with health-related quality of life (rrho = −0.224, $p < 0.01$). The CCA produced a significant association between neurocognitive domain scores and 12 individual dyskinesia items in the MIMS. Only the first canonical variate pair was significant (rc1 = 0.360, Wilk's $\lambda = 0.643$, $\chi 2$ (65), $p < 0.05$). This variate pair linked low scores on immediate memory, visuospatial reasoning, attention, delayed memory, and estimated IQ to jerking movements of the tongue, and lip smacking and pouting (perioral). The redundancy analysis suggested that 6.5% of variance was shared by neurocognition and dyskinesia. The CCA between neurocognition and Parkinsonism did not achieve significance (rc1 = 0.337, Wilk's $\lambda = 0.669$, $\chi 2$ (90), $p = 0.14$).

Conclusions: In schizophrenia, neurocognition demonstrate a modest association with dyskinesia. There appear to be a link between a neurocognitive profile characterized by impairments in immediate memory, visuospatial reasoning, attention, delayed memory, and general intelligence and dyskinesia of the tongue and perioral region. The result of our study is partly consistent with those of prior studies and may establish neurocognitive phenotypes as possible targets for movement disorder risk profiling.

Keywords: tardive dyskinesia; parkinsonism; akathesia; schizophrenia; neuroleptic-induced movement disorder.

Disclosures: A. Ahmed, Nothing to Disclose.

W152. Loss of Neural Signals Related to Cognitive Flexibility in the Rostral Caudate Following Short-term Cocaine Self-administration

Brianna Sleezer*, Benjamin Hayden

University of Rochester, Webster, New York

Background: Drug-induced changes in frontostriatal circuits are believed to play a pivotal role in the transition from voluntary drug use to compulsive drug-associated behaviors in addiction. While much of this work has focused on how addictive drugs enhance striatal-mediated habit learning processes, little has looked at how drugs might disrupt striatal activity related to flexible, goal-directed decision-making. Given that recent work has implicated the rostral striatum in goal-directed processing, it is possible that addictive drugs may promote inflexible behaviors by altering neural signals in this region. To determine if and how addictive drugs alter goal-directed processing in the striatum, we examined neural activity in two monkeys performing a novel monkey adaptation of the Wisconsin Card Sorting Test (WCST), a classic and well-studied paradigm for understanding goal-directed cognition. We recorded the activity of single neurons in the rostral portions of three striatal regions (the caudate, putamen, and ventral striatum) before and during a period of short-term cocaine self-administration.
Methods: On each trial of our task, monkeys are presented with a series of three stimuli that differ in shape (circle, triangle, or star) and color (cyan, magenta, or yellow). Monkeys must choose one of these stimuli based on a rule (a specific color or a shape). Once the rule is learned, it must be maintained for 10, 15, or 20 trials. Correct choices are followed by positive visual feedback and a reward, while incorrect choices are followed by negative visual feedback and no reward. Rule changes are not cued and new rules must be learned through trial and error. Following training, we recorded firing rate activity in the rostral regions of the caudate, putamen, and ventral striatum while two monkeys performed this task. Our analyses were designed to detect three types of task-related activity: (1) associative encoding, (2) rule signals, and (3) switching signals. After baseline data collection was complete, we implemented a cocaine self-administration paradigm (4 mg/kg/day, 5 days/wk), and collected a second set of physiology data from the caudate, putamen, and ventral striatum. In this experiment, behavior and/or physiology data was collected in the morning, while self-administration occurred in the afternoon. Physiology data were collected from self-administration days 21 to 45.
Results: *Cocaine Naïve Condition* Prior to cocaine exposure, we found that monkeys were able to rapidly learn, maintain, and switch between rule sets. Neurons throughout all three striatal regions exhibited activity related to associative encoding, rule maintenance, and behavioral switching. While the ventral striatum and caudate appeared to contribute primarily to associative encoding and switching, the putamen appeared to contribute less to associative coding and switching and more to rule maintenance. *Cocaine Exposed Condition* Although task performance was similar between the cocaine naïve and cocaine exposed conditions, accuracy and motivation were subtly impaired. Changes in neural responses were clearer. The most prominent signals were a substantial reduction in the prevalence of shift signals in the caudate. We also found a reduction in the strength of associative encoding signals in the caudate, but no change in the number of cells contributing to associative encoding or rule maintenance in the caudate or any of the three measures in putamen or ventral striatum.
Conclusions: Results from our pre-cocaine experiments indicate that, under normal conditions, all three regions of the rostral striatum contribute to cognitive flexibility through their involvement in associative encoding, rule maintenance, and switching. These findings are inconsistent with the commonplace notion that striatum is specialized for habit learning, but are consistent with recent work implicating the rostral striatum in goal-directed behaviors. Our cocaine experiments suggest that drug-induced changes in striatal activity may occur prior to the development of any strong behavioral impairments. Specifically, our data suggest that cocaine exposure reduces neural signals related to flexible behavior (shift signals) in the caudate. At first glance, these findings appear somewhat inconsistent with previous work, which has suggested that long-term psychostimulant exposure promotes a shift in the control of behavior from the ventral striatum to the dorsal striatum (rather than away from the dorsal striatum, as our data would suggest). However, given that the majority of work in this area has looked at long-term rather than short-term drug exposure and the putamen rather than the caudate, our findings are not entirely inconsistent previous work. More specifically, it is possible that, rather than promoting a shift from the ventral striatum to the dorsal striatum (ie from motivational to habit-related domains), addictive drugs may instead promote a shift from the rostral caudate, to the ventral striatum, to the putamen (ie from cognitive to motivational to habit-related domains). These findings provide further insight into how the motivational aspects of drugs are able to initially gain control of behavior prior to the development of drug-related compulsions.
Keywords: striatum, macaque, single-unit, addiction, self-administration.
Disclosures: B. Sleezer, Nothing to Disclose; B. Hayden, Nothing to Disclose.

W153. Long-term Reduction of Cocaine Seeking in Rats and Monkeys by Viral Vector-delivered Cocaine Hydrolase (CocH)

Marilyn E Carroll*, Natalie E Zlebnik, Yang Gao, Stephen Brimijoin, PhD

University of Minnesota, Minneapolis, Minnesota

Background: Cocaine dependence is a chronic disorder that is devastating to individuals and costly to society, and there are currently no FDA approved treatments. Our research has focused on a novel therapeutic approach to cocaine addiction through protein therapeutics. Human butyryl-cholinesterase, the native enzyme that metabolizes cocaine when ingested, was modified to create a more catalytically efficient enzyme called cocaine hydrolase (CocH) that is encoded in a helper-dependent adenoviral (HdAd) or adeno-associated viral (AAV) vector and delivered by iv injection. After transduction of the virus in the liver, the organism produces CocH, which interferes with cocaine-

seeking in a rat model of relapse for up to 6 months (Anker *et al*, 2012). In the present study we tested this enzyme delivery system (*vs* control injections; eg, saline) with rats and rhesus monkeys using the latest and most potent versions of the HdAd and AAV vector delivery in animals exposed to cocaine for several hours a day, 7 days per week. We found marked reductions in cocaine self-administration that endured for several months. Specificity of CocH was confirmed by examining CocH effects (*vs* saline) on methamphetamine self-administration.

Methods: Rats were trained to self-administer 0.4 mg/kg/infusion cocaine during 2 h daily sessions, 7 days per week. After behavior stabilized for at least 10 days, the vector group received HdAd vector encoding CocH in 1 iv. injection in the tail vein, and a control group received iv. saline. Cocaine self-administration was monitored daily for 1–2 months, and subsequently all rats were pre-treated ip. with iso-OMPA (1.5 mg/kg). This esterase inhibitor was given 12 h before the next self-administration session for 8 consecutive days to determine whether the decreases in cocaine self-administration could be reversed by inactivation of CocH. Following iso-OMPA treatment, rats were allowed 7 days to re-establish baseline responding. Next, iv. cocaine was replaced with methamphetamine (0.05 mg/kg/infusion) to establish that reduced cocaine self-administration was due to self-administration of the CocH substrate, cocaine, and not a general reduction in motivation for drug. Blood was collected at weekly then bi-weekly intervals during cocaine self-administration and daily during iso-OMPA treatment to evaluate the serum enzyme levels in relation to the amount of cocaine self-administered or treatment with iso-OMPA. Monkeys were trained to orally self administer cocaine HCl (0.4 mg/ml) mixed in tap water under a fixed-ratio 4 (FR 4) schedule with water also available during 3-h daily sessions, 7 days per week, according to Meisch *et al* (1990). When behavior stabilized for 2–3 weeks, the monkeys were injected with the HdAd or AAV vector with 1 iv. injection in the femoral vein (under general anesthesia). Behavior was then allowed to stabilize, and daily cocaine intake was monitored for up to 6 months. The monkeys had previously been trained to self-administer phencyclidine and/or ethanol, and they will subsequently be tested with these drugs again to establish that reductions in cocaine intake are not due to general reductions in motivation. Food and water intakes were also monitored, and blood was collected weekly, and later bi-weekly and monthly to analyze the relationship between serum CocH levels and cocaine intake. The monkeys served as their own controls in a repeated measures study with a pre-vector cocaine baseline. Several cocaine doses will be compared from before to after vector administration.

Results: In the rat experiment there was an initial attempt to surmount the blockade by CocH, and responding nearly doubled for several days, then it declined to nearly 0 and remained stable for 1–2 months. Iso-OMPA reversed this effect and increased responding to the higher levels of saline-treated controls. Vector-treated rats returned to low levels of cocaine self-administration after iso-OMPA was discontinued indicating that the esterase inhibitor temporarily suppressed effects of the CocH vector. Furthermore, methamphetamine self-administration was at levels close to the saline-treated group's cocaine infusions, and it was unaffected by the CocH vector. In the monkey study 4 monkeys have been injected with low doses of CocH vector to assure a safe and effective dose for eventual use in humans. This initial group of monkeys ranged from low to moderate cocaine responders. Initial results indicated a suppression in cocaine intake (from baseline) and elevated CocH serum levels that have endured for several weeks to 2 months. There were no adverse effects of either type of vector delivery to the monkeys; thus, the study continues with higher HdAd and AAV doses in monkeys that are moderate to high cocaine responders.

Conclusions: Initial results indicate that viral-vector delivery of cocaine hydrolase reduced iv. cocaine self-administration in rats to near zero levels, and that effect lasted for 2–3 months of testing to date. Oral cocaine self-administration in monkeys was also reduced to about half of baseline levels for several weeks to 2 months. Initial data with monkeys was obtained with low CocH doses, but higher doses are currently being tested. These results suggest that the CocH vector may be a viable option to treat cocaine addiction in humans. Supported by DP1 DA031340 (SB) and RO1 DA002486 (MEC).

Keywords: effective treatment cocaine abuse viral vector-delivered enzyme CocH rats monkeys.

Disclosures: M. Carroll, Nothing to Disclose; N. Zlebnik, Nothing to Disclose; Y. Gao, Nothing to Disclose; S. Brimijoin, Ph.D., Nothing to Disclose.

W154. The Neurokinin-1 Receptor Mediates Stress-induced Reinstatement to Alcohol and Cocaine Seeking

Jesse R Schank*, Courtney King, Kejun Cheng, Kenner C Rice, David Weinshenker, Jason P Schroeder, Markus Heilig

NIAAA/NIH, Bethesda, Maryland

Background: Substance P (SP) and its preferred neurokinin-1 receptor (NK1R) mediate reward for alcohol and opiates in rodents, and play an important role in stress and anxiety-related behaviors. We have shown that NK1R antagonism inhibits footshock stress-induced reinstatement of alcohol seeking in non-dependent animals and suppresses escalated alcohol self-administration in alcohol preferring rats. To date, no studies have indicated a direct role for NK1R in cocaine reward or reinforcement. However, some work has suggested that intracranial SP infusion can trigger reinstatement of cocaine seeking following extinction.

Methods: Here, we explored the effect of the NK1R antagonist L822429 on yohimbine-induced alcohol and cocaine seeking. For these experiments we used operant self-administration followed by extinction and reinstatement procedures. Yohimbine is an alpha-2 adrenergic antagonist that is considered to be a pharmacological stressor. Yohimbine triggers reinstatement of drug seeking following extinction and induces escalated alcohol self-administration.

Results: In our studies, we found that L822429 suppresses the escalation of alcohol self-administration that is induced by yohimbine pretreatment and attenuates yohimbine-induced reinstatement of alcohol seeking after extinction. The latter finding parallels the effects of NK1R antagonists on footshock-induced reinstatement of alcohol seeking. We

also observed a suppression of yohimbine-induced reinstatement of cocaine seeking by L822429 at a dose that had no effect on baseline cocaine self-administration or self-administration of saccharin.
Conclusions: To our knowledge, this is the first demonstration that an NK1R antagonist can affect cocaine seeking behavior. Our findings suggest that the NK1R influences stress-induced seeking of both alcohol and cocaine. It is hypothesized that this role in stress-elicited reinstatement of drug seeking would extend to other classes of drugs, such as opiates. Future experiments will explore this, and will attempt to determine the neuroanatomical locus that mediates the role of NK1R on stress -induced drug seeking.
Keywords: stress, reinstatement, neurokinin, cocaine, alcohol.
Disclosures: J. Schank, Nothing to Disclose; C. King, Nothing to Disclose; K. Cheng, Nothing to Disclose; K. Rice, Nothing to Disclose; D. Weinshenker, Nothing to Disclose; J. Schroeder, Nothing to Disclose; M. Heilig, Nothing to Disclose.

W155. Chronic Nicotine Treatment Differentially Alters the Discriminative Stimulus Effects of Nicotine and Varenicline in Rhesus Monkeys

Colin S Cunningham, Lance McMahon*

UT Health Science Center, San Antonio, Texas

Background: The clinical effectiveness of a low efficacy agonist treatment for drug dependence is proposed to result from partial substitution for and antagonism of the effects of the dependence-inducing drug. The nicotinic acetylcholine receptor (nAChR) agonist varenicline (Chantix) has lower efficacy (intrinsic activity) than nicotine at receptor subtypes implicated in the abuse and dependence liability of nicotine. Under conditions of limited nicotine treatment, ie, rhesus monkeys ($n = 5$) discriminating 1.78 mg/kg of nicotine base, varenicline was able to fully mimic the effects of nicotine. Nicotine and varenicline were equipotent, had a similar duration of action, and the discriminative stimulus effects of both were antagonized by mecamylamine (Cunningham *et al*, 2012). Under these conditions, the desired profile of a low efficacy agonist was not apparent. However, drug history is expected to be an important determinant of the effects of low *vs* high efficacy agonists.
Methods: In the current study, the effects of nicotine and varenicline were examined under conditions expected to be more predictive of clinical use, ie, heavy cigarette smoking. A second group of rhesus monkeys ($n = 5$) was trained to discriminate 1.78 mg/kg of nicotine base; however, these monkeys received nicotine base (8.9 mg/kg) daily after discrimination sessions.
Results: Nicotine dose-dependently increased drug-appropriate responding and the potency of nicotine was not significantly different from that obtained in monkeys discriminating nicotine without chronic nicotine treatment. In contrast, in the chronic nicotine treatment group, varenicline was no longer able to mimic the effects of nicotine (maximum 28% nicotine-lever responding) up to a dose that disrupted behavior. When varenicline and nicotine were combined, the potency of nicotine was increased. To examine the extent to which chronic nicotine treatment resulted in tolerance, the hypothermic effects of nicotine were assessed. Nicotine base (3.2 mg/kg) significantly decreased rectal temperature by 1°C in monkeys not receiving chronic nicotine, but did not significantly alter rectal temperature in the chronic nicotine treatment group.
Conclusions: Collectively, these results demonstrate that chronic nicotine treatment results in profound cross-tolerance to the nicotine-like discriminative stimulus effects of varenicline but not the behaviorally disruptive effects of varenicline. That cross-tolerance to varenicline was greater than tolerance to nicotine is highly consistent with the prediction of receptor theory that a loss of sensitivity to a low efficacy agonist is greater than loss of sensitivity to a high efficacy agonist. However, these data suggest that any partial nicotine-like effects of varenicline in cigarette smokers are due to administration of relatively small doses to avoid side effects, as opposed to low nAChR efficacy. Moreover, there is no evidence from this study that varenicline antagonizes the effects of nicotine. Supported by USPHS grant DA25267.
Keywords: nicotine varenicline partial agonist substitution therapy Chantix drug discrimination rhesus monkey.
Disclosures: C. Cunningham, Nothing to Disclose; L. McMahon, Nothing to Disclose.

W156. Antidepressant-like Effects of Buprenorphine are Primarily Mediated Through the Kappa Opioid Receptor

Edgardo Falcon*, Irwin Lucki

University of Pennsylvania, Philadelphia, Pennsylvania

Background: Major Depressive Disorder (MDD) is one of the leading causes of disability worldwide. The limited therapeutic effectiveness and long latency to onset of current antidepressant medications support the need to develop newer and more efficacious drugs. Buprenorphine (BPN) is a drug of mixed pharmacology, with actions as a high affinity partial agonist at *mu*-opioid receptors (MORs) and antagonist at *kappa*-opioid receptors (KORs) (Lufty *et al*, 2004). In a limited number of clinical studies, BPN has been shown to produce antidepressant effects in treatment-resistant patients (Bodkin *et al*, 1995; Nyhuis *et al*, 2008). Few studies have been carried out with BPN using antidepressant and anxiolytic-like responses in rodents. The goal of this study was to identify the mechanisms associated with the antidepressant-like effects of BPN using the forced swim test (FST) in C57BL/6J mice. We examined BPN's effects in the FST in mice following pharmacological blockade of *kappa* opioid receptors and in mice with genetic deletion of MORs or KORs to determine the role of these opioid receptors in the antidepressant-like behavioral effects of BPN.
Methods: Male C57BL/6J mice (8 weeks old) were purchased from Jackson Laboratories. *Oprk1*−/− mice and *Oprm1*−/− mice were bred and genotyped in our laboratory. Buprenorphine (0.125–0.5 mg/kg) was provided by the NIH (RTI International). Nor-BNI (10 mg/kg) was purchased from Tocris Biosciences. All drugs were injected i.p. at a volume of 10 ml/kg. Mice were subjected to the forced swim test (FST), a behavioral test in mice that is sensitive to clinically effective antidepressants, as described previously (Lucki *et al*, 2000). Briefly, mice were injected with BPN and tested

2 h post-treatment to avoid complications from locomotor hyperactivity. Mice were placed in a cylinder (20 cm diameter) filled with water (15 cm deep) at room temperature (25 ± 1°C). Immobility scores were measured throughout a 6 min session from videotapes. Data is presented as immobility time during the last 4 min of the swim session. For the antagonist studies, animals were first injected with nor-BNI, followed 24 h later by BPN and the FST session 24 h post-BPN.

Results: BPN (0.25 and 0.5 mg/kg) significantly reduced immobility in the FST when tested 24 h post-injection. At this timepoint, BPN had no discernible effects in locomotor activity that might account for the behavior observed in the FST. To evaluate the role of *kappa* opioid receptors in the antidepressant-like response, we tested BPN in the FST after pretreatment with the KOR antagonist nor-BNI (10 mg/kg) and also in mice with genetic deletion of KORs (*Oprk1−/−* mice). Although pretreatment of mice with either nor-BNI or BPN alone reduced immobility, BPN failed to produce a further decrease of immobility in mice pretreated with nor-BNI. In addition, BPN failed to produce a reduction of immobility in the *Oprk1−/−* mice. In an effort to assess a role for *mu* opioid receptors in the antidepressant-like response, we tested BPN in *Oprm1−/−* mice. Interestingly, BPN (0.125 and 0.25 mg/kg) produced a greater reduction of immobility in the *Oprm1−/−* mice when compared to their WT littermates. In contrast, the KOR antagonist nor-BNI produced a similar reduction of immobility in the *Oprm1−/−* mice as that observed in WT mice.

Conclusions: Buprenorphine produced a significant reduction of immobility in C57BL/6J mice 24 h post-injection. Pretreatment of WT mice with a selective KOR antagonist recapitulated the antidepressant-like behavioral phenotype observed in the *Oprk1−/−* mice and blocked the behavioral effects of BPN. The results of this study suggest that a primary component mediating the antidepressant-like response produced by BPN in the FST is the *kappa* opioid receptor. In contrast, the antidepressant-like response to BPN was amplified in the *Oprm1−/−* mice. The reason for the hypersensitive response of *Oprm1−/−* mice to BPN is not known, and will be studied further along with the potential interaction of BPN with delta opioid or ORL receptors. These results in the mouse FST agree with previous reports on the potential of KOR antagonists as antidepressants. Furthermore, they support additional clinical testing of BPN for treatment-resistant depression because BPN is one of the few agents medically available that is an effective KOR antagonist.

Keywords: buprenorphine, depression, kappa receptors, animal models, forced swim test.

Disclosures: E. Falcon, Nothing to Disclose; I. Lucki, **Part 1:** Consultant for Alkermes.

W157. Antidepressant and Anti-inflammatory Properties in the Action of Agomelatine

Raffaella Molteni, Flavia Macchi, Andrea Carlo Rossetti, elisa colombo, Mario Dell'Agli, Marco A Riva, Giorgio Racagni*

University of Milan, Milan, Italy

Background: Agomelatine is a novel antidepressant molecule approved by the European Medicines Agency in 2009 and now registered in 88 countries, with a unique receptor profile different from all other antidepressants used acting as a MT1/MT2 melatonergic agonist and 5-HT2C receptor antagonist. The antidepressant properties of this drug have been shown in different animal models and rely on the ability to regulate several systems known to be altered in depression. Indeed, through a synergistic activity between melatonergic and 5HT2C receptors agomelatine regulates neurogenesis and neurotrophic-related mechanisms. In addition, agomelatine is able to resynchronize disturbed circadian rhythms which are modified in depressed patients. More recently, growing evidence suggests that the activation of the inflammatory/immune system contributes to depression pathogenesis. Accordingly, aim of this work was to evaluate the effect of agomelatine on systems that at molecular and functional level might be relevant for the relationship and mutual modulation of depression and inflammation.

Methods: Adult male rats received agomelatine (40 mg/kg, p.o.) or vehicle for 21 days before being challenged with an acute injection of lipopolysaccharide LPS (250 mg/kg; i.p.) 16 h after the last drug administration. Rats were sacrificed 2, 6, or 24 h after the inflammatory challenge, in order to establish the ability of agomelatine to interfere with the initial (2–6 h) or the later phase (24 h) of the inflammatory response. Real time PCR was used for gene expression analyses at central levels whereas ELISA was employed for protein analyses at central and plasma level.

Results: Our results demonstrate that agomelatine attenuates the inflammatory response induced by LPS injection, showing for the first time *in vivo* that chronic agomelatine treatment may affect different components of the immune/inflammatory system. Specifically, we found that agomelatine significantly reduced the LPS-induced up-regulation of pro-inflammatory cytokines (IL-1β and IL-6) in the brain as well as in periphery and was able to regulate microglia activation caused by the inflammatory challenge. Indeed, the antidepressant limited the LPS-induced up-regulation of the marker for this cellular phenotype CD11b, with a parallel increase of CD68, suggesting the induction of active phagocytosis that might contribute to the anti-inflammatory property of agomelatine. The attenuation of microglia activation by agomelatine involves neuron-glia cross-talk. Indeed, LPS reduced the expression of neuronal CX3CL1 thus leading to increased microglia activation, an effect normalized by agomelatine. In addition, we found that agomelatine was also able to alter basal and LPS-induced changes of the kynurenine pathway, which represents a point of convergence between inflammatory and neurotransmitter defects associated with depression. In particular, agomelatine prevented the LPS-dependent increase of KMO and was also able to reduce *per se* the expression of this enzyme. Given that KMO converts kynurenine to quinolinic acid, an N-Methyl-D-aspartate agonist, it is feasible to postulate that diminished KMO expression after agomelatine reduces NMDA receptor activation, which may also relevant for antidepressant properties. Preliminary data shown that agomelatine, acutely, is also able to modulate LPS-induced pro-inflammatory cytokines increase.

Conclusions: In summary, our results provide novel evidence on the ability of the antidepressant agomelatine to interfere with molecular systems involved in inflamma-

tory response. Since, in a translational perspective, inflammation may contribute to the development of depression in a significant number of patients and may be responsible for residual symptoms that impair or limit clinical remission, we suggest that the ability of agomelatine to modulate or interfere with immune/inflammatory system may represent an add-on value for its therapeutic activity.
Keywords: agomelatine; mood disorders; synergism; inflammatory response.
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W158. Agomelatine Treatment Induces Early and Time-dependent Modulation of Rat Hippocampal MiRNome

Daniela Tardito, Mara Seguini, Alessandra Mallei, Maurizio Popoli, Giorgio Racagni*

University of Milan, Milan, Italy

Background: MicroRNAs (miRNAs) are small non-coding RNAs that mediate post-transcriptional silencing of gene expression via the recognition by base-pairing of specific sequences in target messenger (mRNA). miRNAs regulate key processes in the central nervous system including development, homeostasis and neuroplasticity McNeill *et al*, (2012). Recently, dysregulation in miRNA expression was reported in different neuropsychiatric disorders, including major depression and a few studies have suggested a role for miRNAs in the action of mood stabilizers and antidepressants (Tardito *et al* (2013)). We therefore sought to determine whether treatment for 3 and 21 days with agomelatine, an antidepressant with a unique receptor profile as MT1/MT2 receptors agonist and 5-HT2C receptor antagonist Racagni *et al*, (2011) can affect the miRNome expression profile in rat hippocampus.
Methods: Adult male Sprague-Dawley rats (9 for group) were treated with vehicle or agomelatine (40 mg/kg/day i.p, two hours before dark phase) for 3 or 21 days. MiRNA expression analysis was carried out in total hippocampus by Quantitative Real Time PCR reactions by using TaqMan Array rodent MicroRNA A + B Cards Set v3.0 (Life Technologies). Statistical analysis was carried out with the Significance Analysis of Microarrays software, v.4.0, with FDR for multiple testing at $<5\%$. In order to identify miRNA putative target genes and molecular pathways potentially involved, bioinformatic analyses were performed. Meta-predictions based on integration, filtering and re-ranking of outputs produced by different target prediction tools was performed and followed by annotation analyses with Gene Ontology subcategories (Biological Processes, Molecular Function and Cellular Component), and KEGG pathways (exact Fisher test with $p<0.05$; and multiple testing correction).
Results: A total of about 400 miRNAs were detected in all samples (mean Ct value <35). The expression analysis showed that hippocampal miRNome was significantly modulated by agomelatine treatment. In particular, 3 days of treatment induced a marked effect by modifying the expression levels of 37 miRNAs with 7 up-regulated and 30 down-regulated, whereas 21 days of treatment regulated a lower number of miRNAs (increasing the expression of 4 and reducing that of 2). The bioinformatic analysis suggested that agomelatine treatment for 3 days could induce primary regulation of pathways involved in epigenetic mechanisms, inflammation, and morphological and functional neuroplasticity among the others. The same analysis on miRNAs regulated by 21 days of treatment highlighted the possible involvement of downstream mechanisms, mainly related to neuroplasticity and neurotransmission. Interestingly, several of the effector-coding genes have been previously associated to both depression pathophysiology and antidepressant mechanism of action, including agomelatine (ie VEGF, SNARE proteins, Bcl2/6, Erk1/2, glutamatergic receptors, Bmal1, Per2, Interleukins, MAP1b). Experiments are in progress in order to validate some of the putative identified targets and to compare the effects of agomelatine on hippocampal miRNome to those of classical antidepressants.
Conclusions: The results of the present work show for the first time that agomelatine induces time-dependent modifications in rat hippocampal miRNome expression profile. The main effect was found after 3 days of treatment thus suggesting that miRNAs could represent early mediators of agomelatine action. The bioinformatics analyses revealed that pathways involved in epigenetic mechanisms, inflammation and neuroplasticity could be affected by the modulated miRNAs. Although further work is needed to get further insight, these results suggest that miRNA modulation might be involved in the early effects of agomelatine.
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W159. Lurasidone Exerts Antidepressant Properties in the Chronic Mild Stress Model Through the Regulation of Synaptic and Neuroplastic Mechanisms in the Prefrontal Cortex

Marco A Riva*, Flavia Macchi, Mariusz Papp, Giorgio Racagni, Raffaella Molteni

University of Milan, Milan, Italy

Background: IIt is well established that stress exposure represents the major environmental contributor to the development of psychiatric conditions. Stress may indeed affect multiple systems that contribute to the dysfunction associated with mood disorders. With this respect, it is

important to establish to what extent long-term treatment with psychotropic drugs is able to normalize genes and proteins whose expression and function is altered by stress exposure in key brain regions. In the present study we have therefore investigated the antidepressant properties of the novel antipsychotic drug lurasidone in the chronic mild stress (CMS) model of depression and we tried to identify some of the molecular mechanisms through which lurasidone may exert its therapeutic activity.
Methods: Male Wistar rats were exposed to CMS for 2 weeks and sucrose consumption was used to distinguish between susceptible and non-susceptible animals. Control and CMS-susceptible rats were then randomized to receive chronic vehicle or the novel antipsychotic drug lurasidone (3 mg/kg/day) for 5 more weeks, while continuing the stress procedure, in order to evaluate the antidepressant properties and molecular changes set in motion by chronic drug treatment.
Results: After 2 weeks of CMS, we observed decreased expression of BDNF only in rats developing anhedonia, suggesting the contribution of the neurotrophin to the behavioral dysfunction produced by CMS. The down-regulation of BDNF expression persisted until the end of the stress procedure (7 weeks) in vehicle-treated rats, whereas chronic lurasidone treatment was able to revert the depressive-like behavior and normalized the BDNF mRNA levels in the prefrontal cortex of CMS rats. We also found that stressed rats display, within the prefrontal cortex, synaptic deficits, as shown by reduced expression of PSD-95, as well as glial pathology, with a significant reduction of GFAP mRNA levels, which may contribute to the overall neuroplastic impairment. Once again, the treatment with lurasidone was able to normalize the alterations produced by CMS.
Conclusions: Our results demonstrate that lurasidone show antidepressant properties in the CMS model and this may occur through the modulation synaptic and neuroplastic proteins as well as through the regulation of glial function. The adaptive changes set in motion by repeated treatment with lurasidone may contribute to the amelioration of functional capacities, closely associated with neuronal plasticity, which are deteriorated in patients with major depression and stress-related disorders.
Keywords: brain derived neurotrophic factor; astrocyte; adaptive mechanisms; antipsychotic drug; anhedonia.
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W160. Selective Orexin-2 Receptor Antagonism as Adjunctive Therapy for Insomnia in Depression

Timothy Lovenberg*, Jonathan Shelton, SuJin Yun, Pascal Bonaventure, Brock Shireman, Christine Dugovic

Janssen Pharmaceutical R&D, LLC, San Diego, California

Background: Depression is often associated with disturbances in sleep initiation and continuity that may be the result of insufficient reduction in activity of the wake-promoting system. Recently developed dual orexin-1 and orexin-2 receptor (OX1/2R) or selective OX2R antagonists have been shown to promote sleep in various species by inhibiting the output of wake active neurons, as opposed to zolpidem which promotes sleep by stimulating sleep active neurons via enhanced GABA signaling. While selective blockade of OX2R seems to be sufficient to initiate and prolong sleep, the beneficial effect of additional inhibition of OX1R remains controversial. We investigated the effects of additional blockade of OX1R in the presence of OX2R antagonism specifically on REM sleep since REM sleep alterations in depressed patients are characterized by a shortened latency to REM sleep associated with an increase in REM sleep during the first part of the night.
Methods: Male Sprague-Dawley rats were implanted with telemetric devices for EEG/EMG recordings. Two separate groups of animals received the dual OX1/2R antagonist SB-649868 (10–30 mg/kg po) or the OX1R antagonist GSK-1059865 (10 mg/kg sc) in combination with the OX2R antagonist JNJ-10397049 (10 mg/kg sc) at dark onset. Sleep-wake parameters were analyzed during the subsequent 12-h dark phase.
Results: As expected, the dual OX1/2R antagonist SB-649868 was effective in promoting NREM and REM sleep. However, a disruption of REM sleep was evidenced by a more pronounced reduction in the onset of REM as compared to NREM sleep, a marked enhancement of the REM/total sleep ratio and the occurrence of a few episodes of direct wake to REM sleep transitions (REM intrusion). The OX2R antagonist JNJ-10397049 increased NREM duration whereas the OX1R antagonist GSK-1059865 did not alter sleep. REM sleep was not affected either by OX2R or OX1R blockade alone, but administration of the OX1R antagonist in combination with the OX2R antagonist induced a significant reduction in REM sleep latency and an increase in REM sleep duration at the expense of the time spent in NREM sleep.
Conclusions: These results indicate that additional blockade of OX1R to OX2R antagonism elicits a dysregulation of REM sleep by shifting the balance in favor of REM sleep at the expense of NREM sleep. This may exacerbate REM sleep disinhibition (advanced REM sleep latency and increased REM sleep duration) in depressed patients. Translation of this hypothesis remains to be tested in the clinic.
Keywords: orexin sleep insomnia.
Disclosures: T. Lovenberg, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2:** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4:** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC; J. Shelton, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2:** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4:** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC; S. Yun, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC; P. Bonaventure, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2:** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4:** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC; B. Shireman, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2:** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4:** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC; C. Dugovic, **Part 1:**

Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC.

W161. Selective Blockade of 2-arachidonoylglycerol Hydrolysis Affects Learning and Memory Performance While Slowing Down Epileptogenesis in Rodents

Guy Griebel*, Philippe Pichat, Sandra Beeské, Bruno Biton, Dominique Françon, Richard Alonso, Dmitri Wiederschain, Heike Arlt, Bingzhi Zhang, Patrick Avenet, George F Koob, Johanna Escoubet

Sanofi, Chilly-Mazarin, France

Background: Endocannabinoids (eCBs) play a key neuromodulatory role in the central nervous system, regulating appetite, cognition, emotion, mood and pain by activation of cannabinoid (CB1) receptors. Alterations in the eCB system has been associated with disease in several major therapeutic areas. In particular, changes in tissue concentrations of their natural lipid ligands, N-arachidonoylethanolamine (anandamide) and 2-arachidonoyglycerol (2-AG) have been observed in neurological and psychiatric disorders, observations which have fueled considerable pharmaceutical interest in developing eCB-manipulating drugs to treat these conditions. These include compounds that act at CB1 receptors or inhibit anandamide degradation by fatty acid amide hydrolase (FAAH). Much less attention focused on the identification of drugs that modify 2-AG levels via manipulation of the serine hydrolase monoacylglycerol lipase (MAGL), its principal degradative enzyme. Here we report the pharmacological profile of a potent and selective MAGL inhibitor, Compound A.
Methods: MAGL selectivity and activity of Compound A were determined in *in vitro* and *ex vivo* biochemical assays, followed by a pharmacokinetic study to measure its brain exposure in mice. The effects of Compound A were then evaluated using rodent models with a focus on cognition and epilepsy tests since there is recent evidence that the modulation of 2-AG levels affects cognitive processes and seizure activity.
Results: Compound A behaves as a highly selective and competitive reversible inhibitor of mouse and human MAGL (IC50 = 29 and 3.8 nM, respectively). It does not affect the activities of other human serine hydrolases or interact with a panel of selected kinases, neurotransmitter transporters, ion channels and receptors, including the binding of a high-affinity ligand to CB1 and CB2 receptors. *Ex vivo* assays confirmed the degree of selectivity across eCBs as Compound A decreased MAGL activity (ID50 = 2.7 mg/kg po), while increasing levels of 2-AG (MED = 3 mg/kg po) in the absence of effect on other serine hydrolase substrates. Compound A demonstrated excellent brain permeability in the mouse (brain/plasma 4.3 at 10 mg/kg po). In a preliminary experiment, Compound A was found to decrease *in vitro* long term potentiation in rat hippocampus. This finding is in line with those of behavioral experiments showing that Compound A affected learning performance in the novel object recognition task (NOR), the Y-maze (YM) and the Morris water maze test, suggesting alterations in episodic, working and spatial memory. The effects of Compound A in the NOR and YM were antagonized by rimonabant suggesting that they were mediated by CB1 receptors. It is noteworthy that no tolerance to the effects of the drug was observed in the latter test upon repeated administration for 5 days. Interestingly, the effects of Compound A in the NOR were similar to those observed following genetic deletion of MAGL, supporting further the role of 2-AG in the intrinsic modulation of cognitive processes. In acute seizure tests in mice, Compound A was inactive over a wide dose-range in the 6-Hz model and after the administation of the convulsants pentylenetetrazole and kainate. However, in the mouse corneal kindling model of partial epilepsy, repeated administration of Compound A for 2 weeks delayed the acquisition and decreased the expression of kindled seizures, suggesting antiepileptogenic and anticonvulsant activities.
Conclusions: These findings demonstrate that selective pharmacological or genetic blockade of 2-AG hydrolysis affects memory performance, suggesting that MAGL inhibitors may be of limited utility as therapeutic agents for CNS disorders. However, it cannot be totally excluded that they may serve to treat psychopathologies hallmarked by an inability to extinguish maladaptive behaviors, such as post-traumatic stress syndrome and obsessive-compulsive disorder. Finally, our study reveals a previously unsuspected role of 2-AG in epileptogenesis process, a finding which deserves further investigation to determine the therapeutic potential of MAGL inhibitors as antiepileptic drugs.
Keywords: Endocannabinoids, learning, memory, epilepsy, monoacylglycerol lipase.
Disclosures: G. Griebel, **Part 5:** Sanofi; P. Pichat, **Part 5:** Sanofi; S. Beeské, **Part 5:** Sanofi; B. Biton, **Part 5:** Sanofi; D. Françon, **Part 5:** Sanofi; R. Alonso, **Part 5:** Sanofi; D. Wiederschain, **Part 5:** Sanofi; H. Arlt, **Part 5:** Sanofi; B. Zhang, **Part 5:** Sanofi; P. Avenet, **Part 5:** Sanofi; G. Koob, Nothing to Disclose; J. Escoubet, **Part 5:** Sanofi.

W162. Behavioral Effects of the Cannabinoid CB1 Receptor Negative Modulator ORG27569 in Rats

Yuanyuan Ding, Yanyan Qiu, Yanan Zhang, Jun-Xu Li*

University at Buffalo, Buffalo, New York

Background: Blockade of the cannabinoid CB1 receptor signaling is implicated in energy homeostasis and psychiatric disorders and the CB1 receptor antagonist/inverse agonist Rimonabant® was used clinically for treating obesity. However, its serious side effects (eg, depression) led to forced withdrawal from the clinic. Recently, a new CB1 receptor modulating site has been described which may achieve functional CB1 receptor antagonism without directly inhibiting CB1 receptor signaling. Such a strategy might be able to retain similar therapeutic potential as orthosteric CB1 receptor antagonists such as Rimonabant® but with better safety profile. However, no *in vivo* functional studies exist to characterize the pharmacological effects of CB1 receptor modulators. This study examined the behavioral effects of a purported CB1 receptor negative modulator ORG27569 in rats.
Methods: Different groups of rats were used to evaluate the hypothermic and cataleptic effects induced by the synthetic CB1 receptor agonist CP55940 and the endogenous CB1 receptor agonist anandamide, alone or in combination with ORG27569. Separate groups of rats were used to examine the

observational effects (grooming and scratching behaviors) induced by the CB1 receptor antagonist/inverse agonist rimonabant alone or in combination with ORG27569. Animals used in these studies were maintained in accordance with the Institutional Animal Care and Use Committee, University at Buffalo, and the Guide for the Care and Use of Laboratory Animals(8th edition, Institute of Laboratory Animal Resources on Life Sciences, National Research Council, National Academy of Sciences, Washington DC).
Results: CP55940 (0.1–1 mg/kg) and anandamide (3.2–32 mg/kg) dose-dependently and markedly decreased the rectal temperature in rats, with varied duration of action. When studied alone, ORG27569 had no effect on the rectal temperature. However, ORG27569 (3.2 and 10 mg/kg) markedly antagonized CP55940- and anandamide-induced hypothermic effects. CP55940 (0.032–1 mg/kg) produced marked cataleptic effects. Although rimonabant markedly antagonized the cataleptic effects of CP55940, ORG27569 did not alter such an effect. Rimonabant dose-dependently increased the frequency of grooming and scratching behaviors in rats. ORG27569 alone did not increase the frequency of these observational behaviors and did not alter the potency of rimonabant in this assay.
Conclusions: ORG27569 attenuates the hypothermic but not the cataleptic effects induced by CB1 receptor agonists. ORG27569 did not alter the observational behaviors induced by CB1 receptor antagonists. This effect was likely achieved through negative allosteric modulation of the CB1 receptors because ORG27569 does not bind to the orthosteric binding site but has high affinity at a recently described CB1 receptor allosteric modulating site and has been shown to decrease the maximal effects of CB1 receptor agonists in an *in vitro* binding assay. These data extend the preliminary observations by confirming that ORG27569 is a CB1 receptor negative modulator and can function as a CB1 receptor antagonist *in vivo*. To the extent that ORG27569 has differential antagonism on CB1 receptor agonist-induced behavioral effects, a better understanding of the antagonism profiles is warranted which may eventually lead to useful therapeutic actions via biased CB1 receptor blockade.
Keywords: CB1 receptor negative modulators, hypothermia, catalepsy, observational behavior, rats.
Disclosures: Y. Ding, Nothing to Disclose; Y. Qiu, Nothing to Disclose; Y. Zhang, Nothing to Disclose; J. Li, Nothing to Disclose.

W163. Discovery and Characterization of a G Protein-biased Agonist That Inhibits β-arrestin Recruitment to the D2 Dopamine Receptor

David R Sibley*, R Benjamin Free, Lani Chun, Amy Moritz, Brittney Miller, Trevor Doyle, Jennie Conroy, Adrian Padron, Julie Meade, Jingbo Xiao, Yang Han, Lihua Duan, Marc Ferrer, Jonathan Javitch, Noel Southall, Juan Marugan

NINDS, Bethesda, Maryland

Background: The D2 dopamine receptor is a highly validated drug target in neurology and psychiatry. For instance, all receptor-based antiparkinsonian drugs work via stimulating the D2 receptor, whereas all FDA-approved antipsychotic agents are antagonists of this receptor. However, most drugs targeting the D2 receptor are problematic as they are either less efficacious than desired or possess adverse side effects due to either cross reactivity with other receptors or activation of non-desired D2 receptor-mediated signaling pathways. A novel approach for attaining greater selectivity of therapeutic agents targeting the D2 receptor is to identify and develop ligands that exhibit functional selectivity, or biased agonism. In contrast to most agonists, which activate all signaling pathways in parallel with equal efficacy, functionally biased agonists favor one transduction pathway over another. Here we report the discovery of a novel D2 receptor agonist that is fully biased for G-protein-linked signaling while having no ability to activate β-arrestin-based signaling. We have also generated preliminary structure-activity relationship (SAR) data for this compound.
Methods: We performed a high throughput-screening (HTS) campaign to interrogate a 380 000 + small molecule library to identify novel D2 dopamine receptor agonists. The primary HTS assay utilized a cell line expressing the D2 receptor coupled to a chimeric Gqi5 protein, thereby linking receptor activation to robust Ca2 + mobilization. We also conducted secondary assays to measure orthogonal D2 receptor signaling activities including inhibition of cAMP accumulation (G protein-linked) and stimulation of β-arrestin recruitment (non-G protein-linked).
Results: The primary HTS screen resulted in the identification of 2288 compounds with agonist activity. While the majority of the confirmed hit agonist compounds activated all subsequent signaling pathways tested, some compounds showed a diminished ability to stimulate β-arrestin recruitment to the D2 receptor. One such compound, MLS1547, was identified as a full agonist at D2 receptor-mediated G-protein-linked signaling (Ca2 + and cAMP assays). In contrast, the compound demonstrated no ability to activate β-arrestin recruitment (enzyme complementation-based as well as BRET-based assays), but instead *antagonized* dopamine-stimulated β-arrestin recruitment to the D2 receptor. Furthermore, the compound failed to stimulate β-arrestin recruitment to any other dopamine subtype (D1-D5). In an effort to characterize this chemical scaffold further, and identify a preliminary SAR that imparts this unique pharmacological profile, 20 analogs of MLS1547 were obtained or synthesized and analyzed in our assays. Interestingly, some of these analogs gained the ability to stimulate β-arrestin recruitment without losing G-protein signaling, thereby losing their functional selectivity. Other analogs, however, maintained their full G-protein signaling bias. From these data, a preliminary SAR for the functionally selective properties of MLS1547 was obtained.
Conclusions: MLS1547 is the first example of a G-protein-biased agonist of the D2 receptor. While activating G protein-mediated signaling, the compound fails to promote β-arrestin recruitment to the receptor. Rather, through occupancy of the receptor, MLS1547 functions as an antagonist of β-arrestin recruitment to the D2 receptor. Administration of compounds with this pharmacological profile to animals should stimulate G protein-based signaling of the D2 receptor while simultaneously inhibiting signaling through the β-arrestin pathway. Such compounds may also engender less arrestin-mediated receptor desensitization or internalization. Importantly, MLS1547 appears to be a full agonist at G protein-mediated signaling outputs for the D2 receptor. Functionally selective

probes, such as MLS1547, will help to elucidate the specific roles of D2 receptor signaling pathways in normal physiology and behavior and also elucidate their involvement in the therapeutic effects of various pharmaceutical agents.

Keywords: dopamine receptor functional-selectivity biased-agonism cAMP β-arrestin D2.

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W164. Morphine-induced Conditioned Place Preference and Effects of Morphine Pre-exposure in Adolescent and Adult Mice

Wouter Koek*

UT Health Science Center, San Antonio, Texas

Background: Given evidence for age-related differences in the effects of drugs of abuse, surprisingly few preclinical studies have explored effects of opioids in adolescents (*vs* adults). The present study compared conditioned rewarding effects of morphine, without (experiment 1) and with morphine pre-exposure (experiment 2), in adolescent and adult mice.

Methods: Experiment 1: on each of three consecutive days, one of the two conditioning sessions was preceded by an injection of morphine (0.1–100 mg/kg, i.p.) and the other by saline; place preference was tested on day 4. Experiment 2: mice received once daily injections of saline or morphine (17.8–56 mg/kg) for 4 days, and 3 days later, place conditioning with morphine (0.32–10 mg/kg) began.

Results: In both experiments, morphine induced conditioned place preference along similar inverted U-shaped dose-response curves in adolescent and adult mice, with maximal effects between 0.32–10 mg/kg. Morphine pre-exposure did not sensitize morphine-induced conditioned place preference; instead, tolerance occurred, but only in adults. Adolescents were more sensitive than adults to morphine-induced locomotor stimulation, and were less sensitive to the body weight-decreasing effects of intermittently administered morphine (32–100 mg/kg).

Conclusions: The rewarding effects of morphine were similar in adolescent and adult mice, but showed differential tolerance after morphine pre-exposure. In agreement with previous findings, adolescents were more sensitive than adults to the acute locomotor stimulating effects of morphine, consistent with overactivity of dopamine systems during adolescence, and were less sensitive to a withdrawal-related effect of morphine, consistent with evidence that adolescents exhibit less withdrawal with several drugs of abuse.

Keywords: morphine conditioned place preference withdrawal adolescence mouse.

Disclosures: W. Koek, Nothing to Disclose.

W165. Therapeutic Potential of Selective Orexin-1 Receptor Antagonists

Pascal Bonaventure*, Diane Nepomuceno, Brian Lord, Leah Aluisio, Ian Fraser, James R Shoblock, Tamara Berdyyeva, Brock Shireman, Christine Dugovic, SuJin Yun, Jonathan Shelton, Nicholas Carruthers, Timothy Lovenberg

Janssen Pharmaceutical R&D, LLC, San Diego, California

Background: Over the last 15 years, interest in the orexin (hypocretin) system, which plays a prominent role in the maintenance of wakefulness, has led to at least four dual orexin receptor antagonists entering human trials for the treatment of sleep related disorders. The orexin-1 receptor and orexin-2 receptors (OX1/2R) are co-located or selectively located in unique areas of the CNS suggesting differentiated roles. OX1Rs are selectively expressed in the bed nucleus of the stria terminalis, amygdala, cingulate cortex and locus coeruleus. Conversely, OX2Rs are exclusively in histaminergic neurons in the tuberomammillary nuclei and play a critical role in wake promotion. Previously we have shown that antagonism of the OX2R is sufficient to initiate and prolong sleep in rodents. Evidence has accumulated that demonstrate the involvement of the OX1R in reward pathways associated with drug dependence. The role of OX1Rs in more complex emotional behavior (panic, anxiety) is also emerging. Recent studies have demonstrated that orexin and glutamate interact at the synaptic level and that orexin facilitates glutamatergic actions. There is evidence for the overactivation of the OX1R pathway in hyper-excited or hyper-active states, and consequently a selective OX1R antagonist might normalize overexcited networks without inducing sedation. In order to further validate the role of the OX1 receptor, characterization of improved tool compounds in animal models is required.

Methods: The affinity and potency of OX1R for Compound 1 (5-Bromo-N-({1-[(3-fluoro-2-methoxyphenyl)carbonyl]-5-methylpiperidin-2-yl}methyl)pyridin-2-amine) and Compound 2(N-({3-[(3-Ethoxy-6-methylpyridin-2-yl)carbonyl]-3-azabicyclo[4.1.0]hept-4-yl}methyl)-5-(trifluoromethyl)-pyrimidin-2-amine) has been determined by radioligand binding and *in vitro* functional assays. Receptor occupancy in the rat brain was determined using *ex vivo* autoradiography. Neurochemistry experiments monitoring levels of glutamate in prefrontal cortex of freely moving mice were performed using enzyme coated biosensors. Effects on sleep/wake stages in rats were measured using EEG/EMG.

Finally, the effect of pharmacological blockade of the OX1R was tested in a model of psychological stress (cage exchange and social interaction).
Results: Compound 1 and 2 are high affinity potent selective OX1R antagonists. Both compounds are highly selective *vs* the OX2R and a panel of over 50 different receptors. *Ex vivo* receptor binding studies demonstrate that after systemic administration of low doses, both compounds cross the blood brain barrier and occupy OX1Rs in rat brain. In wild-type mice, pharmacological blockade of OXR1 (Compound 1) significantly attenuated excessive cortical glutamate release elicited by the non-selective NMDA antagonist MK-801 (~50%). Mice lacking the OX1R had a blunted glutamate release response to MK-801 compared to the wild-type control. MK-801 still induced an increase in glutamate release in OX1R knockout mice but this increase in glutamate release was about half of the release observed in wild type animals, consistent with the pharmacological data. In agreement with previous studies, Compounds 1 and 2 had no effect on spontaneous sleep. OX1R blockade (Compound 1) attenuated the NREM promoting effect of an OX2R antagonist by increasing REM sleep duration and reducing REM latency. In a model of psychological stress, Compound 2 was found to reverse prolongation of sleep onset (NREM and REM latency) induced by cage exchange without affecting the sleep duration. The compound was shown to reverse the disruption of social interaction induced by bright light.
Conclusions: Compounds 1 and 2 are selective OX1R antagonists with an excellent selectivity profiles. Both compounds engaged the OX1 receptor in rodent brain after systemic administration. Our neurochemical data indicates that the OX1R interferes with glutamate system function in cortex and suggest that selective OX1R antagonist may normalize overactive networks and thus may represent a novel therapeutic strategy for the treatment of various psychiatric disorders. Reversal of the effects of a mild psychological stress using a selective OX1 antagonist is in agreement with this hypothesis.
Keywords: orexin, glutamate, panic.
Disclosures: P. Bonaventure, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2:** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4:** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC; D. Nepomuceno, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2:** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4:** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC; B. Lord, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2:** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4:** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC; L. Aluisio, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2:** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4:** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC; I. Fraser, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2:** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC; J. Shoblock, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2:** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4:** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC; T. Berdyyeva, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2:** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4:** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC; B. Shireman, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2:** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4:** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC; C. Dugovic, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2:** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4:** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC; S. Yun, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4:** Janssen Pharmaceutical R&D, LLC, **Part 5** Janssen Pharmaceutical R&D, LLC; J. Shelton, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2:** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC; N. Carruthers, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2:** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4:** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC; T. Lovenberg, **Part 1:** Janssen Pharmaceutical R&D, LLC, **Part 2:** Janssen Pharmaceutical R&D, LLC, **Part 3:** Janssen Pharmaceutical R&D, LLC, **Part 4:** Janssen Pharmaceutical R&D, LLC, **Part 5:** Janssen Pharmaceutical R&D, LLC.

W166. Improved Neuritogenesis and Mitochondrial Dynamics by Levetiracetam Might Explain Cognitive Improvement in Brain Aging and Animal Models of Alzheimer's Disease

Walter E Mueller*, Davide Miano, Carola Schiller, Kristina Leuner

Johann Wolfgang Goethe-University, Frankfurt, Germany

Background: The antiepileptic levetiracetam (Lev) has been shown to improve hippocampal hyperactivity associated with Mild Cognitive Impairment (MCI) in patients and cognitive deficits in an animal model of Alzheimer's disease (AD). These effects have been explained by improvement of synaptic function but the mechanism has not yet been clarified, also effects on mitochondrial function seem to be involved.
Methods: Since loss of synapses and neurites associated with impaired mitochondrial function and dynamics (fission and fusion) are typical for brain aging and early AD, we assessed the effects of Lev on neurite outgrowth and mitochondrial parameters. Human neuroblastoma cells (SY5Y) and PC12 cells were used under conditions imitating aging and SY5Y cells expressing slightly higher beta-amyloid levels typical for very early stages of AD.
Results: These cells exhibit impaired neuritogenesis and mitochondrial dynamics already under baseline conditions and/or after treatment with rotenone. Lev at a concentration

as low as 20 micromolar improves neuritogenesis and mitochondrial dynamics in both cell lines following oxidative stress either induced by rotenone treatment (complex I inhibition) or by sodium nitroprusside (SNP). At similar concentrations levetiracetam also had beneficial effects on both parameters in SY5Y cells overexpressing beta-amyloid.
Conclusions: Improved neuroplasticity followed by improved neuronal communication might be associated with the beneficial effects of Lev on cognitive functions in Alzheimer patients and Alzheimer mice independent of its anticonvulsant properties.
Keywords: Alzheimer disease, mitochondrial dysfunction, neuritogenesis, levetiracetam.
Disclosures: W. Mueller, **Part 1:** Speaker honorarium and research grant by UCB; D. Miano, Nothing to Disclose; C. Schiller, Nothing to Disclose; K. Leuner, **Part 1:** Speaker honorarium and research grant by UCB.

W167. Fluoxetine Exposure During Adolescence Alters Responses to Aversive Circumstances in Adulthood

Sergio Iñiguez*, Vincent Vialou, Brandon L Warren, Lace Riggs, Mary Kay Lobo, Raisa Ahmed, Bryan Cruz, Eric Nestler, Carlos A Bolanos-Guzman

California State University, San Bernardino, California

Background: Little is known regarding the mechanisms underlying the neurobiological consequences of antidepressant exposure during adolescence. Here, we assessed for long-lasting effects of adolescent exposure to Fluoxetine (FLX), a selective serotonin reuptake inhibitor, on behavioral reactivity to emotion-eliciting stimuli in adulthood.
Methods: We administered FLX (20 mg/kg/day) to male adolescent C57BL/6 mice (postnatal days 35–49), and assessed their behavioral reactivity to the social defeat procedure, forced swim test (FST), and the elevated plus-maze (EPM), 21 days after drug administration. We also examined the role of extracellular signal-regulated kinase (ERK1/2)-signaling within the ventral tegmental area (VTA) in mediating the FLX-induced behaviors using a virus-mediated gene transfer approach.
Results: Repeated exposure to FLX induced a decrease in sensitivity to the negative behavioral consequences resulting after exposure to the social defeat procedure and the FST (ie, antidepressant-like behavior), along with an increased sensitivity to the anxiety-eliciting environment of the EPM, in adulthood. FLX exposure during adolescence also resulted in decreased ERK2 mRNA expression within the adult VTA. To determine a causal relationship between ERK activity and behavior, we increased ERK2 levels, using viral vectors, in adult mice pretreated with FLX during adolescence. We found that increasing ERK2 levels within the VTA reversed the antidepressant-like effects observed after FLX exposure.
Conclusions: Exposure to FLX during adolescence results in enduring functional outputs indicative of decreased sensitivity to stress, while enhancing reactivity to anxiety-eliciting environments, in adulthood. This complex behavioral phenotype is mediated, at least in part, via decreases in ERK2 signaling within the VTA. Overall, these findings underscore the need for a better understanding of the effects of FLX exposure on the developing nervous system.
Keywords: Prozac, ERK, ventral tegmental area, juvenile, MAPK1.
Disclosures: S. Iñiguez, Nothing to Disclose; V. Vialou, Nothing to Disclose; B. Warren, Nothing to Disclose; L. Riggs, Nothing to Disclose; M. Lobo, Nothing to Disclose; R. Ahmed, Nothing to Disclose; B. Cruz, Nothing to Disclose; E. Nestler, Nothing to Disclose; C. Bolanos-Guzman, Nothing to Disclose.

W168. A Role for Innate Immune Signaling in Microglia in Behavioral Changes Induced by Repeated Social Defeat Stress in Mice

Xiang Nie, Shiho Kitaoka, Kohei Tanaka, Eri Segi-Nishida, Yuki Imoto, Atsubumi Ogawa, Shuh Narumiya, Tomoyuki Furuyashiki*

Medical Innovation Center, Kyoto, Japan

Background: Previous studies using rodent stress models have suggested that repeated stress causes microglial activation in multiple brain areas. However, a role for stress-induced microglial activation and its mechanism remain to be established. Recent evidence implicates innate immune molecules, such as Toll-like receptors (TLRs), in detecting endogenous damage-associated molecular pattern molecules (DAMPs) to evoke inflammation upon cellular damage or stress. Since TLR agonists can activate microglia *in vitro* and *in vivo*, we hypothesize that TLR signaling may be involved in microglial activation and behavioral changes induced by repeated stress.
Methods: We examined mRNA expression of several endogenous DAMPs in mouse brains before and after repeated social defeat stress by quantitative RT-PCR. We subjected male wild-type mice and mice lacking TLR of 8-week old to agonistic encounters from aggressor ICR mice for 10 min daily for 10 consecutive days, and examined the level of social avoidance from a novel ICR mouse and the level of anxiety-like behaviors in the elevated plus maze test. We also examined histological markers associated with microglial activation, such as increased Iba-1 immunoreactivity, and expression of c-Fos, a marker for neuronal response, upon stress exposure in wild-type and TLR-deficient mice. To examine microglial expression of innate immune molecules, we isolated fluorescently labeled microglia and non-labeled non-microglial cells from CX3CR1-EGFP mice before and after repeated stress, and subjected total RNA extracted from these cells to quantitative RT-PCR.
Results: Repeated social defeat stress increased mRNA levels of several, but not all, DAMPs that are known to activate TLR2 and TLR4 in the brain. To examine a role for these receptors in stress-induced behavioral changes, we subjected mice lacking either TLR2 or TLR4 to repeated social defeat stress, but these mice normally developed social avoidance after repeated stress. Given that TLR2 and TLR4 partly share signaling pathways, we examined mice lacking TLR2 and TLR4 in combination (TLR double knockout; TLR-DKO), and found that TLR-DKO mice failed to show social avoidance

and elevated anxiety-like behavior after repeated stress. mRNA expression of TLR2 and TLR4 was enriched in microglia, and stress-induced microglial activation was abolished in TLR-DKO mice. As neuronal correlate of behavioral changes, social defeat stress induced c-Fos expression in mPFC neurons, and this c-Fos induction was diminished with repetition of stress. TLR-DKO mice showed less attenuation of c-Fos expression in mPFC neurons after repeated stress, compared with wild-type mice.
Conclusions: Collectively, these results demonstrate that innate immune signaling through TLR2 and TLR4 is critical for microglial activation and mPFC dysfunction induced by repeated stress as well as concomitant behavioral changes. Given enriched expression of TLRs in microglia, our study suggests that TLR-mediated microglial activation induces functional alteration in the mPFC, thereby leading to behavioral changes. Our study also highlights innate immune signaling as a potential pharmaceutical target for stress-related pathophysiology in psychiatric disorders. Since repeated stress induces expression of several DAMPs that can activate TLR2 and TLR4 in the brain, it is plausible that TLR in microglia senses cellular damage through DAMPs released upon repeated stress. To identify a DAMP involved in stress-induced behavioral changes and the mechanism underlying stress-induced release of DAMPs remain for future investigation.
Keywords: mouse, stress, depression, microglia, Toll-like receptor.
Disclosures: X. Nie, Nothing to Disclose; S. Kitaoka, Nothing to Disclose; K. Tanaka, Nothing to Disclose; E. Segi-Nishida, Nothing to Disclose; Y. Imoto, Nothing to Disclose; A. Ogawa, Nothing to Disclose; S. Narumiya, Nothing to Disclose; T. Furuyashiki, Nothing to Disclose.

W169. Effects of Ethanol and Antidepressant on the Platelet BDNF Release Function in the Peripheral Blood: Implication in the Pathogenesis of Psychiatric Disease

Toshikazu Saito*, Eri Hashimoto, Wataru Ukai, Takao Ishii, Yoshiyasu Kigawa, Kengo Furuse, Hanako Tsujino

Sapporo Medical University, School of Medicine, Sapporo, Japan

Background: Recently, it has been elucidated that the alterations of neural circuit including synapse/neuro-genesis are the key mechanism of various clinical conditions of psychiatric diseases such as schizophrenia, depression, and alcohol related disorders, and the neurogenic and neurotrophic effects of psychotropics are considered to contribute to the prevention of morphological and functional brain change induced by those psychiatric diseases. In relation to these evidences, implication of brain-derived neurotrophic factor (BDNF) in the pathogenesis of these diseases has been also demonstrated. Previously, we have investigated the effect of psychotropic agent such as antidepressant and BDNF on the neurogenetic change induced by alcohol and endoplasmic reticulum (ER) stress, and alterations of trophic factor signaling and transcription factors, CREB and NRSF/REST play an important role in the neoruprotective/neurogenetic effects of these psychotropic agents. In addition, there are some reports demonstrated that serum BDNF levels in patients of these disorders are lower than control subjects, and antidepressant and antipsychotic treatment increases its serum levels in responder. However, its mechanism and biological meaning in the pathophysiology of schizophrenia, depression, and alcohol related problems have not yet understood.
Methods: In this study, we investigated the effect of antidepressant, antipsychotic, and ethanol on the BDNF release function of platelet from peripheral blood *in vitro*. We also analyzed the related cellular signaling using pharmacological inhibitors including trkB cascade, and alteration of BDNF release function using platelets derived from chronic corticotrophin-treated depression model rats. All experimental protocols in animal studies were approved by Animal Care and Use Committee of Sapporo Medical University and were conducted in accordance with the Sapporo Medical University Guide for the Care and Use of Laboratory Animals.
Results: In the *in vitro* study, it was indicated that ethanol significantly suppressed the platelet BDNF release which was promoted by antidepressant and antidepressant-induced BDNF release was inhibited by trkB blockade. In the *in vivo* study, it was demonstrated that serum BDNF was increased by the intravenous treatment of antidepressant and antidepressant-induced platelet BDNF release function was dramatically decreased in depression model rat.
Conclusions: These results suggested that alterations of trophic factor functions not only in the brain but also in the peripheral blood possibly underlies the pathogenetic mechanism of neural circuit repair and maintenance dysfunction of the brain damage in these psychiatric disorders, those might be the key role of psychotropic drugs for their clinical efficacies.
Keywords: ethanol, antidepressant, platelet, BDNF, blood.
Disclosures: T. Saito, **Part 4:** SHIONOGI PHARMA Inc., MOCHIDA PHARMACEUTICAL CO., LTD., Eli Lilly Japan K. K.; E. Hashimoto, Nothing to Disclose; W. Ukai, **Part 4:** SHIONOGI PHARMA Inc., MOCHIDA PHARMACEUTICAL CO., LTD., Eli Lilly Japan K. K.; T. Ishii, Nothing to Disclose; Y. Kigawa, Nothing to Disclose; K. Furuse, Nothing to Disclose; H. Tsujino, Nothing to Disclose.

W170. The M1 Muscarinic Receptor Subtype Regulates the Antidepressant-like Effects of the Rapidly-acting Antidepressant Scopolamine

Jeffrey M Witkin*, Carl Overshiner, John Catlow, Douglas Schober, Beverly Heinz, Alexander Nikolayev, Tolstikov Vladimir, Wesley Anderson, Richard Higgs, Kuo Ming-Shang, Christian Felder

Lilly Resarch Labs, Indianapolis, Indiana

Background: Scopolamine produces rapid improvement in patients with depression, in some cases in patients who do not respond to standard of care antidepressants. However, there has been little progress in understanding the mechanisms of action of scopolamine that are responsible for these effects. Scopolamine is a muscarinic receptor antagonist that displays a relatively similar affinity across all five muscarinic receptor subtypes (M1-M5). One major question addressed by the present series of studies was to

identify the receptor subtype(s) scopolamine might be blocking to trigger its fast-action and large effect size.
Methods: The approach we took to answer this question made use of a combination of pharmacological tools and mice without specific muscarinic receptor subtypes. The behavioral assay utilized in this study to measure an antidepressant phenotype was the mouse force swim test. Mice with genetic deletion of each muscarinic receptor subtype (M1-M5) were evaluated in the forced swim assay with scopolamine and several other muscarinic receptor antagonists. Behavioral effects were compared to those obtained in wild-type mice with the full complement of muscarinic receptors. Selectivity of the muscarinic antagonists was established in a binding assay utilizing [3H]-N-methylscolpolamine. Plasma from wildtype and M1 receptor −/− mice was also evaluated for multiple analytes to ascertain potential differences after scopolamine treatment.
Results: Scopolamine produced a robust antidepressant response in wild-type mice with minimal effective dose of 1 mg/kg, the quaternary analog, N-methyl-scopolamine, was without effect. Interestingly, mice without M1 or M2 receptors had a blunted response to scopolamine but not to the non-muscarinic anti-depressant imipramine in the forced-swim assay that detects standard of care antidepressants. Other muscarinic drugs with a preference for M1 (pirenzepine and VU 0255035) also demonstrated activity in the forced-swim test and these effects were deleted in mice without M1 receptors. As with the rapidly-acting anti-depressant ketamine, effects in the forced-swim assay were attenuated by blockade of AMPA receptors. Metabolomic analysis of plasma from mice given scopolamine revealed a significant 2-fold difference in ribose-5-phophate levels between M1 receptor −/− and wild-type mice.
Conclusions: Taken together, these data establish muscarinic M1 receptors as the likely target against which scopolamine interacts to engender antidepressant-like effects. The involvement of AMPA receptor facilitation to these behavioral effects of scopolamine add to the literature on fast-acting antidepressants (eg, ketamine) that signal through this receptor. The effects of scopolamine might be related to M1 receptor-directed modulation of pyridine neucleotide regulation of nervous system function.
Keywords: scopolamine antidepressant M1 muscarinic receptors forced swim mouse.
Disclosures: J. Witkin, Nothing to Disclose; C. Overshiner, Nothing to Disclose; J. Catlow, Nothing to Disclose; D. Schober, Nothing to Disclose; B. Heinz, Nothing to Disclose; A. Nikolayev, Nothing to Disclose; T. Vladimir, Nothing to Disclose; W. Anderson, Nothing to Disclose; R. Higgs, Nothing to Disclose; K. Ming-Shang, Nothing to Disclose; C. Felder, Nothing to Disclose.

W171. Adolescent Cannabis Exposure Differentially Affects Heroin Reinforcement and Accumbens Dopamine Transmission in Lewis and Fisher344 Rats

Gaetano Di Chiara*, Cristina Cadoni, Daniele Lecca, Sandro Fenu

University of Caligari, Cagliari, Italy

Background: Vulnerability to drug addiction depends on acquired as well as genetic factors (Swendsen and Le Moal, 2010). Among acquired factors is previous exposure to other drugs of abuse. Thus, exposure to Cannabis has been suggested to predispose to heroin abuse and dependence (Gateway Hypothesis) (Kandel *et al*, 2006). Previous studies have failed to provide evidence for increase in heroin reinforcement after cannabinoid pre-exposure (Solinas *et al*, 2004). However, Cannabis use often begins in adolescence, a critical phase of brain development. Indeed, Ellgren *et al* (2007) did report an increased taking of iv heroin after adolescent THC exposure and suggested a reduction of heroin reward. Genetic risk factors also play a major role in drug addiction. Given these premises, we thought to mimick more closely the human condition by introducing genetic background as a variable. Therefore we studied the influence of adolescent cannabinoid exposure on heroin reward and reinforcement and on *in vivo* dopamine stimulantion by heroin in two inbred rat strains differentially vulnerable to drugs of abuse, the addiction prone Lewis and the addiction resistant Fisher344 strain.
Methods: Male Fischer344 and Lewis inbred rats aged 6 weeks (38–42 postnatal day, mid adolescence, Charles River, Calco, Italy) were utilized. One group received for 3 days, twice daily, increasing doses of THC (2, 4 and 8 mg/kg i.p.) while the other received vehicle (3 ml/kg i.p.). All experiments were performed at least 30 days after last THC exposure. Microdialysis of DA was performed according to Cadoni *et al* (2009) in rats implanted with probes in the n.accumbens (NAc) shell and core. CPP was performed in a two compartment apparatus according to Fenu *et al* (2006). On day 1 (*pre-conditioning*), unconditioned preference was recorded for 15 min. On days 2–7 (*conditioning*) rats received heroin (0.5 mg/kg s.c.) or saline on alternate days and were confined to a given compartment for 30 min. Testing of place *preference* was performed for 15 min. On the extinction phase, rats were re-exposed to the apparatus for 6 times. For reinstatement, heroin (0.5 mg/kg) was given in the home cage and after 40 min rats were tested for place preference. After 21 days rats were retested for preference three times. Behavior was scored as time (sec) spent performing each behavioral item. The items recorded for extinction were: jumping, sniffing, gnawing, locomotion and rearing.; for reinstatement: stupor, licking, sniffing, gnawing and locomotion. Fixed ratio (FR) heroin iv SA was performed according to Lecca *et al* in 1 h daily sessions (except for weekends). Following acquisition on FR1, SA was switched to FR3 and then to FR5. After 29 FR sessions, progressive ratio (PR 3–4) was introduced for 13 sessions of 4 h each, followed by 8 extinction sessions. Finally, reinstatement was performed by presentation of drug-cues and, next day, by a priming injection of heroin (0.5 mg/kg sc). Cumulative active and inactive nose-pokes per session, heroin intake and breaking points per session were recorded and analyzed by ANOVA and *post-hoc* test.
Results: THC (1.0 mg/kg ip) stimulated shell DA in Lewis but not in Fischer 344 rats. Adolescent THC exposure potentiated DA stimulant effects of heroin (0.5 mg/kg sc) in the shell and core of Lewis and only in the core of Fischer344 rats. Control Lewis rats developed stronger CPP to heroin and resistance to extinction compared with Fischer344 strain. In Lewis rats, THC exposure did not increase heroin CPP but potentiated the effect of heroin priming. In Fischer344 rats, THC exposure increased heroin

CPP and made it resistant to extinction. Lewis rats showed marked seeking behavior during extinction and hedonic reactions on heroin priming. Fisher344 rats showed no seeking behavior during extinction and stereotypies on heroin priming. THC pre-exposure increased responding for iv heroin and heroin intake on FR3 and FR5 and on PR sessions and increased breaking points in Lewis but not in Fisher344 rats. After extinction, presentation of discriminative stimuli associated with drug availability as well as priming by passive heroin exposure, reinstated low levels of responding on the active lever in Lewis and in Fisher344 rats but pre-exposure to THC selectively increased reinstatement in the Lewis strain.
Conclusions: Adolescent THC pre-exposure potentiated heroin stimulatory effects on NAc shell and core DA of LEW rats and only in the core of F344 rats. In a CPP paradigm, adolescent THC pre-exposure also allowed the induction of reinstatement of preference for the heroin-paired compartment in Lewis but not in Fisher344 rats. Upon heroin priming, Lewis rats showed marked seeking behavior while Fisher344 rats showed typical sensitized stereotyped behavior. In a FR and PR iv SA paradigm, THC pre-exposure increased heroin reinforcement, as indicated by PR responding, only in Lewis rats. These observations suggest that, in genetically predisposed individuals, adolescent Cannabis exposure can increase vulnerability to heroin addiction by augmenting heroin reinforcing properties.
Keywords: THC heroin dopamine reinforcement addiction.
Disclosures: G. Di Chiara, Nothing to Disclose; C. Cadoni, Nothing to Disclose; D. Lecca, Nothing to Disclose; S. Fenu, Nothing to Disclose.

W172. Therapeutic Effects of TrkB Ligands on Depression-like Behaviors and Dendritic Changes in the Hippocampus and Nucleus Accumbens After Inflammation

Kenji Hashimoto*, Ji-Chun Zhang, Jin Wu, Qian Ren, Suxia Li, Yukihiko Shirayama

Chiba University Center for Forensic Mental Health, Chiba, Japan

Background: Brain-derived neurotrophic factor (BDNF)-TrkB signaling plays a central role in the therapeutic mechanisms of antidepressants. The purpose of this study is to examine whether 7,8-dihydroxyflavone (7,8-DHF; TrkB agonist) and ANA-12 (TrkB antagonist) could show antidepressant effects in depression-like behaviors and changes of spine density in mice after inflammation.
Methods: Twenty three hours after administration of lipopolysaccharide (LPS) or saline, vehicle, 7,8-DHF, or ANA-12 were administered. Behavioral tests, including locomotion, tail suspension test (TST) and forced swimming test (FST), were performed 1, 4, 6-h after drug administration of TrkB ligands, respectively. Furthermore, Western blot analysis of BDNF and Golgi staining were performed.
Results: Administration of 7,8-DHF or ANA-12 did not alter the locomotor activity in mice. In the TST and FST, 7,8-DHF attenuated an increase of the immobility time of LPS-treated mice, in a dose dependent manner. Antidepressant effects of 7,8-DHF was blocked by treatment with ANA-12. Surprisingly, ANA-12 alone showed antidepressant-like effects in LPS-treated mice. Furthermore, 7,8-DHF significantly attenuated LPS-induced reduction of BDNF and spines density in CA3 and dentate gyrus (DG), whereas ANA-12 significantly attenuated LPS-induced increase of BDNF and spine density in nucleus accumbens (NAc). Moreover, bilateral injection of ANA-12 into NAc showed antidepressant effects in LPS-treated mice.
Conclusions: This study shows that LPS-induced inflammation caused depression-like behavior, as well as alterations in BDNF protein and spine density within the hippocampus and NAc. The present results suggest that TrkB agonists could represent effective therapeutic drugs for depressive patients with decreased BDNF levels in the hippocampus, and that TrkB antagonists may act as therapeutic agents for patients, who show increased BDNF-TrkB signaling in NAc.
Keywords: BDNF; depression; inflammation; spine; TrkB.
Disclosures: K. Hashimoto, Nothing to Disclose; J. Zhang, Nothing to Disclose; J. Wu, Nothing to Disclose; Q. Ren, Nothing to Disclose; S. Li, Nothing to Disclose; Y. Shirayama, Nothing to Disclose.

W173. Chronic Methamphetamine-induced Recognition Memory Deficits are Associated with Impaired Long-term Depression and Decreased glun2b Surface Expression in the Perirhinal Cortex

Michael D Scofield, Heather Trantham-Davidson, Marek Schwendt, Ronald E See, Carmela M Reichel*

Medical University of South Carolina, Charleston, South Carolina

Background: Methamphetamine (meth) is one of the most prevalent drugs of abuse worldwide and can result in pronounced cognitive deficits in many users. Of these impairments, episodic memory is particularly at risk. In rats, we have demonstrated pronounced deficits in recognition memory following extended access to self-administered meth. Recognition for novelty is dependent on intact perirhinal cortex function. Since repeated presentation of familiar objects results in long-term depression (LTD) within the perirhinal cortex, we predicted that chronic meth might prevent the induction of LTD as the objects become recognizable. Further, we assessed surface expression of GluN2b receptors, since perirhinal LTD is dependent upon activation of this receptor.
Methods: Male Sprague-Dawley rats self-administered meth (0.02 mg/infusion, i.v.) on an FR1 schedule of reinforcement or received yoked saline infusions. After 7 daily 1-h sessions, rats were switched to 6-h daily access sessions for 14 days, and then underwent drug abstinence. On abstinence day 7, rats were tested for object recognition memory using a two-item object recognition task. Immediately after the test, rats were decapitated and tissue was used to evaluate LTD or surface expression of GluN2b in the perirhinal cortex.
Results: Rats escalated meth intake over the long access period. Predictably, only control rats spent more time interacting with the novel over the familiar object. In contrast, meth rats spent equal amounts of time with both objects, indicating a deficit in object recognition memory. GluN2b surface expression was reduced approximately 40%

in meth rats and LTD was absent in perirhinal slices of meth rats only. Specifically, 30 min after LTD induction in slices from control animals, the PS amplitude was reduced to 85.68 ± 2.65% of baseline amplitude. In contrast, the PS amplitude in slices from meth rats was similar to baseline (114.12 ± 1.4%), indicating an absence of LTD in this group.
Conclusions: Taken together, the consistency of results across the behavioral and cellular levels of analysis suggests a possibility that chronic self-administered meth impairs recognition memory by reducing GluN2b receptor mediated LTD in the perirhinal cortex. Further investigations will test this notion and determine whether these meth-induced changes in synaptic plasticity can be rescued via pharmacological manipulations *in vivo* and bath application of glutamate receptor agonists.
Keywords: methamphetamine, memory, glutamate, self-administration, LTD.
Disclosures: M. Scofield, Nothing to Disclose; H. Trantham-Davidson, Nothing to Disclose; M. Schwendt, Nothing to Disclose; R. See, Nothing to Disclose; C. Reichel, Nothing to Disclose.

W174. Methylphenidate Enhancement of Early-stage Sensory Signal Processing

Barry Waterhouse*, Rachel Navarra, Gerard Zitnik, Brian Clark

Drexel University College of Medicine, Philadelphia, Pennsylvania

Background: Methylphenidate (MPH) is a prescription stimulant used to treat attention deficit hyperactivity disorder (ADHD). The drug is also rapidly gaining popularity among healthy individuals for its ability to promote wakefulness, focus attention, and improve concentration. The off-label use of MPH by non-ADHD patients for performance enhancement raises significant health and ethical concerns. Extensive work has focused on the reinforcing effects and abuse liability of psychostimulants, but understanding the mechanisms through which these agents regulate neural circuit functions that govern cognitive and sensorimotor processes and result in performance enhancement has received less attention. Optimal detection of sensory information within complex, dynamic environments is critical for appropriate decision making and executive actions. As such, overall performance enhancement may significantly rely on more efficient processing of incoming sensory stimuli. MPH increases catecholamine neurotransmission through the blockade of dopamine and norepinephrine (NE) reuptake transporters. The ascending locus coeruleus (LC)-NE system regulates behavioral state and modulates state dependent transmission of sensory signals and low dose MPH has been shown to improve performance in sensory guided behavioral tasks. The rat dorsal lateral geniculate nucleus (dLGN) is the primary thalamic relay for visual information from the retina to the visual cortex and is densely innervated by fibers from the LC-NE system. The goal of the present study was to examine the effects of MPH and atomoxetine (ATX), a NE specific reuptake blocker, on visually evoked responses of single neurons in the rat dLGN.
Methods: Extracellular recordings using bundles of fine wires were made from the dLGN of anesthetized rats during presentation of variable intensity light stimuli (high, intermediate, low), before and after systemic administration of vehicle, MPH, or ATX. Peri-event histograms were generated from the spike train discharge of single neurons and used to measure the effects of MPH and ATX on the magnitude and timing of dLGN neuronal responses to light stimuli.
Results: Preliminary experiments in anesthetized animals identified the dose range and time course of MPH-mediated effects on dLGN neuronal responses to light stimuli. In these studies 2.0 mg/kg ip was identified as the optimal dose of MPH for altering the magnitude and timing of stimulus evoked discharges in dLGN neurons. Maximal drug effects were observed between 15–30 min post-drug. In a majority of cells tested ($n = 132$, 9 animals) in subsequent studies, both MPH (2.0 mg/kg, i.p.) and ATX (0.5 mg/kg, i.p.) increased the magnitude (90–140% over control) and decreased the latency (MPH only, avg = 1–2 msec shorter than control) of dLGN unit responses to light stimuli. In addition, 54% of cells that were unresponsive to low level light stimuli prior to drug treatment displayed a 'gating effect,' wherein prominent responses to these stimuli were evident following administration of either MPH or ATX. These effects were similar to those observed previously in noradrenergic terminal fields after local application of NE or LC electrical stimulation. Preliminary evidence further suggests that the facilitating effects of MPH were due to activation of $\alpha 1$ adrenergic receptors.
Conclusions: Acute administration of MPH or ATX produces noradrenergic-like modulatory effects in the visual thalamus of rat brain. Prior studies have shown that rodent performance in a visually guided sustained attention task is enhanced by the same dose of MPH (2.0 mg/kg, ip). Our working hypothesis is that the observed effects on early-stage visual signal processing are the result of blockade of NE reuptake and elevated extracellular levels of NE in the dLGN, and that these actions lead to net enhancement of sensory signal processing. Such alterations in sensory circuit perations may be a factor in the performance enhancing effects of MPH and therefore contribute to its desirability as a performance enhancing drug (PED).
Keywords: methylphenidate atomoxetine performance enhancement sensory signal processing norepinephrine.
Disclosures: B. Waterhouse, Nothing to Disclose; R. Navarra, Nothing to Disclose; G. Zitnik, Nothing to Disclose; B. Clark, Nothing to Disclose.

W175. Optogenetic Control of Central Serotonergic Neurons Affects Anxiety and Impulsivity

Yu Ohmura*, Kenji Tanaka, Iku Tsutsui-Kimura, Akihiro Yamanaka, Tomomi Tsunematsu, Mitsuhiro Yoshioka

Hokkaido University, Sapporo, Japan

Background: It has generally been thought that serotonin release in the forebrain attenuates anxiety and impulsivity. However, there is so far no direct evidence proving this hypothesis because there has been no method that

reversibly, selectively, and temporally-specifically controls serotonergic activity. Although there is extensive indirect evidence using pharmacological agents or electrolytic lesion, it is mixed. Therefore, in the present study, we aimed to obtaining direct evidence about the effects of acute serotonin release on anxiety and impulsivity using recently developed optogenetic tools.
Methods: We obtained transgenic mice expressing channelrhodopsin-2 (ChR2) mutant (C128S) only in central serotonergic neurons by crossing tetO-ChR2(C128S)-EYFP knock-in mice with Tph2-tTA BAC transgenic mice. We applied blue light to the median raphe nucleus (MRN) to open ChR2, and recorded behavioral changes. The elevated plus maze and dark/bright maze were used to assess anxiety, and the 3-choice serial reaction time task (3-CSRTT) was used to assess impulsivity.
Results: Blue light illumination to the MRN increased anxiety-like behavior in both the elevated plus maze and dark/bright maze tests without affecting locomotor activity. Moreover, the anxiogenic effects were reversed by the microinjection of 5-HT2C receptor antagonist (SB242084, 0.5 μg) to the ventral hippocampus. Optogenetic activation of serotonergic neurons in the MRN suppressed impulsive action without affecting other cognitive parameters in the 3-CSRTT.
Conclusions: Although the present results regarding serotonin and anxiety are inconsistent with generally accepted hypothesis, our findings could account for why increased anxiety is sometimes observed during the initial phase of treatment of selective serotonin reuptake inhibitors (SSRIs). Moreover, our findings support a recently suggested therapeutic strategy recommending the co-administration of 5-HT2C receptor antagonists with SSRIs because the blockade of 5-HT2C receptor abolished anxiogenic response induced by serotonergic activation. As for impulsivity, serotonergic activation reduced impulsive action, consistent with generally accepted hypothesis. Thus, serotonergic activation of the MRN enhanced anxiety but suppressed impulsivity. Whether the serotonergic neurons involving in anxiety are different from those controlling impulsivity should be elucidated in future studies.
Keywords: optogenetics; impulsivity; anxiety, serotonin; median raphe nucleus.
Disclosures: Y. Ohmura, Nothing to Disclose; K. Tanaka, Nothing to Disclose; I. Tsutsui-Kimura, Nothing to Disclose; A. Yamanaka, Nothing to Disclose; T. Tsunematsu, Nothing to Disclose; M. Yoshioka, Nothing to Disclose.

W176. Ketamine Is a Potent Antidepressant in Two Rodent Models of Depression

Aleksander Mathé*, Vasco Sousa, Christina Weide Fischer, Tiberiu Loredan Stan, Gregers Wegener, Andreas Lennartsson, Per Svenningsson

Karolinska Institutet, Stockholm, Sweden

Background: Mood disorders are the major cause of 'Years of life lived with disability' and a major cause of 'Years of life lost because of premature death'. The problem is growing due to the increased life-span and higher depression frequency with increasing age. Methods to prevent the onset of disease have not been developed and there has been no major clinical breakthrough since introduction of monoaminergic antidepressants in 1950ies, perhaps as a consequence of the prevailing hypothesis of dysregulation in neurotransmission of biogenic amines. Using currently available drugs, 30–40% of patients are only partial- or non-responders and additional compounds based on same mechanisms will not solve the problem. Thus there exists a major unmet medical need to develop treatments based on different modes of action. Changes in the monoaminergic systems may be a sufficient but not a necessary pathophysiological factor and converging evidence indicates that other systems, such as the glutamatergic are of paramount importance. Indeed, work at the Yale University, NIMH, and Icahn School of Medicine at Mount Sinai demonstrated the marked antidepressant effects of intravenously infused ketamine to treatment resistant patients diagnosed with major depressive disorder. In order to better understand the mechanisms of ketamine effects we decided to test it on animal models.
Methods: All experiments were approved by the Karolinska Institutet's Committee for Animal Protection. Two rat models, bred at the Karolinska Institutet, the Flinders Sensitive Line (FSL) and their controls, the Flinders Resistant Line (FRL), and the SERT KO (homozygous, heterozygous, and the wild type rats) were used. Male animals were injected 10 mg ketamine/kg body weight or vehicle and the Open Field Test (OF) and Forced Swim Test (FST) carried out 30 respectively 40 min later. The tests were recorded and subsequently scored blindly using the NOLDUS system. One hour after the injection the animals were euthanized, the brains harvested, immediately deep-frozen, and stored for future analyses. Brains were dissected into frontal cortex, hippocampus and striatum and rtPCR and Western blot used for NMDA, AMPA, and mGlu2/3 receptors, neuropeptide Y and NPY-Y1 receptor, BDNF and mTor analyses. 2-way ANOVA and Bonferroni *post-hoc* test were used for statistical analysis.
Results: Behavioral results are presented in this abstract.I.FSL and FRL. (1) OF: FSL moved significantly more (distance/cm) and faster (cm/sec) than FRL in the test (p's < 0.01). Ketamine had no significant effect on locomotion. (2) FST: FSL showed greater baseline immobility compared to FRL ($p < 0.05$). Ketamine markedly reduced immobility time, both total and when assessed minute by minute, in both strains ($p < 0.0001$), more so in the FSL.II. SERT KO. (1) OF: no significant differences in either distance covered or speed of movement were found between the homozygous, heterozygous, and the wild type rats in the test. Ketamine had no significant effect on locomotion. (2) FST: Homozygous rats showed larger mean immobility time, though not reaching the statistical level of significance, compared with the heterozygous and the wild type rats. Ketamine markedly reduced the immobility time, both total and when assessed minute by minute, in all three subgroups ($p < 0.001$).
Conclusions: This first study of ketamine in two animal models of depression shows that a single injection of the compound has marked behavioral effects in the FST, generally accepted as a tool to assess antidepressant action of putative antidepressants. The results are consistent with the effects of single ketamine injection in treatment

resistant depressed patients. Of note, since the FSL and SERT KO models are phenotypically similar but represent different brain pathologies, analysis of ketamine effects on brain molecular and cellular levels will likely contribute to identification of the pathways necessary for antidepressant effects, independent of the underlying pathologies.
Keywords: ketamine, depression, animal models, forced swim test, open field test.
Disclosures: A. Mathé, Nothing to Disclose; V. Sousa, Nothing to Disclose; C. Weide Fischer, Nothing to Disclose; T. Stan, Nothing to Disclose; G. Wegener, Nothing to Disclose; A. Lennartsson, Nothing to Disclose; P. Svenningsson, Nothing to Disclose.

W177. What's Serotonin Got to do with It? Studies on the Actions of SSRIs and Cocaine in SERT M172 Mice

Linda D Simmler*, Alexander G Nackenoff, Sonja J Stutz, Noelle Anastasio, Kathryn Cunningham, Randy D Blakely

Vanderbilt University School of Medicine, Nashville, Tennessee

Background: Many drugs with therapeutic or addictive effects interact with the presynaptic serotonin transporter (SERT), including SSRIs, cocaine, and MDMA. As these agents often show activity after chronic administration and/or act at multiple targets in the brain and periphery, the specific role played by different signaling systems in drug action continues to be an important point of discussion. For example, we and our colleagues (Bonnin *et al*, *Neuropsychopharmacology*, 2012) have recently shown that the high-affinity SERT antagonist citalopram acts to influence axonal trajectories via sigma-1 receptors, a serotonin-independent action. Additionally, we have reported (Prosser *et al*, SFN abstract, 2012) that the action of cocaine to shift the circadian rhythm of suprachiasmatic neurons is mediated by serotonin and not by dopamine. Both of these observations have been achieved using a novel mouse model in which the *Slc6a4* gene has been mutated to eliminate high-affinity interactions of multiple drugs with SERT.
Methods: A previously described mutation (Ile172Met, ie, I172M) in the *Slc6a4* gene leads to loss of high-affinity cocaine recognition at SERT (Henry *et al*, *J Biol Chem*, 2006). Aiming to optimize performance in behavioral assays, we backcrossed the I172M mutation onto a C57BL/6J background (SERT M172 mice). We assessed SERT protein expression by western blot and effects of cocaine, citalopram, fluoxetine, and paroxetine in synaptosomal uptake inhibition experiments. In a 2-bottle choice paradigm we compared the preference for cocaine in drinking water (0.1 mg/ml) over pure drinking water between WT (SERT I172) and transgenic mice over a period of seven weeks. All animal studies were performed under approved protocols reviewed by the Vanderbilt University or University of Texas Medical Branch Institutional Animal Care and Use Committee and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.
Results: As with our original mice on the 129S6/S4 background (Thompson *et al*, *PNAS*, 2011), we find that the introduced mutation (I172M) does not disturb normal SERT protein expression or functional activity, nor does it influence interactions with serotonin or paroxetine, a drug previously found to be insensitive to the mutation in transfected cells. In contrast, significant potency shifts are evident for cocaine, citalopram and fluoxetine, affording opportunities to evaluate the contribution of serotonin signaling to the actions of these drugs. Moreover, whereas WT animals (SERT I172) display reduced immobility to citalopram, SERT M172 animals fail to respond to the antidepressant. We then examined the impact of the cocaine-insensitive SERT in cocaine-induced behavior after either acute or chronic cocaine administration in the 2-bottle choice paradigm. We find that WT animals display a stable preference for cocaine administered in the drinking water over several weeks. In contrast, in the first week of administration, SERT M172 animals exhibited a blunting of preference for cocaine. At week two, this effect reverses, leading to an exaggerated preference for cocaine over WT animals.
Conclusions: These data support the efficacy of the SERT M172 mouse model as a tool to establish the serotonin-dependence of actions elicited by antidepressants and cocaine. Our findings support an essential role for SERT in the acute actions of SSRIs and suggest that SERT antagonism plays a significant, time-dependent role in the plasticity underlying the drive to consume cocaine. In ongoing studies, we are evaluating the actions of cocaine in these mice in other behavioral tests known to be sensitive to acute and chronic cocaine administration and evaluating serotonin-dependent transcriptome responses that contribute to cocaine action.
Keywords: serotonin, cocaine, SSRI, neuroplasticity, transgenic mouse model.
Disclosures: L. Simmler, Nothing to Disclose; A. Nackenoff, Nothing to Disclose; S. Stutz, Nothing to Disclose; N. Anastasio, Nothing to Disclose; K. Cunningham; R. Blakely, **Part 1:** Lundbeck Inc Scientific Advisory Board, NeuroscienceDetectives, Inc Research Contract, **Part 4:** NeuroscienceDetectives, Inc Research Contract.

W178. Endocannabinoid Elevation Reverses Social Withdrawal and Normalizes Neuronal Activation Patterns in the PCP Rat Model of Schizophrenia

Julien Matricon, Alexandre Seillier, Andrea Giuffrida*

UT Health Science Center, San Antonio, Texas

Background: The negative symptoms of schizophrenia, which include anhedonia, reduced affect and social withdrawal, are not adequately treated by current antipsychotic therapies. Recent studies suggest a link between negative symptoms and dysfunctional endocannabinoid transmission in the brain. In particular, increased levels of the endocannabinoid anandamide (AEA) have been reported in the cerebrospinal fluid of drug-naive schizophrenics and inversely correlated to the severity of negative symptoms (Giuffrida *et al*, 2004; Leweke and Koethe, 2008). In line with these observations, we previously showed that URB597-induced elevation of brain AEA in the phencyclidine (PCP) rat model of schizophrenia reverses PCP-induced social

withdrawal via activation of CB1 receptors. The same drug, however, produced a social deficit in control rats via a CB1-independent mechanism (Seillier *et al*, 2013). In this study we assessed the ability of JZL184, an inhibitor of monoacyl glycerol lipase, to selectively elevate the other major endocannabinoid, 2-arachidonoyl glycerol (2-AG), and reverse PCP-induced social withdrawal. Finally, to identify the neuronal correlates underlying the effects of PCP and endocannabinoid-enhancing drugs on social behavior, we analyzed c-Fos expression, a marker of neuronal activity, in several cortical, limbic and sub-cortical regions of PCP- and saline-treated rats undergoing social interaction following an injection of URB597 (or vehicle).
Methods: Dose-response curves of the effects of JZL184 on brain 2-AG levels and motor activity were constructed to identify an effective dose for the social interaction test. Quantification of 2-AG was carried out by isotope-dilution mass spectrometry as previously described (Hardison *et al*, 2006). Motor activity (horizontal and vertical) was monitored for 60 min using the ActiMot Activity Measuring System (TSE Systems GmbH, Germany) following an acute injection of vehicle (Tween-80/PEG/saline, 10/10/80, 1 ml/kg), or JZL184 (5, 15 and 30 mg/kg, i.p.). For the c-Fos experiments, brain samples were collected from rats treated with PCP (5 mg/kg, i.p., 2 injections/day for 7 days followed by 1-week washout) and saline-treated controls tested for social interaction in the presence of URB597 (0.3 mg/kg, i.p.) or vehicle. Rats exploring the arena alone were used as controls.
Results: Systemic administration of JZL184 (15 and 30 mg/kg, i.p.) produced a 2-fold increase of 2-AG in the hippocampus, striatum and prefrontal cortex without affecting AEA levels. As the highest dose of JZL184 produced significant motor inhibition, all subsequent experiments were carried out using the dose of 15 mg/kg. Like URB597, JZL184 reversed the social deficit observed in PCP-treated rats. This drug, however, had no effect on social behavior in control rats. Social interaction produced neuronal activation in the orbitofrontal cortex of saline-treated rats, but not in PCP-treated rats. URB597 administration prevented the activation of this brain area in saline-treated rats, whereas it restored neuronal activation in the PCP-treated group. Interestingly, we found an opposite activation pattern in the central amygdala, suggesting critical contribution of the prefrontal cortex-amygdala pathway to the deficits observed in our model. Other brain areas showing different activation patterns between control and PCP rats included the infralimbic cortex, the dorso-medial striatum, the septum, the stria terminalis and the basolateral amygdala.
Conclusions: These findings indicate that PCP-induced social withdrawal results from reduced endocannabinoid transmission and that elevation of endocannabinoid tone can beneficially affect the negative symptoms of schizophrenia. The deleterious effects of URB597, but not JZL184, in control rats may depend on the ability of the former drug to elevate AEA, which in turn may activate non-CB1 targets when reaching supra-physiological concentrations. Our data also show that PCP-treated rats have altered patterns of neuronal activation in several cortico-limbic regions after the social interaction challenge, and that URB597 can reverse these abnormalities. Further investigations are ongoing to assess the effects of JZL184 on cFos expression.
Supported by NIMH grant RO1MH91130 (*AG*).

Keywords: cannabinoid, cFos, CB1, anandamide, 2-AG, social interaction.
Disclosures: J. Matricon, Nothing to Disclose; A. Seillier, Nothing to Disclose; A. Giuffrida, Nothing to Disclose.

W179. Inhibition of Select Bromodomain Proteins Attenuates Cocaine Reward

Gregory C Sartor*, Shaun P Brothers, Claes Wahlestedt

University of Miami Miller School of Medicine, Miami, Florida

Background: Several recent studies have demonstrated that histone-modifying proteins are involved in behavioral and molecular responses to cocaine. Representing an unexplored yet promising target for the treatment of addiction, epigenetic 'reader' proteins, such as bromodomain containing proteins, are key regulators of chromatin state. Bromodomain proteins bind acetyl-lysine residues in histones and recruit histone acetyltransferases, histone deacetylases and protein complexes leading to chromatin remodeling and transcriptional activation or repression. Given the ability of bromodomain proteins to alter chromatin and influence gene expression, they have long been appreciated in cancer research, but have garnered little attention in the field of neuroscience and especially addiction research. Thus, we investigated the role of a select family of bromodomain proteins, called bromodomain with extraterminal bromodomains (BETs: Brd2, Brd3, Brd4, and Brdt), in cocaine reward.
Methods: Using the conditioned place preference (CPP) paradigm, male C57bl/6 mice received 3 conditioning sessions where cocaine (15 mg/kg) and saline were paired with distinct contexts in the CPP apparatus. Before each cocaine conditioning injection, the BET bromodomain inhibitor, JQ1 (10, 25, or 50 mg/kg, i.p.), or vehicle was injected. As a control, iBET-151 (50 mg/kg), a similar BET inhibitor that does not penetrate the blood brain barrier, was also administered prior to cocaine injections in a different group of mice. JQ1-induced (50 mg/kg) CPP was also measured to determine the drug's rewarding or aversive properties. Similar to the cocaine CPP paradigm, JQ1 (50 mg/kg) or vehicle was injected prior to LiCl (150 mg/kg) conditioning in the conditioned place aversion (CPA) paradigm. In additional experiments, male Sprague Dawley rats were implanted with guide cannula above the nucleus accumbens and received intra-accumbal injections of JQ1 (1 μM) before each cocaine CPP session. Preference scores were calculated as the time spent in the drug-paired side during post-test minus the time spent in the drug-paired side during pre-test.
Results: In cocaine conditioned mice, systemic administration of the BET bromodomain inhibitor, JQ1, prior to each cocaine conditioning session dose-dependently reduced cocaine CPP (25 and 50 but not 10 mg/kg, i.p.), whereas a similar bromodomain inhibitor that does not cross the blood brain barrier (I-BET 151, 50 mg/kg, i.p.) had no affect on cocaine CPP. JQ1 (50 mg/kg, i.p.) alone did not induce a conditioned place preference or aversion. Additionally, JQ1 (50 mg/kg, i.p.) did not attenuate LiCl-induced (150 mg/kg, i.p.) conditioned place aversion, indicating that JQ1 does not affect all

contextual learning paradigms. Lastly, intral-accumbal injections of JQ1 significantly reduced acquisition of cocaine CPP.
Conclusions: In the current study, we revealed that systemic and intra-accumbal inhibition of BET bromodomain proteins attenuates cocaine reward-related behaviors, but does not affect other types of contextual learning, such as conditioned place aversion. Results from these studies shed light on novel epigenetic processes involved in cocaine use, which could ultimately lead to new therapeutic avenues for drug addiction.
Keywords: cocaine, conditioned place preference, bromodomain, JQ1, behavior.
Disclosures: G. Sartor, Nothing to Disclose; S. Brothers, Nothing to Disclose; C. Wahlestedt, **Part 1:** I am a consultant to Opko Health and a founder of Epigenetix.

W180. Suppression of Drug-evoked Nucleus Accumbens Dopamine by Somatic Hyperpolarization

James E McCutcheon*, Samantha M Fortin, Jackson J Cone, Christopher G Sinon, Ilana B Witten, Karl Deisseroth, Garret D Stuber, Mitchell F Roitman

University of Leicester, Leicester, United Kingdom

Background: Dopamine signaling in forebrain regions such as the nucleus accumbens (NAc) is held under tight control by several membrane proteins. Importantly, the dopamine transporter (DAT) clears dopamine from the extracellular space, and the dopamine D2 autoreceptor (D2R) provides negative feedback to suppress activity of dopamine cells. Several addictive drugs, for example the psychostimulants, act as DAT blockers; many therapeutic compounds, particularly anti-psychotics, are antagonists of D2Rs. Antagonism of DATs and D2Rs evokes phasic dopamine release from terminals in the forebrain (Venton *et al*, 2007). Both proteins are expressed on dopamine cell bodies in the ventral tegmental area (VTA), as well as at the terminals. Therefore, it is unclear whether drug effects are due to influencing dopamine release via action at the terminals or if there are additional effects on cell excitability at the cell body. Indeed, DAT and D2R blockade has been shown to increase the firing rate of dopamine neurons (Shi *et al*, 2004). Understanding the mechanism of action of these different drugs relies on determining how DATs and D2Rs that reside in different cellular compartments contribute to activity in dopamine cells. In addition, this will give insight into how dopamine cell firing can be modulated under normal conditions.
Methods: Transgenic rats (Long-Evans background) that express Cre recombinase under the control of the tyrosine hydroxylase promoter (TH-Cre rats; Witten *et al*, 2011) were used to express halorhodopsin (eNpHR3.0-YFP) specifically in dopamine cells. Halorhodopsin is a light-activated chloride channel that hyperpolarizes cells in response to light. This strategy allows manipulation of activity with high temporal precision in a specific subpopulation of cells within the VTA, ie dopamine cells. Halorhodopsin or control virus (eYFP) was infused unilaterally into the VTA of TH-Cre or wild-type rats, and six to ten weeks was allowed for expression and transport. Then, rats were anesthetized with urethane and implanted with a guide cannula directed towards the nucleus accumbens shell (from Bregma: AP +1.7, ML +0.9), a reference electrode in contralateral cortex, and an optic fiber lowered into ipsilateral VTA (AP −5.8, ML +0.7, DV −8.0 from skull). A carbon fiber electrode was lowered into NAc shell and phasic dopamine release events (transients) were measured using fast-scan cyclic voltammetry. Following the experiment, rats were perfused and brains were processed using immunohistochemistry to confirm expression levels of virus.
Results: Histological examination of brains revealed robust expression of virus (halorhodopsin or eYFP) in the VTA and NAc of TH-Cre rats, but not of wild-type controls. As previously shown, occurrence of spontaneous dopamine transients before drug administration was extremely low. However, intraperitoneal injection of cocaine (DAT blocker; 10 mg/kg) and raclopride (D2R antagonist; 1 mg/kg) caused a dramatic increase in dopamine transients. During 30 s trials, light (532 nm; 10–15 mW) was applied directly to the VTA for 5 s. In TH-Cre rats expressing halorhodopsin, light administration significantly decreased dopamine concentration (-17.25 ± 4.63 nM *vs* baseline) and reduced the probability of dopamine transients with high temporal precision only while the light was on. No effect of laser was observed in controls—eg wild-type rats infused with halorhodopsin or TH-Cre rats infused with eYFP—demonstrating the specificity of this approach.
Conclusions: Here, we show that the increase in dopamine release generated by blockade of DATs and D2Rs is primarily dependent on the drug action at the cell body. As such, somatic hyperpolarization via light-activated channels expressed specifically in dopamine cells, strongly suppressed cocaine/raclopride-evoked dopamine release. In addition, we show the utility of this technique for dissecting the role of specific circuit elements *in vivo* and for determining mechanism of action of drugs. Future experiments will use this approach to explore the role of dopamine suppression in mediating aversive learning.
Keywords: dopamine nucleus accumbens cocaine optogenetics aversion.
Disclosures: J. McCutcheon, Nothing to Disclose; S. Fortin, Nothing to Disclose; J. Cone, Nothing to Disclose; C. Sinon, Nothing to Disclose; I. Witten, Nothing to Disclose; K. Deisseroth, Nothing to Disclose; G. Stuber, Nothing to Disclose; M. Roitman, Nothing to Disclose.

W181. Glucagon-like Peptide-1 Receptor Activation Reduces Cocaine Reward

Gregg Stanwood*, Devon Graham, India Reddy, Lynette Daws, Aurelio Galli

Vanderbilt University, Nashville, Tennessee

Background: The glucagon-like peptide-1 (GLP-1) is both an incretin hormone and neuropeptide that regulates energy homeostasis and feeding behavior through stimulation of high affinity GLP-1 receptors (GLP-1R). GLP-1 and GLP-1Rs are expressed within the brain, including within brain reward circuitry. In fact, GLP-1R activation within the mesolimbic dopamine (DA) system reduces the hedonic components of food intake and GLP-1R activators are

successful treatments for diabetes mellitus. Given the overlap between food and drug reward circuitries, we tested the hypothesis that activation of the GLP-1R would alter dopamine homeostasis and diminish cocaine reward.
Methods: Brain slices (300 μm) of male wild-type C57Bl/6J mice were prepared with a vibratome in an ice-cold oxygenated buffer and used for dopamine uptake, biotinylation and immunoblotting studies using standard methods. High-speed chronoamperometry was used to measure DA clearance *in vivo*. Cocaine reward was assessed using a conditioned place preference (CPP) procedure consisting of three phases: preconditioning (Day 1), conditioning (Days 2–9), and testing (Day 10). One mouse from each cage was randomly assigned a drug treatment (pretreatment/treatment) for the CPP procedure: Ex4/SAL, SAL/Cocaine, or Ex4/Cocaine (10–100 μg/kg Ex4 and 20 mg/kg cocaine). During the conditioning phase of the test, the treatments were alternated with SAL/SAL pairings, and treatments were counter-balanced across cohorts. Statistical tests were typically ANOVA accompanied by *post hoc* comparisons and were conducted using Prism and SAS.
Results: GLP-1 and a long-lasting GLP-1R agonist, exendin-4 (Ex-4), dose-dependently increased DA uptake and DAT cell surface expression in slices containing the nucleus accumbens and lateral septum, brain regions which contain significant levels of GLP-1 receptors. Local application of GLP-1 increased DA clearance rate as detected by high speed chronoamperometry. As expected, cocaine alone (20 mg/kg) induced robust CPP. Peripheral Ex-4 significantly blunted the rewarding properties of cocaine. Importantly, Ex-4 alone did not produce an aversion, as these mice had no significant change in chamber preference over the course of the study.
Conclusions: Our data indicate that activation of GLP-1Rs attenuates the rewarding properties of cocaine, likely due to effects on DA homeostasis. The fact that Ex-4 is already FDA-approved for the treatment of type 2 diabetes (Byetta and Bydureon) demonstrates that there are fundamental neurobiological circuitries common to drug addiction and food 'addiction', and that the potential utility of Ex-4 as a treatment for psychostimulant abuse and addiction could immediately be translated to clinical studies.
Keywords: GLP-1, addiction, cocaine, dopamine, transporter.
Disclosures: G. Stanwood, Nothing to Disclose; D. Graham, Nothing to Disclose; I. Reddy, Nothing to Disclose; L. Daws, Nothing to Disclose; A. Galli, Nothing to Disclose.

W182. Lithium Ameliorates Rotenone-induced Methylation and Hydroxymethylation of DNA in Cortical Primary Neurons

Gustavo Scola, L Trevor Young, Helena Kim, Mirian Salvador, Ana Andreazza*

University of Toronto, Ontario, Canada

Background: Mitochondrial complex I dysfunction is consistently reported in bipolar disorder (BD). Alterations in methylation levels have also been reported in BD, and lithium was found to cause modifications in epigenetic factors in patients and in cells. One of the mechanisms by which lithium may exert its effects in BD is by improving mitochondrial function. Therefore, in this study, we aimed to evaluate the role of mitochondrial complex I dysfunction in methylation and hydroxymethylation of DNA using a cellular model by treating rat cortical primary neurons with rotenone, which inhibits complex I and cause mitochondrial dysfunction. Furthermore, we also examined the ability of lithium to ameliorate rotenone-induced alterations to global 5mc and 5hmc levels.
Methods: Rat E18 cortical neurons (PC35102 Neuromics, US) were treated with 0.75 mM of lithium for 7 days and then treated with different concentrations of rotenone (5, 10, and 50 nM) for 30 min. After washout, cells were cultured additionally for 24 h. Cell viability was verified by levels of MTT and ATP; complex I activity was measure by standard ELISA assay and DNA methylation and hydroxymethylation levels were assessed by immunocytochemistry.
Results: Here, we report decreased complex I activity, ATP production, cell viability and increased methylation and hydroxymethylation following rotenone treatment, suggesting a relationship between complex I function and levels of 5mc and 5hmc. Importantly, lithium was found to prevent rotenone-induced alterations to 5mc and 5hmc levels as well as damage to mitochondrial function produced by rotenone, suggesting that lithium may be ameliorating alterations to 5mc and 5hmc levels by preventing rotenone-induced damage to the mitochondria.
Conclusions: The findings of this study demonstrated that mitochondrial complex I dysfunction induced by rotenone treatment decreases ATP levels and cell viability while increasing methylation and hydroxymethylation of DNA. Notably, lithium pre-treatment was able to prevent complex I dysfunction and, therefore, decrease methylation and hydroxymethylation levels. Decreased expression of complex I subunits and its activity are consistent findings in BD, while lithium is one of the most commonly prescribed and effective drugs to treat this disease. Therefore, our result demonstrating that complex I dysfunction induced by rotenone leads to changes in methylation and hydroxymethylation levels implies a possible interaction between these two systems. Furthermore, our findings suggest that lithium treatment may benefit patients with mitochondrial dysfunction in part by ameliorating complex I function and preventing its consequences on DNA methylation and hydroxymethylation.
Keywords: lithium, oxidative strees, mitochondrial dysfucntion, DNA methylation.
Disclosures: G. Scola, Nothing to Disclose; L. Young, Nothing to Disclose; H. Kim, Nothing to Disclose; M. Salvador, Nothing to Disclose; A. Andreazza, Nothing to Disclose.

W183. A Microarray and Proteomics Study of Lithium-treated Mice and Knockout Mice with Lithium-like Behavior Reveals a Common Effect on Mitochondrial Function and Autophagy

Galila Agam*, Lilach Toker…, Yuly Bersudsky, RH Belmaker, Inbar Plaschkes, Vered Chalifa-Caspi, Dieder Moechars, Roberto Buccafusca, Gerard T Berry

Ben-Gurion University of the Negev, Beer-Sheva, Israel

Background: The inositol-depletion hypothesis proposes that lithium attenuates phosphatidylinositol signaling.

Knockout-mice of two genes (*IMPA1*, *Slc5a3*) encoding for proteins related to inositol-metabolism exhibit a lithium-like behavioral phenotype1.
1Agam *et al*, Biochem Soc Trans. 2009.
Methods: We performed a DNA-microarray study searching for pathways commonly affected by chronic lithium-treatment and by the knockout of each of the genes. Data was analyzed using three different bioinformatics approaches. Commonly differentially-expressed genes were verified by real-time PCR. A parallel proteomics study was also carried out, confirmed by Western blotting. We sought a potential behavioral correlate of the bioinformatics results using a pharmacological intervention examined in the bipolar-related forced-swim test and amphetamine-induced-hyperlocomotion paradigm.
Results: All bioinformatics analyses revealed up-regulation of mitochondrial-function. Three of seven genes commonly differentially-expressed in the three paradigms, *Cox5a*, *Ndufs7* and *Ndufab*, all members of the mitochondrial electron-transfer-chain, have been reported as associated with bipolar-disorder and/or lithium treatment. Their differential-expression was verified by real-time PCR analysis. The proteomics study indicated that the mitochondrial function-related process, autophagy, is enhanced. The result of the bioinformatics analysis was consistent with an observed interrelationship between treatment with lithium and rotenone, an inhibitor of mitochondrial-function. Lithium and rotenone counteracted each other's effects in the forced-swim test and the amphetamine-induced-hyperlocomotion paradigm.
Conclusions: The results support reports of mitochondrial dysfunction in bipolar-disorder and its amelioration by lithium as well as reports of lithium-induced autophagy upregulation, all mediated via lithium's effect on inositol metabolism.
Keywords: lithium-treatment, knockout-mice, inositol, DNA-microarrays, proteomics, bioinformatics, mitochondrial-function, autophagy, behavior, forced-swim-test, amphetamine-induced hyperlocomotion, beclin1/P62 protein levels.
Disclosures: G. Agam, Nothing to Disclose; L. Toker…, Nothing to Disclose; Y. Bersudsky, Nothing to Disclose; R. Belmaker, Nothing to Disclose; I. Plaschkes, Nothing to Disclose; V. Chalifa-Caspi, Nothing to Disclose; D. Moechars, Nothing to Disclose; R. Buccafusca, Nothing to Disclose; G. Berry, Nothing to Disclose.

W184. ORM-12741: Receptor Pharmacology of a Novel Alpha2C-Adrenergic Receptor Subtype Selective Antagonist with Multi-therapeutic Potential

Jukka Sallinen, Juha Rouru, Jyrki Lehtimaki, Päivi Marjamäki, Merja Haaparanta-Solin, Eveliina Arponen, Semi Helin, Olof Solin, Frank Tarazi, Mohammed Shahid*

Orion Pharma, Nottingham, United Kingdom

Background: The α2-adrenergic receptors (α2A, α2B, α2C) act as inhibitory auto- and hetero- receptors, which modulate the neuronal release of monoamines (norepinephrine [NE], serotonin [5-HT], and dopamine [DA]) as well as other neurotransmitters (eg acetylcholine). Blockade of these receptors has been proposed as a promising approach for psychiatric therapeutics especially treatment of affective disorders and cognitive impairment. However the α2A- and α2B- adrenergic receptor subtypes show a widespread peripheral distribution and raise the potential for autonomic system related side effects. In contrast, the α2C-adrenergic receptor shows more brain restricted expression, primarily in the striatum and hippocampus. Selective α2C-adrenergic receptor antagonism may therefore provide an approach to boost central NE activity with lower incidence of peripheral side effects. This study describes the receptor pharmacology of ORM-12741, a novel α2C-adrenergic receptor selective antagonist, currently in clinical development.
Methods: *In vitro* binding and functional properties of ORM-12741 were examined in cloned human α2-adrenergic receptors (α2A, α2B, α2C) expressed in HEK cells. Potential for activity at other molecular targets was examined in a 126 receptor *in vitro* screen with 1 μM ORM-12741. *In vivo* receptor occupancy of ORM-12741 in rat brain was determined using a proprietary α2C-adrenergic receptor PET tracer (11C-ORM-13070). Brain sections from rats treated with vehicle or ORM-12741 (2, 10 or 50 μg/kg, s.c.) were collected and the striatal regions were analysed for specific binding by autoradiography.
Results: ORM-12741 shows very high affinity for the human α2C-adrenergic receptor with a Ki value of 0.08 nM. It has a much lower affinity for the human α2A- and α2B-adrenergic receptors with Ki values of 8.3 and 0.8 nM, respectively. Furthermore, ORM-12741 also showed high selectivity against 126 receptors examined (including α1-adrenergic receptor). In functional assays, ORM-12741 blocked NE-induced and α2-adrenergic receptor mediated Ca2 + responses in a concentration-related manner. The Kb values for α2A-, α2B- and α2C-adrenergic receptors were 41, 5.6 and 0.01 nM, respectively. In rat brain, ORM-12741 produced a dose-related displacement of 11C-ORM-13070 binding in the striatum with ∼70% α2C-adrenergic receptor occupancy at the highest dose tested.
Conclusions: The current data show that ORM-12741 is a highly selective α2C-adrenergic receptor antagonist with a 100 fold and 3800 fold selectivity over the α2A-adrenergic receptor in binding and functional assays, respectively, suggesting a low potential for α-adrenergic receptor related cardiovascular side-effects. Consistent with its sub-nanomolar affinity for the α2C-adrenergic receptor, ORM-12741 showed high potency for displacing 11C-ORM-13070 with ∼45% receptor occupancy at plasma exposure levels considered to be necessary for efficacy in antidepressant, antipsychotic and cognition assays. The combination of ORM-12741 and 11C-ORM-13070 presents a powerful tool to translate and test preclinical properties of ORM-12741 and the therapeutic of utility of dialling in α2C-adrenergic receptor activity towards the treatment of cognitive and affective dysfunction.
Keywords: ORM-12741 α2C-adrenergic receptor antagonist antidepressant cognition.
Disclosures: J. Sallinen, **Part 5:** Orion Pharma; J. Rouru, **Part 5:** Orion Pharma; J. Lehtimaki, **Part 5:** Orion Pharma; P. Marjamäki, Nothing to Disclose; M. Haaparanta-Solin, Nothing to Disclose; E. Arponen, Nothing to Disclose; S. Helin, Nothing to Disclose; O. Solin, Nothing to Disclose; F. Tarazi, Nothing to Disclose; M. Shahid, **Part 5:** Orion Pharma.

W185. Differential Effects of Vilazodone *vs* Citalopram and Paroxetine on Serotonin Transporters and Receptors

Yong Kee Choi, Ronald Oosting, Pradeep Banerjee, Frank Tarazi*

Harvard Medical School, Belmont, Massachusetts

Background: Vilazodone is a novel antidepressant drug approved for treatment of major depressive disorder in adults. It exhibits an unique mechanism of action as a selective serotonin reuptake inhibitor (SSRI) and a 5-HT1A partial receptor agonist. Evidence suggests that vilazodone lacks sexual side effects in male rats and in humans, which is a major adverse event of clinically used SSRIs.
Methods: We compared the effects of chronic administration (2 weeks) of therapeutically relevant doses of vilazodone (3 and 10 mg/kg, IP) *vs* two other established SSRIs; citalopram and paroxetine (both 10 mg/kg, IP) on serotonin transporters (5-HTT), and on serotonin 5-HT1A and 5-HT2A receptors in different rat forebrain regions using autoradiographic assays.
Results: Chronic vilazodone administration (3 and 10 mg/kg) reduced 5-HTT levels in medial prefrontal cortex (MPC; by 28 and 49%, respectively), nucleus accumbens (NAc; by 48 and 51%), caudate putamen (CPu; by 38 and 42%), hippocampal CA1 (by 42 and 46%) and CA3 (by 51 and 55%) regions. In contrast, citalopram and paroxetine induced more profound reductions in 5-HTT levels in MPC (by 82 and 91%, respectively), NAc (by 83 and 90%), CPu (by 71 and 79%), hippocampal CA1 (by 55 and 72%) and CA3 (by 71 and 87%) regions. In addition, vilazodone (3 and 10 mg/kg) treatment decreased 5-HT1A receptor levels in hippocampal CA1 (by 49 and 50%, respectively) and CA3 (by 49 and 42%) regions. In contrast, citalopram and paroxetine treatment increased 5-HT1A receptors in hippocampal CA1 (by 21 and 19%) and CA3 (by 33 and 34%) regions. The three drugs increased 5-HT2A receptors in cerebral cortex.
Conclusions: The observed differential effects of vilazodone *vs* citalopram and paroxetine on 5-HTT and 5-HT1A receptors may potentially result in vilazodone's stimulation of serotonergic neurotransmission in forebrain regions to achieve adequate clinical efficacy, but without certain adverse events, including sexual side effects, commonly associated with SSRI treatment.
Keywords: citalopram, depression, paroxetine, serotonin transporters, serotonin receptors, vilazodone.
Disclosures: Y. Choi, Nothing to Disclose; R. Oosting, **Part 4:** Research Grant: Forest Labs; P. Banerjee, **Part 5:** Forest Laboratories Inc.; F. Tarazi, **Part 4:** Research Grants from Forest Labs, Shire Pharmaceuticals, Lundbeck and Novartis.

W186. A Progressive Ratio Determination of the Relative Reinforcing Effect of Methylphenidate *vs* Cocaine by Intravenous Self-administration Testing in Rats

David J Heal*, Niki Buckley, Emma L Johnson, Jane Gosden, Sharon L Smith

RenaSci Ltd, Nottingham, United Kingdom

Background: *dl-threo*-Methylphenidate (methylphenidate) is widely used to treat attention deficit hyperactivity disorder (ADHD). This drug is highly unusual because although it is a relatively weak catecholamine reuptake inhibitor, intracerebral microdialysis experiments have revealed that it has the ability to evoke rapid, large increases in the overflow of dopamine and noradrenaline in the brain without a dose-effect ceiling (Heal *et al*, 2012, Curr Top Behav Neurosci, 9: 361–390). The pharmacodynamics of methylphenidate's actions on the catecholamine neurotransmitters are similar to those of the β-phenylethylamine releasing agents, eg *d*-amfetamine, and clearly differentiated from those of conventional catecholamine reuptake inhibitors, eg bupropion or atomoxetine (Heal *et al*, 2012). In a recent evaluation of the discriminative and reinforcing properties of various stimulants used to manage ADHD, methylphenidate was found to cross-generalise to the *d*-amfetamine cue in a 2-choice, rat drug-discrimination procedure and to serve as a positive reinforcer in rats trained to self-administer low dose cocaine intravenously (Heal *et al*, 2013, Neuropharmacology, Jun 6; 73C: 348–358). We have now conducted a second study to confirm the positive reinforcing effect of methylphenidate in rats trained to self-administer cocaine on a fixed ratio (FR) schedule of drug reinforcement. The relative reinforcing efficacy of methylphenidate was then determined by measuring its break-point using a progressive ratio (PR) schedule and comparing it to the break-points of two reinforcing doses of cocaine.
Methods: Eleven, adult, male Sprague-Dawley rats (Charles River UK) were used in the study. They were individually housed on a 12 h/12 h light/dark cycle, at $21 \pm 4^{\circ}$C and $55 \pm 20\%$ humidity. The rats were allowed free access to water, but were mildly food restricted. The self-administration protocol is described in detail by Heal *et al* (2013). In summary, rats were trained to lever-press for food rewards on a FR2 reinforcement schedule. Once operant responding for food pellet rewards was stable under the FR 2 schedule, a chronic in-dwelling intravenous (iv) catheter was implanted into the jugular vein of each rat under gaseous anaesthesia. After implantation of the intravenous catheter, rats were switched to self-administer multiple injections of a low reinforcing dose of cocaine (0.32 or 0.1 mg/kg/injection, iv) on a FR2 reinforcement schedule. Test sessions were initiated by a non-contingent injection of drug and rats were allowed to lever-press for a maximum of 20 injections/1.0 h session/day. After establishing consistent and robust self-administration of cocaine (average $\geqslant$15 cocaine infusions over 3 consecutive sessions), it was substituted by saline (iv) to demonstrate extinction of self-administration (average $\leqslant$8 saline infusions over 3 consecutive sessions). Rats were then given access to the most robustly reinforcing dose of methylphenidate (0.1 mg/kg/injection, iv) in the model (Heal *et al*, 2013) or they were re-established on cocaine (0.32 or 0.1 mg/kg/injection, iv). When stable positive reinforcement had been attained on the FR2 schedule, the rats were given access to the reinforcer on an ascending PR schedule (Richardson and Roberts, 1996, J Neurosci Meth, 66: 1–11) in a 2 h session to determine the break-point for operant responding.
Results: The placebo control, saline (iv), maintained only low levels of operant responding (mean total infusions/session $\pm$ SEM $= 4.4 \pm 0.4$ [$n = 11$]). Both doses of cocaine served as positive reinforcers in this group of rats (mean

total infusions/session ± SEM: 0.1 mg/kg/injection = 18.2 ± 0.8 [$n = 6$], $p < 0.001$ *vs* saline; 0.32 mg/kg/injection = 16.2 ± 0.6 [$n = 7$], $p < 0.001$ *vs* saline). Methylphenidate (0.1 mg/kg/injection, iv) also served as a positive reinforcer in cocaine-maintained rats (mean total infusions/session ± SEM = 18.4 ± 0.7 [$n = 6$], $p < 0.001$ *vs* saline). When rats were given access to cocaine on the PR schedule of reinforcement, the break-point of operant responding for the higher dose of cocaine (mean lever-presses ± SEM = 43.0 ± 2.8 [$n = 7$]) was significantly greater ($p < 0.01$) than for the lower dose of cocaine (mean lever-presses ± SEM = 14.1 ± 1.7 [$n = 6$]). The break-point for methylphenidate (mean lever-presses ± SEM = 37.8 ± 7.8 [$n = 6$]) was not significantly different from the break-point for the higher dose of cocaine, but it was significantly greater ($p < 0.01$) than the lower dose.
Conclusions: The findings confirm that methylphenidate served as a positive reinforcer in rats trained to self-administer low doses of cocaine. The break-point analysis revealed that the relative reinforcing effect methylphenidate was equal to that of a highly reinforcing dose of cocaine. These results provide additional evidence to demonstrate that methylphenidate and cocaine possess similar pharmacological and reinforcing characteristics.
Keywords: reinforcing effects, cocaine, methylphenidate, self-administration, progressive ratio.
Disclosures: D. Heal, Nothing to Disclose; N. Buckley, Nothing to Disclose; E. Johnson, Nothing to Disclose; J. Gosden, Nothing to Disclose; S. Smith, Nothing to Disclose.

W187. Neonatal SSRI Exposure Alters Neurodevelopment and Risk for Depression in Model Rats

Sarah M Clinton*, Matthew E Glover, Phyllis C Pugh, Joshua Cohen, Huda Akil

University of Alabama Birmingham, Alabama

Background: Depression is the most common psychiatric illness afflicting women during their child-bearing years. Pregnant women and new mothers who struggle with depression need to be treated for their own benefit as well as for the benefit of their children, and this treatment frequently involves administration of selective serotonin reuptake inhibitor (SSRI) antidepressants. SSRIs are generally considered safe as they do not increase risk for miscarriage or major birth defects. However, evidence in humans and rodents suggests that early-life SSRI exposure can have serious long-term behavioral and physiological consequences for offspring exposed in utero or infancy via breast milk. Unfortunately, we have only limited knowledge of how neonatal SSRI exposure impacts brain development and ultimately adult behavior, particularly within individuals at high-risk for developing depression due to biological predisposition. Our study addressed two critical questions on this topic: (a) are certain individuals particularly susceptible to the deleterious effects of neonatal SSRI exposure; and (b) if so, what neurobiological factors place them at risk.
Methods: Our study utilized Sprague-Dawley rats bred for low *vs* high behavioral response to novelty—traits that, in turn, predict several other aspects of emotional behavior, including propensity to anxiety- and depression-like behavior. Rats bred for low response to novelty (LRs) also display high anxiety- and depressive-like behavior compared to High Novelty-Responders (HRs), which vigorously explore novelty and show high aggression and impulsivity. The bred LR/HR model offers a unique opportunity to test the interplay between genetic predisposition for depression and neonatal SSRI exposure on neurodevelopment and emotional behavior. For the 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. HR and LR male offspring were weaned on postnatal day (P)21, and as adults were tested to assess locomotor response to novelty, anxiety- and depression-like behavior. A second study used genome-wide expression profiling to examine the impact of neonatal SSRI exposure on limbic system (hippocampus and amygdala) development. Another group of LR females was treated with paroxetine (or normal tap water) throughout pregnancy and the postpartum period. LR pups exposed to neonatal SSRI or vehicle treatment were sacrificed and brains collected at three early developmental time points (P7, P14, P21) and adulthood (P75). The amygdala and hippocampus were dissected and RNA was extracted for analysis via NimbleGen Rat Gene Expression 12 × 135 K Arrays.
Results: Neonatal SSRI exposure enhanced adult LR offspring's already high levels of depression-like behavior (Forced Swim Test immobility), while HRs' behavior was unaffected. Neonatal SSRI exposure did not affect LR or HR anxiety-like behavior on the Elevated Plus Maze. Microarray analysis revealed dramatic region- and age-specific gene expression changes in both the hippocampus and amygdala. Thousands of genes were altered by neonatal SSRI exposure in both brain areas, although the developmental pattern varied by region. There were far greater changes in the P7 hippocampus (~9000 genes) compared to later ages (4000–6000 genes each at P14, P21 and P75), whereas in the amygdala, there were fewer changes at P7 (~1500 genes) and much greater changes occurring later (~4000 genes each at P14, P21, and P75). Therefore, in addition to the known neonatal SSRI exposure-induced perturbations of the serotonin system itself, these data demonstrate dramatic drug-induced alterations in developing limbic structures that receive serotonergic input. Ongoing analyses are using bioinformatics approaches to hone in on specific molecular pathways that are changed following neonatal SSRI exposure. Preliminary results indicate significant changes in epigenetic mechanisms (such as DNA methylation and histone modification), as well as perturbations in the Nerve Growth Factor system.
Conclusions: Prior studies suggest that LR rats represent a novel animal model of comorbid anxiety and depression. Compared to HRs, LR rats show a high level of anxiety- and depression-like behavior that is reduced by anxiolytic and antidepressant agents, respectively, when administered in adulthood. The LR/HR traits are heritable, with both behavioral and neurobiological LR/HR differences emerging very early in life. The current data show that 'depression-prone' LR offspring are especially susceptible to the deleterious effects of neonatal SSRI exposure (leading to exacerbated depression-like behavior), while HRs are unaffected. Furthermore, neonatal SSRI exposure induced massive gene expression differences in the developing and adult hippo-

campus and amygdala of LR rats. Overall our data suggest that individuals genetically predisposed to depression may be particularly vulnerable to the negative effects of early-life SSRI exposure, which has important clinical implications for treating depressed pregnant and nursing women.
Keywords: SSRI, neurodevelopment, amygdala, hippocampus, depression.
Disclosures: S. Clinton, Nothing to Disclose; M. Glover, Nothing to Disclose; P. Pugh, Nothing to Disclose; J. Cohen, Nothing to Disclose; H. Akil, Nothing to Disclose.

W188. Effects of Buprenorphine and ALKS 33, Alone and in Combination, on Monoamine Release within the Nucleus Accumbens Shell and Medial Prefrontal Cortex of Male Wistar Rats

Daniel R Deaver*, Jacobi I Cunningham, Reginald L Dean, Mark Todtenkopf, David J Eyerman

Alkermes, Inc., Waltham, Massachusetts

Background: ALKS 5461 represents a novel treatment for depression that combines buprenorphine (BUP), a partial mu agonist, with ALKS 33, a potent mu antagonist. ALKS 5461 was recently studied as adjunctive therapy in subjects having an inadequate response to antidepressants in a phase 2, sequential parallel comparison design trial, and was found to be superior to placebo on a range of primary and secondary measures of depressive symptoms. These clinical findings are consistent with a substantial body of non-clinical and pharmacologic research indicating that endogenous opioid systems regulate mood and are dysregulated in depressive illness [Belluzzi and Stein, 1977; Garriock, 2010; Gross-Isseroff, 1990; Kennedy, 2006]. Antidepressants are known to affect monoamine release in the mesolimbic system, which may contribute to their clinical utility. These non-clinical studies were designed to investigate the effects of BUP and ALKS 33, alone and in combination, on extracellular concentrations of monoamines in the nucleus accumbens shell (NAc-sh) and the medial prefrontal cortex (mPFC).
Methods: These experiments were approved by Alkermes IACUC and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 2011). All studies utilized male Wistar rats (275–375 g). Rats were maintained on a 12-h light/dark cycle with unrestricted access to food and water. Sterile saline was used as the vehicle for each study. Drugs were administered subcutaneously at a dose volume of 1 ml/kg. For the NAc-sh, three sets of experiments were conducted to determine the effects of: (a) BUP alone (0.001–1.0 mg/kg; (b) ALKS 33 alone (0.1–10 mg/kg); and (c) a fixed dose of BUP (0.1 mg/kg) with three doses of ALKS 33 (0, 0.3, 1.0 and 3 mg/kg) on extracellular concentrations of dopamine (DA), its metabolites (DOPAC and HVA) and 5-hydroindole acetic acid (5-HIAA), with the exception that only DA was determined in the BUP dose-response study. For the mPFC, two sets of microdialysis experiments were conducted to determine the effects of ALKS 33 (0.1–10 mg/kg) and in combination as one of three doses (0, 0.3 and 3.0 mg/kg) with a fixed dose of BUP (0.1 mg/kg) on release of DA, norepinephrine (NE) and serotonin (5-HT). Microdialysis probes were inserted into the NAc-sh or mPFC using the following coordinates from bregma: A/P +1.70; M/l ±0.8; D/V −7.8 or A/P +3.4; M/l ±0.80; D/V −6.0 respectively. Each microdialysis experiment lasted 6 h which included baseline and post-drug treatment periods. Probe placement was verified in all rats at the end of the study. Bioanalytical analysis of DA, DOPAC, HVA, NE, 5-HT and 5-HIAA was performed by HPLC-EC using either a single channel (UHPLC) or a dual channel (HPLC) Antec Leyden ALEXYS system. The amount of each analyte was quantified as pg per 10 µl of sample. Raw data were converted to absolute and percent change from baseline and analyzed using a repeated measures 2-way ANOVA using GraphPad Prism 6.0 or SAS 9.2.
Results: Within the NAc-sh, BUP resulted in a dose-dependent elevation in extracellular DA with increases above baseline of approximately 190, 250 and 375% observed at doses of 0.01, 0.1 and 1.0 mg/kg, respectively. Administration of ALKS 33 had no effect on baseline extracellular concentrations of DA or the monoamine metabolites DOPAC, HVA or 5-HIAA at any dose. When the lower doses of ALKS 33 (0.3 and 1.0 mg/kg) were co-administered with BUP, average increases in extracellular DA, DOPAC, HVA and 5-HIAA were attenuated, but not entirely blocked. The higher dose of ALKS 33 (3.0 mg/kg) completely blocked BUP-induced increases in extracellular DA, DOPAC, HVA and 5-HIAA. In the mPFC, treatment with ALKS 33 had no effect on extracellular DA, NE or 5-HT. BUP (0.1 mg/kg) resulted in maximal increases in DA, NE and 5-HT of approximately 160, 150 and 190%, respectively. ALKS 33 dose-dependently attenuated BUP-induced increases in extracellular DA and 5-HT within this region. In contrast, even the high dose ALKS 33 (3.0 mg/kg) only partially attenuated BUP-induced increases in NE within this region.
Conclusions: Dysregulation of the endogenous opioid system has been postulated to play an important role in mood disorders. Exogenous opioids, including BUP, have been shown to have beneficial effects in treating depression. In these studies, BUP caused increased extracellular concentrations of monoamines and their metabolite in the NAc-sh and mPFC. In contrast ALKS 33, an opioid modulator with potent mu receptor antagonist activity, had no effect on monoamine release within these regions of the mesolimbic system. Regional differences in the magnitude of the monoamine response to BUP were noted, as well as regional differences in the effect of BUP when given in combination with ALKS 33 at clinically relevant doses. Differential modulation of monoamine release within the mesolimbic system may contribute to the efficacy of ALKS 5461 in the treatment of depression. Endogenous opioids are known to affect other CNS neurotransmitter systems including acetylcholine, GABA and glutamate. Future non-clinical investigations of ALKS 5461 will evaluate effects on these additional neurotransmitters.
Keywords: buprenorphine, ALKS 33, depression, microdialysis, monoamines.
Disclosures: D. Deaver, **Part 1:** Alkermes, Inc. and own stock in the company., **Part 2:** Yes**Part 5:** full-time employee of Alkermes.; J. Cunningham, **Part 1:** full time employee of Alkermes, Plc, **Part 2:** Yes, **Part 3:** Yes, **Part 5:** Alkermes, Plc; R. Dean, **Part 5:** Alkermes, Inc.; M. Todtenkopf, **Part 5:** Alkermes, Inc.; D. Eyerman, **Part 1:** full-time employee of Alkermes, plc., **Part 2:** Alkermes plc., **Part 3:** Alkermes plc., **Part 5:** Alkermes, plc.

W189. Investigating the Interaction between Lisdexamfetamine and S-citalopram on Monoamine Neurochemistry by Dual-Probe Microdialysis in Freely Moving Rats : Evidence for Synergistic Augmentation of Serotonin and Dopamine Efflux

Pete Hutson*, Helen Rowley, Rajiv Kulkarni, David J Heal

Shire Pharmaceuticals, Wayne, Pennsylvania

Background: The SSRIs are the most widely prescribed antidepressants. Although SSRIs are therapeutically beneficial, they take several weeks to produce their maximum antidepressant effect, a substantial proportion of patients do not achieve remission, and 30–40% of subjects are unresponsive to SSRIs. To address these shortcomings, various pharmacological approaches to augment the therapeutic effects of the SSRIs and/or accelerate their onset of action have been tried which include adjunctive administration of 5-HT1A receptor antagonists or atypical antipsychotics. Lisdexamfetamine dimesylate (LDX), a prodrug of d-amphetamine that is pharmacologically inactive until converted into d-amphetamine, is currently approved only for use in patients six years and older with attention-deficit/hyperactivity disorder (ADHD). Intracerebral microdialysis has shown that therapeutically relevant doses of LDX produce substantial and prolonged increases in extracellular concentrations of noradrenaline and dopamine in the prefrontal cortex (PFC) and striatum (Rowley *et al*, 2012, J Psychopharmacol 26 [Abst Suppl], A73). These neurochemical effects would be predicted to complement the therapeutic actions of the SSRIs if the compounds were co-administered. We have, therefore, performed dual-probe, intracerebral, microdialysis experiments in rats to investigate the interactions between LDX and S-citalopram on extracellular 5-HT (serotonin), noradrenaline and dopamine in hippocampus, nucleus accumbens, prefrontal cortex (PFC) and striatum of freely-moving rats to determine whether neurochemical mechanisms exist that would support the clinical investigation of co-therapy in depression.

Methods: Male Sprague Dawley rats (250–350 g; Charles River UK) were maintained on a 12 h/12 h light/dark cycle, at 21 ± 4°C and 55 ± 20% humidity with free access to food and water. Under gaseous anesthesia, two concentric microdialysis probes (CMA, Sweden) were stereotaxically implanted into hippocampus (4 mm tip, AP: −4.8 mm; L: +/−4.8 mm relative to bregma; V: −7.8 mm relative to the skull surface) and nucleus accumbens (2 mm tip, AP: + 2.2 mm; L: +/−1.5 mm; V: −8.0 mm) or into PFC (2 mm tip, AP: +3.2 mm; L: +/−2.5 mm; V: −4.0 mm) and striatum (4 mm tip, AP: +0.2 mm; L: +/−3.0 mm; V: −7.8 mm according to the stereotaxic atlas of Paxinos and Watson (1986). The following day groups of rats ($n = 7$–8) were given vehicle (2 ml/kg, po)/vehicle (2 ml/kg, ip), LDX (1.5 mg/kg *d*-amphetamine base po)/vehicle (ip); vehicle (po)/S-citalopram (5 mg/kg, ip) or LDX (1.5 mg/kg, po)/S-citalopram (5 mg/kg, ip). Following collection of 3 basal samples, LDX was administered at $t = -20$ min followed by S-citalopram (5 mg/kg ip) at $t = 0$ min. Samples were collected for a further 3 h. Noradrenaline, dopamine and 5-HT in the dialysis samples were quantified by reverse-phase, ion-pair, HPLC coupled with electrochemical detection (ALEXYS, Antec, The Netherlands).

Results: S-Citalopram significantly increased the overall efflux of 5-HT in the hippocampus (AUC[0–3.0 h] = 427%; $p < 0.01$), nucleus accumbens (392%; $p < 0.05$) and striatum (288%; $p < 0.01$), but it did not significantly enhance 5-HT overflow in PFC. S-Citalopram's effect was relatively slow in onset taking 60–80 min to reach its peak, but 5-HT efflux remained elevated thereafter. S-Citalopram had no effect on extracellular noradrenaline or dopamine. LDX produced complementary monoaminergic changes by increasing extracellular noradrenaline and dopamine in the hippocampus (AUC [0–3.0 h] = 403%; $p < 0.05$ and 268%, $p < 0.001$, respectively) and PFC (194%; $p < 0.05$ and 195%, $p < 0.001$, respectively). It also increased dopamine efflux in nucleus accumbens (253%; $p < 0.001$) and striatum (229%; $p < 0.001$), but it had no effect on noradrenaline in these regions. LDX did not enhance 5-HT efflux. Combining LDX with S-citalopram significantly increased extracellular concentrations of noradrenaline and dopamine in the PFC, all three monoamines in the hippocampus, and dopamine and 5-HT in the nucleus accumbens and striatum. Thus, the complementary actions of these two compounds on monoaminergic neurotransmission were realised when they were administered in combination. There was clear evidence of synergistic augmentation of S-citalopram-induced 5-HT efflux in the hippocampus ((AUC [0–3.0 h] = 1089%; greater than additive $p < 0.001$), nucleus accumbens (521%; greater than additive $p < 0.05$) and striatum (561%; greater than additive $p < 0.001$). Conversely, S-citalopram profoundly augmented the enhancing effect of LDX on dopamine efflux in the striatum (536%; greater than additive $p < 0.001$).

Conclusions: The observations that LDX potentiated the effect of S-citalopram on 5-HT efflux in the hippocampus, nucleus accumbens and striatum, and increased noradrenergic and dopaminergic neurotransmission to complement SSRI-enhanced serotonergic neurotransmission provide viable neurochemical mechanisms to support the hypothesis that adjunctive LDX administration may augment antidepressant efficacy in SSRI-treated depressed subjects. It must be emphasised that this preclinical study allows for insight and hypothesis generation, and ongoing Phase III clinical trials will help confirm this hypothesis.

Keywords: lisdexamfemaine, S -citalopram, serotonin, dopamine microdialysis.

Disclosures: P. Hutson, **Part 5:** shire pharmaceuticals; H. Rowley, **Part 5:** Renasci Ltd; R. Kulkarni, **Part 5:** Renasci Ltd; D. Heal, **Part 5:** Renasci Ltd.

W190. GLYX-13, a NMDA Receptor Glycine-site Functional Partial Agonist, Produces Long Lasting Antidepressant-like Effects through Modulation of Long-term Synaptic Plasticity

Jeffrey Burgdorf*, Xiao-lei Zhang, Amanda Gross, Roger Kroes, Patric Stanton, J David Leander, Ronald M Burch, Joseph Moskal

Northwestern University, Evanston, Illinois

Background: GLYX-13, a glycine-site functional partial agonist at the NMDAR, is currently in phase II clinical development as an adjunctive therapy for major depressive

disorder (clinicaltrials.gov identifier NCT01684163). GLYX-13 has been shown to: (a) preferentially enhance conductance of NR2B-containing NMDARs at rat Schaffer collateral-CA1 synapses *in vitro*; (b) enhance the magnitude of long-term potentiation (LTP) of synaptic transmission while simultaneously reducing that of long-term depression (LTD), which differentiates GLYX-13 from other NMDAR modulators such as D-cycloserine. In animal studies, GLYX-13 has been shown to: (a) enhance performance in several hippocampus-dependent learning tasks in both young adult and learning-impaired aged rats; and (d) produce an antidepressant-like effects in a variety of models without ketamine-like dissociative, addictive or sedative side effects.
Methods: Chronic Unpredictable Stress (CUS) Procedure: Male Sprague-Dawley (SD) rats (2–3 Months old) received 21 days of CUS: 9 different CUS stressors were used (2 stressors per day). Animals in the CUS groups received a single optimal dose of GLYX-13 (3 mg/kg IV; $n = 10$) previously shown to produce a robust antidepressant-like response or sterile saline vehicle ($n = 10$). Porsolt Test: Animals were placed in a 46 cm tall × 20 cm in diameter clear glass tube filled to 30 cm with tap water ($23 \pm 1^{\circ}C$) for 15 min on the first day (habituation) and 5 min on the subsequent test days (1 h, 24 h, 1 week, 2 weeks post-dosing). Sucrose Preference Test: Rats were exposed to a palatable sucrose solution (1%; Sigma, USA) for 48 h, followed by 4 h of water deprivation and a 1 h exposure to two identical bottles, one filled with sucrose solution and the other with tap water. Novelty Induced Hypophagia (NIH) Test: Animals were food deprived on the night before testing, and lab chow was placed into the center chamber of the open field (40 × 40 × 20 cm) for 10 min under dim-red lighting. Ultrasonic Vocalization (USV) Test: Animals received 3 min of heterospecific rough-and-tumble play consisting of alternating 15 s blocks of heterospecific play and 15 s of no-stimulation. Microarray Analyses: Triplicate microarray analyses were performed using the medial prefrontal cortex (MPFC) isolated from individual non-CUS treated animals injected with GLYX- 13 (3 mg/kg IV), or saline vehicle (1 ml/kg IV). Individual 45-mer oligonucleotides complementary to sequences of 1178 cloned rat CNS mRNAs were synthesized on a PolyPlex™ 96-well oligonucleotide synthesizer (GeneMachines®, USA) and spotted in triplicate onto epoxy coated slides (Telechem, USA) using an OmniGrid™ robotic microarrayer (GeneMachines®). Hippocampal Slice Electrophysiology: Hippocampal slices were prepared from adult male SD rats 24 h after a single injection of GLYX-13 (3 mg/kg IV), ketamine (10 mg/kg IV) or vehicle. LTP at Schaffer collateral-CA1 synapses was measured in response to three submaximal bouts of high-frequency Schaffer collateral stimulation (2 × 100 Hz/800 ms).
Results: A single IV dose of GLYX-13 produced a long-lasting antidepressant-like effect in the Porsolt, sucrose preference, and NIH tests in rats exposed to CUS. GLYX-13 also produced a long-lasting antidepressant-like effect in the USVs test and increased positive emotional learning in rats exposed to CUS. The AMPA/kainate receptor antagonist NBQX, 24 h post-dosing, occluded the antidepressant-like effect of GLYX-13 in the Porsolt test. GLYX-13, 24 h and 2 weeks post-dosing, showed enrichment in LTP- and LTD-associated genes in the medial prefrontal cortex as measured by microarray. GLYX-13 metaplasticly enhances LTP 24 h and 1 week following a single dose, and persistently enhances LTP following multiple bi-weekly doses.
Conclusions: We have hypothesized that the long-lasting antidepressant effects of GLYX-13 are due to metaplastic enhancement in long-term activity-dependent synaptic plasticity. Based on the results reported here we suggest that the *long-lasting* antidepressant-like effects of GLYX-13 are due at least in part to metaplasticity mechanisms associated with NMDAR-triggered induction of LTP. The induction/upregulation of LTD-associated transcripts at 1 h by GLYX-13 may produce a shift in the threshold for future synaptic plasticity processes. This shift may initially favor LTD and be associated with a more sustained rebound that persistently favors future LTP since it is well established that the thresholds for LTD and LTP are plastic.
Keywords: GLYX-13, NMDA receptor, depression.
Disclosures: J. Burgdorf, **Part 1:** Naurex, Inc, **Part 2:** Naurex, Inc, **Part 3:** Naurex, Inc; X. Zhang, Nothing to Disclose; A. Gross, **Part 5:** Naurex, Inc; R. Kroes, **Part 1:** Naurex, Inc, **Part 2:** Naurex, Inc, **Part 4:** Naurex, Inc; P. Stanton, **Part 1:** Naurex, Inc, **Part 2:** Naurex, Inc, **Part 4:** Naurex, Inc; J. Leander, **Part 1:** Naurex, Inc, AgeneBio, Nektar, and CoLucid; R. Burch, **Part 5:** Naurex, Inc; J. Moskal, **Part 1:** Naurex, Inc, **Part 2:** Naurex, Inc, **Part 3:** Naurex, Inc.

W191. Levomilnacipran Inhibits both Norepinephrine and Serotonin Reuptake Across the Clinical Dose Range

Joann O'Connor*, Laishun Chen, Carl Gommoll, Stephen R Zukin

Forest Research Institute, Jersey City, New Jersey

Background: Dysfunction of the norepinephrine (NE) and serotonin (5-HT) systems is thought to play a central role in the etiology and pathophysiology of major depressive disorder (MDD). Some symptoms of depression, such as anxiety, agitation, and irritability have been more closely associated with 5-HT while other depressive symptoms, such as fatigue, lack of motivation, and decreased concentration appear to be more strongly related to NE. Effective treatment options for MDD include the serotonin-norepinephrine reuptake inhibitors (SNRIs) duloxetine, venlafaxine, and desvenlafaxine. These compounds all show greater potency *in vitro* for inhibiting reuptake of serotonin relative to norepinephrine and studies have suggested that duloxetine and venlafaxine may only inhibit NE reuptake in patients at higher doses. In contrast, levomilnacipran extended-release (ER), a potent and selective SNRI recently approved for the treatment of MDD in adults, shows approximately 2-fold greater potency for inhibition of NE relative to 5-HT reuptake, *in vitro*. The objective of this study was to characterize the pharmacokinetic (PK) profile of levomilnacipran ER 40, 80, and 120 mg/d and to use the PK data to estimate NE and 5-HT reuptake inhibition over the levomilnacipran ER dose range.
Methods: PK data were collected from adult patients with MDD who participated in a positive 8-week placebo-controlled, fixed-dosed trial of levomilnacipran ER 40, 80, and 120 mg/day (Asnis *et al*, 2013; *J Clin Psych*; NCT00969709). Approximately 15% of patients in each levomilnacipran ER dose group consented to give serial

blood samples after the first 4 weeks of double-blind treatment. Blood samples were collected predose, and at 2, 4, 6, 8, 12, and 24 h postdose. Plasma sample were analyzed using a validated liquid chromatography-tandem mass spectrometry method. PK analysis was performed using Phoenix WinNonlin (Ver 6.1). Unbound plasma concentrations were estimated based on the previously determined levomilnacipran plasma protein binding value of 22%. To evaluate 5-HT and NE inhibition over the levomilnacipran ER clinical dose range, unbound plasma concentrations obtained over a 24-h period from MDD patients receiving levomilnacipran ER 40, 80, and 120 mg dose were plotted against *in vitro* NE and 5-HT reuptake inhibition data generated using human recombinant transporters (Auclair *et al*, 2013; *Neuropharmacology*). These raw *in vitro* data were curve fitted using SigmaPlot (Ver 12.0) and the equation: $y = \min + (\max - \min)/(1 + [x/IC50]\gamma)$ (Where x = levomilnacipran concentration, y = % of remaining activity, min = minimal remaining activity; max = maximal remaining activity, IC50 = concentration at 50% inhibition, and γ = Hill slope. Concentrations at IC80 and IC90 were calculated based on parameters of the equation derived from the fitting).

Results: Levomilnacipran steady state pharmacokinetics were linear and dose proportional following oral administration of levomilnacipran ER in the dose range of 40–120 mg/day. The mean maximum plasma concentrations (Cmax) were 92.8, 180.4 ng/ml, and 297.2 ng/ml, for the 40, 80, and 120-mg doses, respectively. The mean time to maximum observed plasma concentration(Tmax) was 6 h for all 3 doses. The mean areas under the plasma concentration-time curves from zero to 24 h (AUC0-τ) were 1519.8, 2722.2, and 4675.9 h*ng/ml for levomilnacipran ER 40, 80, and 120 mg/day, respectively. The mean trough plasma concentrations (Cmin) at steady state for levomilnacipran ER were 28.8, 52.3, and 90.7 ng/ml for levomilnacipran ER 40, 80, and 120 mg/day, respectively; these values are equivalent to 109.1, 198.1, and 343.6 nM, respectively. The levomilnacipran IC80 and IC90 values were estimated *in vitro* to be 39 and 86 nM for the NE reuptake transporter, and 88 and 232 nM for the 5-HT reuptake transporter, respectively. For the entire dosing period of 24 h, the average unbound plasma concentrations for levomilnacipran that were reached in MDD patients treated with levomilnacipran ER 40, 80, or 120 mg/day exceeded the concentration that showed 90 and 80% inhibition of NE and 5-HT reuptake, respectively.

Conclusions: Administration of once daily levomilnacipran ER is expected to inhibit NE and 5-HT reuptake more than 90 and 80%, respectively, across the 24-h dosing interval and approved dose range. These data suggest that the clinical benefits of NE reuptake inhibition could potentially be achieved at all 3 clinically approved levomilnacipran ER doses.

Keywords: levomilnacipran, SNRI, MDD, pharmacokinetic.

Disclosures: J. O'Connor, **Part 5:** Employee of Forest Research Institute, a subsidiary of Forest Laboratories, Inc.; L. Chen, **Part 5:** Employee of Forest Research Institute, a subsidiary of Forest Laboratories, Inc.; C. Gommoll, **Part 5:** Employee of Forest Research Institute, a subsidiary of Forest Laboratories, Inc.; S. Zukin, **Part 5:** Employee of Forest Research Institute, a subsidiary of Forest Laboratories, Inc.

W192. Human Neuronal Precursors: Melatonin Abolishes Cytoskeletal Alterations and Promotes Neuronal Development in Olfactory Neuroepithelial Cells Obtained from Schizophrenic Subject

Gloria Benítez-King*, Tania Galván-Arrieta, Carlos Berlanga, Horacio Zamudio-Meza

National Institute of Psychiatry, Mexico City, Mexico

Background: The availability of human neuronal tissue is limited due ethical reasons, which hampers understanding of the etiology and mechanisms of drug action in psychiatric disorders. Olfactory neuroepithelial cells (eg, stem and sustentacular cells, immature and mature olfactory neurons) have been used to study neuronal development in schizophrenic patients (Casella *et al*, J Neurochem 102:587, 2007; Feron *et al*, Schizophr Res 40:211, 1999). Located in the nasal cavity and amenable for collection by biopsy, olfactory neuroepithelium cells are continuously regenerated by active neurogenic and neurodevelopmental processes. Neuroepithelium neuronal precursors can be maintained in culture, and respond to hormones and neurotrophic factors, as shown for central nervous system neurons. Neuroepithelial biopsies have been used to validate the neurodevelopment hypothesis of schizophrenia, however postmortem deterioration, or detrimental effects of anesthesia cannot be excluded. Melatonin, a molecule synthesized at night in the pineal gland, plays a key role in adult brain neurodevelopment as cytoskeleton modulator. In rat hippocampus melatonin increases new neuron formation and dendritogenesis (Ramírez-Rodríguez *et al*, J Pineal Res 50:29, 2011; Domínguez-Alonso *et al*, J Pineal Res 52:427, 2012), and F-actin content (Jiménez-Rubio *et al*, Neurosci Lett 511:47, 2012). Recently, we demonstrated that melatonin-induced differentiation of cloned neuronal precursors obtained from a healthy subject by a non-invasive exfoliation procedure (Benítez-King *et al*, J Neurosci Meth 201:35, 2011). Here we report the effect of melatonin on cytoskeletal organization and development of neuronal precursors in primary cultures obtained from a schizophrenia patient and from a healthy subject.

Methods: The schizophrenia patient was recruited at the Schizophrenia Clinic at the *Instituto Nacional de Psiquiatría*. All experiments were conducted with the understanding and written consent of the subjects and approved by the Institute's Ethics Committee. Cells were obtained by nasal cavity exfoliation, plated and propagated in a selection media up to 4 passages. Neuronal precursors were characterized by IIIβ-tubulin and nestin staining, which are specific neuronal lineage markers. Cells were detached and replated on glass cover slips at 10 000 cells/cm2 and cultured during 4 days. Cultures were incubated with vehicle, or either 100 nM or 10 μM melatonin during 3, 6 or 12 h. Cells were fixed and stained with either an anti-tau1 antibody to label axons, or an anti-IIIβ tubulin to stain neurites. Microfilaments and nucleus were labeled with rhodamine phalloidin and DAPI, respectively. MT1 and MT2 receptor proteins were stained with an anti-MT1 A/B antibody.

Results: Results showed that olfactory neuronal precursors in primary cultures from both healthy and schizophrenia

patients express MT1 and MT2 melatonin receptors. Neuronal precursors obtained from the schizophrenia patient showed an aberrant microtubular organization, an irregular microfilament edge, and randomly oriented thick stress fibers. By contrast, in the presence of melatonin enlarged microtubules were observed in neurites and regular microfilament edges; stress fibers were rearranged and adopted a similar organization as in neuronal precursors obtained from healthy subjects Also, the thickness of stress fibers was reduced. Associated to these changes, melatonin increased axonal formation in both schizophrenia and healthy subject derived cells.
Conclusions: Data indicate that melatonin repairs altered cytoskeletal organization, and stimulates neurodevelopment in schizophrenia olfactory neuronal precursors. Thus, we suggest that melatonin could be a useful agent to reestablish neuronal connectivity in the adult brain of subjects with schizophrenia. Supported by Conacyt/SSA/IMSS/ISSSTE Grant No 86863, and Conacyt/SEP grant No 178075 from México.
Keywords: melatonin, cytoskeleton, neuronal precursors, neurodevelopment, schizophrenia.
Disclosures: G. Benítez-King, Nothing to Disclose; T. Galván-Arrieta, Nothing to Disclose; C. Berlanga, Nothing to Disclose; H. Zamudio-Meza, Nothing to Disclose.

W193. The Effects of Gene Knockout of the Vesicular Monoamine Transporter 2 (VMAT2; SLC18A2) and the Dopamine Transporter (DAT; SLC3A6) on Ethanol Consumption and Escalation in Mice

Frank S Hall*, Alexandra Houston-Ludlam, Zhicheng Lin, George Uhl

NIDA, Baltimore, Maryland

Background: Previous research has implicated monoamine systems in the effects of ethanol and in alcoholism, including both the dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2) genes. Because of the importance of these genes in regulating DA and 5-HT function, and the proposed importance of these genes and those neurotransmitter systems in alcoholism, the present studies were undertaken to examine the effects of transgenic deletion (knockout; KO) of these genes on ethanol consumption.
Methods: Ethanol consumption was compared in male and female wild-type (WT) and knockout (KO) mice with deletions of the DAT or VMAT2 genes on standard mixed C57BL/6J-129S1 backgrounds (DAT and VMAT strains) and congenic strains on C57BL/6J genetic backgrounds (DAT BX and VMAT2 BX strains). Voluntary ethanol (2–32% v/v, the concentration increased every 2 days for 10 days) and water consumption were compared in two-bottle, 24 h access, home-cage preference tests in WT (+/+) and heterozygous KO (+/−) mice of each strain. In separate groups of mice the escalation of ethanol intake (8% ethanol *vs* water, 2 days per week, M/Th, with only water available on intervening days) was measured over 3 weeks.
Results: In the initial concentration study there were no effects of genotype in the DAT, DAT BX or VMAT strains, but VMAT BX +/− mice consumed slightly less ethanol than VMAT BX +/+ mice. In the escalation study availability of ethanol twice per week for 24 h, with no ethanol available during intervening periods, produced escalation of ethanol consumption over the course of 3 weeks. Escalation of intake was reduced in DAT BX +/− mice of both sexes and in female VMAT +/− and VMAT BX +/− mice. Poor escalation, and perhaps floor effects, was observed in male VMAT and VMAT BX mice.
Conclusions: These data indicate that reductions in the expression of both DAT and VMAT may be protective against elevations in ethanol consumption that are produced by intermittent opportunities to consume ethanol in the escalation paradigm. By contrast, these genes seem to have relatively little effect upon initial sensitivity to ethanol even when the consumption of a range of concentrations is assessed. These findings suggest that differences in DAT and VMAT expression may affect the predisposition to alcoholism, but that these influences are only observed in circumstances that model the escalation of ethanol intake associated with initial intermittent use that is a part of the addictive process.(*Support: NIDA-IRP*).
Keywords: alcoholism, pharmacogenetics, dopamine transporter, vesicular monoamine transporter, escalation.
Disclosures: F. Hall, Nothing to Disclose; A. Houston-Ludlam, Nothing to Disclose; Z. Lin, Nothing to Disclose; G. Uhl, Nothing to Disclose.

W194. THC Elicits Temporary or Persistent Changes in Expression of Genes Implicated in Neurodevelopment in Adolescent Rat Brain Regions

Bertha K Madras*, Gregory M Miller, Lisa Ogawa, Josh Zimmer, Eric Vallender, Yasmin Hurd, Susan Westmoreland

Harvard Medical School, Southborough, Massachusetts

Background: Accumulating evidence suggests that the adolescent brain is more vulnerable to the adverse effects of marijuana—higher prevalence of addiction, altered brain morphology, neural circuitry—compared with the adult brain. imaging of human adolescent and adult marijuana users previously revealed anatomical and/or functional abnormalities in specific brain regions. Changes in expression of genes implicated in development of neural circuitry, and dopamine signaling are among the possible underlying mechanisms. Following administration of THC or vehicle to adolescent rats, this study determined whether: (1) THC affects expression levels of genes encoding axonal guidance/cell adhesion molecules, vascular endothelial growth factors, and proteins involved in dopamine signaling. (2) the effects of THC on gene expression persist in brains of treated adolescent rats that have matured into young adults. (3) THC affects markers for dopamine circuitry during adolescence and whether changes persist in young adulthood.
Methods: We treated adolescent rats (PND 28) with THC (10 rats; 1.5 mg/kg i.p., every 3rd day) or vehicle (10 rats). Five drug-treated and five vehicle control rats were euthanized 24 h after the last injection (PND 50) and the remaining 10 rats were euthanized 2 weeks later in early

adulthood (PND 64). Gene expression levels were measured following mRNA isolation from cerebellum, frontal cortex, hippocampus, and striatum. RNA was isolated from the tissues and cDNA was synthesized with a Reverse Transcription Kit. Expression of the selected genes were measured using real-time PCR on a LightCycler 480 using rat-specific, intron-spanning when possible primers and probe-based qPCR assays with the Universal Probe Library system. At least three technical replicates were performed for each gene in every tissue sample. Data were analyzed using the ΔΔCt method, normalizing the raw data to beta actin (ACTB) as a housekeeping gene. The average fold change values with standard error were analyzed for statistical significance, using a two-tailed, paired *t*-test in Excel.

Results: THC promoted significantly different changes in gene expression in animals euthanized after the last dose, compared with animals euthanized two weeks later, or compared with vehicle. (1) in cerebellum, *Dusp6*, *DCC*, *NRP1* and otherswere down-regulated in adult brain of previously treated adolescent- animals; (2) a similar result was observed in hippocampus (*sema3b*. *Sema3a*, *Dusp6*) and in striatum (*DRD3*, *Dusp*). In rats euthanized right after the last dose, gene expression trended above the control while in rats euthanized two weeks after the last dose, gene expression trended below the control. In the cerebellum however, genes appear to be downregulated in all animals despite the time of euthanasia.

Conclusions: In specific brain regions, THC engendered a short-term response resulting in upregulation of certain genes, followed by downregulation of the same genes. However, genes in the cerebellum that were immediately downregulated remained downregulated two weeks following the last dose of THC. Reversal and down-regulation of THC-induced increases in gene expression in various brain regions may be interpreted as compensatory decreases after drug cessation. In the cerebellum, the effects of THC persist into adulthood after THC withdrawal. Our results suggest that THC affects genes critical for neurodevelopment during progression from adolescence to adulthood. The findings have implications for the observed anatomical and/or functional abnormalities detected in brain regions of human adolescent and adult marijuana users.

Keywords: marijuana, adolescent brain, axonal guidance molecules, dopamine, neurodevelopment.

Disclosures: B. Madras, Nothing to Disclose; G. Miller, Nothing to Disclose; L. Ogawa, Nothing to Disclose; J. Zimmer, Nothing to Disclose; E. Vallender, Nothing to Disclose; Y. Hurd, Nothing to Disclose; S. Westmoreland, Nothing to Disclose.

W195. The Role of Efficacy on the Interaction Between Mu Opioid Receptor Agonists and Cannabinoid Receptor Agonists

David R Maguire*, Charles P France

UT Health Science Center, San Antonio, Texas

Background: Pain continues to be a significant clinical problem, and mu opioid receptor agonists (eg, hydrocodone) are the most effective treatment for moderate to severe pain. However, the use of opioids is limited by unwanted effects including constipation and sedation, as well as the development of tolerance and physical dependence following repeated use. Preclinical research confirms that some therapeutic effects of mu opioid receptor agonists (analgesia) are enhanced by cannabinoid receptor agonists (eg, Δ9-tetrahydrocannabinol [Δ9-THC]); however, pharmacological factors that contribute to the interaction are not fully understood. The purpose of this study was to determine whether the antinociceptive effects of agonists that vary in efficacy at the mu opioid receptor were similarly enhanced when combined with cannabinoid receptor agonists.

Methods: The antinociceptive effects of combinations of a mu opioid receptor agonist and a cannabinoid receptor agonist were studied in rhesus monkeys ($n = 4$) using a warm water tail withdrawal procedure.

Results: When administered alone, the high efficacy mu opioid receptor agonist etorphine (0.00056–0.001 mg/kg, s.c.), the moderate efficacy agonist morphine (0.1–10.0 mg/kg, s.c.), and the low efficacy agonist nalbuphine (0.1–32.0 mg/kg, s.c.) dose-dependently increased the latency for monkeys to remove their tails from warm (50 and 54o C) water. Pretreatment with doses of Δ9-THC (1.0 mg/kg, s.c.) or CP55940 (0.032 mg/kg, s.c.), that had no effect in 54°C water when administered alone, enhanced the antinociceptive effects of etorphine and morphine. Doses of morphine and etorphine that were without effect when administered alone, became fully effective when combined with otherwise ineffective doses of Δ9-THC and CP55940, shifting the opioid dose-effect curves leftward up to 16-fold. In contrast, the same doses of Δ9-THC and CP55940 failed to enhance the antinociceptive effects of an equi-effective range of doses of nalbuphine.

Conclusions: The cannabinoid receptor agonists Δ9-THC and CP55940 were more effective at enhancing the antinociceptive effects of a high-efficacy mu opioid receptor agonist (etorphine) and a moderate efficacy agonist (morphine) than those of a putatively lower-efficacy agonist (nalbuphine). These results suggest that the capability of cannabinoids to enhance the antinociceptive effects of mu opioid receptor agonists depends upon efficacy at the mu opioid receptor and, possibly, that opioids with at least moderate efficacy at the mu opioid receptor, such as morphine, are better suited to maximize the effectiveness of a combination-treatment strategy. Drug combinations allow for the possibility that smaller doses of individual drugs can be combined to maintain or improve the therapeutic effects while potentially reducing the likelihood of encountering the unwanted effects associated with larger doses of either drug administered alone. Taken together with research showing that other (eg, abuse-related) effects of mu opioid receptor agonists are not similarly enhanced, this research provides strong support for combining opioids with cannabinoids to treat pain.

Keywords: analgesia, cannabinoids, drug-drug interactions, opioids, pain.

Disclosures: D. Maguire, Nothing to Disclose; C. France, Nothing to Disclose.

W196. Dissecting Nucleus Accumbens Dynorphin Neurons in Aversion and Reward

Ream Al-Hasani, Jordan G McCall, Nicole Capik, Blessan Sebastian, Daniel Hong, Audra Foshage, Michael Krashes, Bradford Lowell, Thomas Kash, Michael R Bruchas*

Washington University, St Louis, Missouri

Background: The adverse effects of stress are well documented, yet many of the underlying mechanisms remain unclear and controversial. The dynorphin/kappa opioid system is implicated in the mediation of stress and resultant vulnerability to drug abuse. It is thought that stress causes dynorphin release activating kappa-opioid receptors (KOR) within both dopaminergic and serotonergic nuclei as well as their striatal targets. Consequently, much attention has focused on these systems in the modulation of KOR-mediated responses. Despite our current knowledge of central dynorphinergic cell body populations, a clear description of the axonal projections of these neurons is unknown.
Methods: We crossed the Cre-dependent tdTomato (Ai9) reporter mouse to a mouse expressing Cre recombinase under the same promoter as dynorphin (Dyn-Cre) so only dynorphinergic cells express tdTomato. This allows complete visualization of dynorphinergic circuitry throughout the brain. We also virally targeted channelrhodopsin-2 to striatal dynorphinergic neurons and optogenetically activated neuronal populations in both the dorsal and ventral NAc shell to measure aversion and reward behaviors using place preference, aversion, and operant conditioning.
Results: Using our dynorphin-cre-tDtomato cross we show robust dynorphin expression in cell bodies throughout the brainstem and forebrain. Clear visualization of intact projections throughout the brain and dynorphinergic projections can be seen from and within the cortex, striatum, amygdala, and numerous monoaminergic nuclei. Dynorphinergic neurons within the striatum are particularly interesting for the study of stress and drug abuse. Prior studies have shown that KOR agonists inhibit dopamine and serotonin release in the nucleus accumbens (NAc), which regulates aversive behaviors. Therefore, we investigated whether specific modulation of dynorphinergic neuronal firing in the NAc is sufficient to induce aversive behaviors. This activation significantly increased c-Fos immunoreactivity in dynorphinergic neurons and inhibited electrically-evoked EPSCs which was reversed by norBNI application. Furthermore, activation of ventral NAc shell induced conditioned and real-time aversive behavior, while dorsal NAc shell stimulation resulted in a place preference which was also shown to be positively reinforcing in an operant task paradigm.
Conclusions: The results presented here for the first time show a discrete subregion of dynorphin-containing cells in the ventral shell of the accumbens are required for aversion mediated by KOR activation. Furthermore, dorsal accumbens dynorphin cell activity is consistent with reward, perhaps via a classical dopamine D1-mechanism, but this requires further study. Understanding the mechanisms by which the dynorphin/kappa opioid system regulates negative affective behaviors will provide valuable insight into potential treatments for stress disorders and drug abuse.
Keywords: optogenetics, dynorphin, nucleaus accumbens, aversion, reward, kappa-opioid.
Disclosures: R. Al-Hasani, Nothing to Disclose; J. McCall, Nothing to Disclose; N. Capik, Nothing to Disclose; B. Sebastian, Nothing to Disclose; D. Hong, Nothing to Disclose; A. Foshage, Nothing to Disclose; M. Krashes, Nothing to Disclose; B. Lowell, Nothing to Disclose; T. Kash, Nothing to Disclose; M. Bruchas, Nothing to Disclose.

W197. Rapamycin, an Inhibitor of mTORC1 Signaling Activity, Improved Measures of Sociability in the BTBR T+ Itpr3tf/J Mouse Model of Autism Spectrum Disorder

Jessica Burket, Andrew Benson, Amy Tang, Stephen Deutsch*

Eastern Virginia Medical School, Norfolk, Virginia

Background: mTOR signaling overactivity is a common pathological point of convergence for several syndromic forms of autism spectrum disorder (ASD), such as Tuberous Sclerosis Complex and fragile X syndrome, stimulating interest in inhibiting mTORC1 activity as a therapeutic strategy for syndromic and nonsyndromic forms of ASDs. The genetically-inbred BTBR T+ Itpr3tf/J (BTBR) mouse has emerged as a model of ASD with upregulated *Ras* signaling activity, an important driver of mTORC1, in frontal cortex and cerebellum, relative to the C57Bl/6J (B6) mouse strain. Thus, we wondered if inhibiting the mTORC1 complex with rapamycin would improve the sociability of this strain. Recently, we reported that D-cycloserine, a partial glycineB site agonist of the NMDA receptor, had prosocial effects in the BTBR mouse. Interestingly, NMDA receptor activation is an important regulator of mTORC1 signaling activity, affecting the cationic amino acid transporters that transport arginine into the cell and the duration of signaling by the phosphorylated form of the Extracellular Signal-Regulated Kinase1/2 (ERK1/2).
Methods: Test mice were experimentally-naïve, 4-week old male, outbred Swiss Webster (Charles River Laboratories, Wilmington, MA) and genetically-inbred BTBR mice (Jackson Laboratories, Bar Harbor, ME). Stimulus mice were 4-week old male ICR mice (Charles River Laboratories, Wilmington, MA). Test mice were individually weighed prior to drug administration and up to 20 mice were tested in each condition. Rapamycin (10 mg/kg) or its 10% DMSO vehicle was injected intraperitoneally on four consecutive days in a volume of 0.01 ml/g of body weight and behavioral testing was conducted 60 min after the last injection. All animal procedures were approved by the Eastern Virginia Medical School Institutional Animal Care and Use Committee and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The laboratory adopted an established mouse behavioral procedure for the quantitative assessment of sociability using a three-compartment apparatus. A two-way ANOVA was used to examine effects of strain (BTBR *vs* Swiss Webster), treatment condition (ie, rapamycin *vs* vehicle), and their interaction on reliably obtained measures of sociability. *Post-hoc* comparisons were made with the Tukey-Kramer

Multiple Comparison Test. Paired *t*-tests were used to determine effects of rapamycin on the salience of the enclosed social stimulus mouse for BTBR and Swiss Webster mice.

Results: Whereas vehicle-treated Swiss Webster mice showed a preference for exploring/sniffing the enclosed stimulus mouse ($p<0.01$), the vehicle-treated BTBR mice did not show this same preference. However, BTBR mice treated with rapamycin spent significantly more time engaged in exploring/sniffing the enclosed stimulus mouse ($p<0.01$). During free interaction between test and stimulus mice, vehicle-treated BTBR and vehicle-treated Swiss Webster mice did not differ from each other in terms of discrete episodes of social approach and anogenital sniffing. However, treatment of the BTBR strain with rapamycin significantly increased their discrete episodes of social approach ($p<0.05$) and anogenital sniffing ($p<0.001$), relative to the vehicle-treated BTBR mice. Rapamycin treatment did not affect episodes of social approach and anogenital sniffing made by the Swiss Webster mice.

Conclusions: Treatment with a centrally-effective dose of rapamycin improved the sociability of the BTBR mouse strain on several reliably-obtained measures (ie, exploring/sniffing of the enclosed stimulus mouse and measures of social approach and anogenital sniffing during free interaction of test and stimulus mice). These data support therapeutic targeting of upregulated mTORC1 signaling activity to improve sociability. Clearly, viable medication strategies for the treatment of ASDs must be safe, tolerable and devoid of serious adverse medical side effects, especially when administered chronically. Thus, less-toxic alternative strategies for inhibiting mTORC1 activity should also be explored in translational clinical trials.

Keywords: rapamycin, mTOR, sociability, autism, mouse model.

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W198. MDPV Has Potent and Atypical Effects on Dopamine Release in Adolescent and Adult Rats

Cynthia M Kuhn*, Sabrina Ergun, Elizabeth Sears, Quentin Walker

Duke University School of Medicine, Durham, North Carolina

Background: 'Bath salts' are a group of novel psychostimulants that are disguised as bath salts used for external purposes only, but are actually snorted, injected or ingested for their euphoria-producing effects. A small but emerging literature suggests that these compounds act mainly as inhibitors of monoamine transporters and/or as monoamine releasing drugs. Methylenedioxypyrovalerone (MDPV) is the molecule most often present in 'bath salts' marketed in the United States. Previous data suggests that it acts as an inhibitor of the dopamine and norepinephrine transporters (DAT and NET). However, its mechanism of action is not understood in detail. Furthermore, its actions have only been described in males. Experience with previous psychostimulants suggests that adolescents and females should exhibit enhanced responses to MDPV. The purpose of the present study was to characterize the behavioral effects of MDPV on adolescent and adult male and female rats, and to begin a mechanistic study of its actions on the DAT. We investigated MDPV-induced locomotion and conditioned place preference, and studied its effects on dopamine neurotransmission using fast-scan cyclic voltammetry in intact animals.

Methods: Adolescent (PN 28) or adult (PN 70 or older) male and female rats from Charles River Laboratories were used in all experiments. Locomotor activity was determined by automated beam crossings and by observational rating after treatment of male and female adult and adolescent rats with saline or by methylenedioxypyrovalerone (MDPV), 0.3 or 1 mg/kg s.c. ($N=8$–12/group). Conditioned place preference in adolescent and adult males was evaluated using an unbiased design. One Day 1, baseline preference for two sides of a chamber which were differentiated by unique visual, tactile and olfactory stimuli. After 3 days of pairing for 20 min (saline in morning, on one side, MDPV, 1.0 mg/kg, in the afternoon on the other side) animals were allowed to explore the entire chamber on the 4th day, and the change in preference from baseline was determined. Finally, separate adolescent and adult males were anesthetized with urethane and dopamine release from the nucleus accumbens shell determined by fast-scan cyclic voltammetry after administration of MDPV (1 mg/kg) or cocaine (15 mg/kg i.p.). Statistics on all results were analyzed by 3 way (sex × age × drug) repeated measures ANOVA (for locomotion), 2 way (age × drug) repeated measures ANOVA or t test (CPP) using NCSS. All experiments were approved by the Duke University IACUC.

Results: MDPV caused a robust, dose dependent and long lasting increase in locomotor activity and stereotypy in all groups ($p<0.001$ for main effect of dose and sex, for dose × time, dose × sex, dose × age, sex × age, dose × age × interval and dose × sex × interval. Females responded more than males, and adolescent males responded more than adult males. MDPV also caused a robust CPP in both adolescent and adult rats ($p<0.001$ for effect of drug). MDPV had opposite effects on phasic and tonic DA release. MDPV caused a significant increase in the number of DA release events (phasic) and the increase in adult males was far more robust following MDPV than cocaine. MDPV increased transient duration more in adolescents than adults. A marked decline in baseline (tonic) DA release was observed from 15 to 90 min after administration of MDPV to adults. Surprisingly, this fall in tonic DA concentration was simultaneous with increased transient frequency (phasic). In adolescents, tonic DA rose for 10 min and then fell slightly by 30 min, but did not decrease dramatically as observed in adults. These findings contrast markedly with the effects of cocaine, which reliably increases both tonic and phasic DA release in adolescent and adult rats.

Conclusions: These results confirm recent findings that MDPV is a very potent and efficacious psychostimulant. We show for the first time that MDPV, like other DAT inhibitors, exerts more locomotor stimulant behavior and more DA release in adolescent than adult male rats. Finally, the findings with tonic DA indicate that MDPV exerts an effect on DA neurotransmission unlike that of other DAT

inhibitors. The functional significance of this imbalance between phasic and tonic release remains to be established, but the increased phasic:tonic ratio could contribute to its high reinforcing efficacy. Supported by DA009079.
Keywords: adolescence, females, bath salts, MDPV, dopamine.
Disclosures: C. Kuhn, Nothing to Disclose; S. Ergun, Nothing to Disclose; E. Sears, Nothing to Disclose; Q. Walker, Nothing to Disclose.

W199. Chronic Lithium Treatment Diminishes the Amplitude of Electrically Evoked Dopamine Concentration Transients in the Nucleus Accumbens Core

Adem Can*, Roger Cachope, Douglas Frost, Joseph Cheer, Todd D Gould

University of Maryland, Baltimore, Maryland

Background: Lithium (Li+) is a prototypical mood stabilizer with efficacy in the treatment of the depressive and manic phases of bipolar disorder and the reduction of suicidal behavior. Increasing evidence indicates that the mesolimbic dopaminergic (DAergic) circuitry is crucial for the expression of depressive and manic behaviors. Li+ treatment attenuates mania-like behaviors elicited by agents that cause elevated DAergic activity in the nucleus accumbens (NAc). While the beneficial clinical effects of Li+ as a mood stabilizer are well established, and its effects on intracellular signaling have been extensively characterized, the physiological mechanism by which Li+ exerts its actions on the mesolimbic DA system are not well understood. This gap in knowledge is a likely obstacle to the development of novel treatments required for mood disorders. It has previously been shown that chronic Li+ treatment lowers extracellular DA concentrations in the rat NAc, as assessed by microdialysis. However, due to the limited temporal resolution of microdialysis, it is unclear whether this finding reflects alterations of basal extracellular DA levels or changes in the magnitude or duration of phasic DA transients. In order to address this issue, we used fast-scanning cyclic voltammetry (FSCV) to record and analyze changes in extracellular DA concentrations in the NAc core evoked by electrical stimulation of the ventral tegmental area (VTA) with sub-second temporal resolution.
Methods: We determined the effects of chronic and acute Li+ treatments on DA release. Mice were 11–12 weeks old at the start of the experiments. For the chronic Li+ treatment experiment, mice were fed 0.4% LiCl containing chow or control chow, *ad libitum*, for 24–40 days resulting in brain Li+ concentrations within the human therapeutic range ($\sim$1 mM). FSCV recordings were made on the last day of treatment. For acute Li+ treatment experiment, 300 mg/kg LiCl or vehicle was injected i.p. five hours prior to making FSCV recordings resulting in brain Li+ levels similar to those obtained following chronic administration, and that were stable and sustained over the course of the experiment. Mice were anesthetized with 1.5 g/kg urethane. Using a stereotaxic frame, a bipolar stimulating electrode was placed in the VTA and a glass-encased carbon fiber electrode was placed in the NAc core. DA transients were evoked by trains of 60 rectangular, biphasic pulses (2 ms/phase; 1 pulse train/3 min), delivered in the VTA. While recording at the depth of maximum DA release, a series of eight pulse trains of increasing amplitude (100 μA to 800 μA in 100 μA steps) was applied to the VTA. Transients evoked by 300 μA pulses were used to assess the release and reuptake kinetics. Recording electrodes were calibrated after the experiment using a DA solution of known concentration.
Results: Mice treated chronically with Li+ manifested a significantly decreased peak amplitude of stimulation-evoked DA transients in the NAc core ($F(1,16)=5.01$, $p<0.05$). There was also a main effect of stimulation amplitude ($F(7,16)=35.24$, $p<0.0001$) and a significant interaction between the stimulation magnitude and treatment ($F(7,16)=4.3$, $p<0.001$). *Post-hoc* pairwise comparisons revealed that chronic Li+ treated mice had lower levels of DA at all stimulation magnitudes from 300 to 700 μA, inclusive, compared to controls ($p<0.05$). Chronic Li+ treatment did not significantly alter the rise time or decay time of the DA transients. Acute Li+ treatment did not significantly alter the peak amplitude or decay constant of stimulus-evoked DA transients.
Conclusions: These results demonstrate that chronic (but not acute) Li+ treatment attenuates phasic DA release in the NAc core evoked by electrical stimulation of the VTA without altering the kinetics of DA release or reuptake. Our data extend prior findings by showing that the amplitude of stimulation-evoked DA transients are diminished by chronic Li+ treatment. DAergic neurotransmission in the NAc has been associated with both mania-like and depression-like behaviors in animal models. The amplitude and timing of DA transients in the NAc are critical in reward prediction, motivational and cognitive control of behavior, and impulsivity. Because impulsivity is strongly associated with suicide, our findings suggest that reduced DA release in the NAc may be part of the mechanism of lithium's anti-suicidal effects. These results are also congruent with the established clinical finding that Li+ treatment is effective only when administered chronically.
Keywords: mood disorders, lithium, dopamine, mouse, voltammetry.
Disclosures: A. Can, Nothing to Disclose; R. Cachope, Nothing to Disclose; D. Frost, Nothing to Disclose; J. Cheer, Nothing to Disclose; T. Gould, Nothing to Disclose.

W200. Effects of Monoamine Releasers with Varying Selectivity to Release Dopamine *vs* Norepinephrine in Assays of Cocaine Discrimination and Cocaine *vs* Food Choice

Matthew L Banks*, Clayton Bauer, Bruce Blough, Richard B Rothman, John Partilla, Steve Negus

Virginia Commonwealth University, Richmond, Virginia

Background: There is currently no Food and Drug Administration-approved pharmacotherapy for the treatment of cocaine dependence. The dopamine (DA)/norepinephrine (NE) *vs* serotonin (5HT)-selective releaser amphetamine has demonstrated efficacy to decrease cocaine-taking behavior in

preclinical and human laboratory studies and clinical trials. However, use of amphetamine as an anti-cocaine addiction pharmacotherapy is hindered by its own high abuse liability. Moreover, the role of DA *vs* NE release in mediating the abuse potential and anti-cocaine taking effects of monoamine releasers is not well understood. Previous studies have suggested a greater role of NE *vs* DA release in mediating the abuse-related effects. The present study determined effects of 5 novel monoamine releasers: 1-(5-chloro-1H-indol-3-yl)-propain-2-amine (PAL-542), 1-(5-fluoro-1H-indol-3-yl)propan-2-amine (PAL-544), 1-(1H-indol-5-yl)propan-2-amine (PAL-571), and (R)-1-(1H-indol-1-yl)propain-2-amine (PAL-569) that varied along a continum in their selectivity for releasing DA *vs* NE in an assay of cocaine discrimination in rats and assays of cocaine discrimination and cocaine self-administration in rhesus monkeys. We hypothesized the DA-selective releaser PAL-542 would not produce abuse-related effects in the assay of cocaine discrimination whereas the NE-selective releaser PAL-569 would produce abuse-related effects in both rats and monkeys. We also hypothesized continous treatment with PAL-542 would produce amphetamine-like decreases in cocaine choices and reciprocal increases in food choices in the cocaine *vs* food choice procedure.

Methods: Rats were trained to discriminate cocaine (5.6 mg/kg, IP) from saline in a two-key, food-reinforced discrimination procedure. Monkeys were trained to discriminate cocaine (0.32 mg/kg, IM) from saline in a two-key food-reinforced discrimination procedure. Test sessions were usually conducted on Tuesdays and Fridays, and training sessions were conducted on Monday, Wednesday, and Thursday. In the assay of cocaine self-administration, rhesus monkeys implanted with a chronic indwelling double-lumen venous catheter, initially responded during daily 2-h sessions under a concurrent schedule of food delivery (1-g pellets, fixed-ratio 100 schedule) and cocaine injections (0–0.1 mg/kg/injection, fixed-ratio 10 schedule). One lumen of the double-lumen catheter was designated as the 'cocaine' lumen and was always filled with the self-administered cocaine solution. The other lumen was designated as the 'treatment' lumen, and saline or PAL-542 (0.032–0.1 mg/kg/h) was continuously infused 23 h/day during 7-day treatment blocks.

Results: In the assay of cocaine discrimination, only PAL-571 produced full substitution in 1 of 5 rats. All other compounds (PAL-542, PAL-544, and PAL-569) failed to produce cocaine-like discriminative stimulus effects up to doses that eliminated rates of responding. In monkeys, the DA-selective releasers PAL-542 and PAL-544 produced full substitution for the discriminative stimulus effects of cocaine in 2 of 5 monkeys, whereas the NE-selective releaser PAL-569 produced full substitution in 3 of 5 monkeys. PAL-571 produced full substitution in a single monkey. Continuous treatment with the DA-selective releaser PAL-542 produced leftward shifts in the cocaine *vs* food choice dose effect function and decreased rates of operant responding.

Conclusions: Selectivity to release DA *vs* NE did not correlate with cocaine-like discriminative stimulus effects in either rats or rhesus monkeys. Furthermore, the DA-selective releaser PAL-542 did not produce an amphetamine-like reduction in cocaine choice. Overall, these results do not support the development of the four novel monoamine releasers tested as candidate anti-cocaine addiction medications.

Keywords: rhesus monkey, cocaine, dopamine, norepinephrine, serotonin.

Disclosures: M. Banks, **Part 1:** His research has been funded by NIH. In the past two years he has received compensation as a collaborator with the pharmaceutical companies Abbott and Purdue for projects related to opioid pharmacology and analgesic drug development. Dr Banks declares that the present study was not related to this professional relationship and should not be perceived as constituting a potential conflict of interest.; C. Bauer, Nothing to Disclose; B. Blough, Nothing to Disclose; R. Rothman, Nothing to Disclose; J. Partilla, Nothing to Disclose; S. Negus, **Part 1:** During the past 2 years, he has received compensation as a consultant for or collaborator with the pharmaceutical companies Abbott and Limerick Biopharma for projects related to opioid pharmacology, analgesic drug development, or assessment of abuse liability. Dr Negus declares that the present study was not related to this professional relationship and should not be perceived as a conflict of interest.

W201. NS1738, a Positive Allosteric Modulator of Alpha7 Nicotinic Receptors, as Adjunctive Treatment in Schizophrenia. An Experimental Study.

Monica M Marcus*, Åsa Konradsson-Geuken, Kristin Feltmann, Vladimir Ivanov, Björn Schilström, Kent Jardemark, Torgny H Svensson

Karolinska Institutet, Stockholm, Sweden

Background: The α7 nicotinic acetylcholine receptor (nAChR) is a potential target for treatment of cognitive deficits in eg schizophrenia. Both preclinical and clinical studies with α7nAChR agonists have shown promising results. Experimentally, selective α7 agonists have been shown to normalize defective sensory gating, and adjunctive treatment with a selective α7 agonist has been found to enhance the antipsychotic-like effect of risperidone in the conditioned avoidance response (CAR) test. Furthermore, add-on treatment with α7 agonists has been found to improve cognitive impairment and negative symptoms in schizophrenic patients. However, directly acting α7 agonists seem to require a specific and rather narrow dose-range to improve cognition and there are concerns about the risk of unwanted side effects. Therefore, positive allosteric modulators (PAMs) of the α7nAChR may provide a novel strategy to avoid some of these problems, since α7 PAMs bind to an allosteric binding site and thereby enhances the effect of the endogenous ligand. Against this background, we have here examined, in rats, the effect of the α7 PAM NS1738 on brain dopaminergic and noradrenergic cell firing *in vivo* and on a measure of cognitive function. Furthermore, adjunctive treatment with NS1738 added to risperidone was examined for antipsychotic-like efficacy. Finally, we studied the effect of these drugs on glutamatergic NMDA receptor-mediated transmission in medial prefrontal cortex (mPFC), both when given alone and in combination.

Methods: We examined antipsychotic efficacy using the CAR test, the effect on cognition using the novel object

recognition (NOR) test, the effect on cell firing in the ventral tegmental area (VTA) and locus coeruleus (LC), respectively, using extracellular single-cell recording *in vivo* and, finally, the effects on NMDA receptor-mediated currents in pyramidal neurons in the mPFC using intracellular electrophysiological recording *in vitro*.
Results: NS1738 increased both firing rate (0.1 mg/kg i.v.) and in particular burst firing (0.05–0.1 mg/kg i.v.) of dopaminergic cells in the VTA, whereas no effect was seen on noradrenergic cells in the LC. Furthermore, NS1738 (1 mg/kg s.c.) significantly improved recognition memory. Addition of NS1738 (1 mg/kg s.c.) to a low dose of risperidone (0.25 mg/kg i.p.) enhanced the antipsychotic-like effect in the CAR model. Whereas neither risperidone (10 nM) nor NS1738 (500 nM) produced any effect on NMDA-induced currents in pyramidal cells in the mPFC when administered alone, the combination of the two drugs potentiated these currents (preliminary results).
Conclusions: Our results show that the α7 PAM NS1738 can enhance cognitive functioning, ie recognition memory, an effect that may be partly related to its stimulatory effect on dopaminergic cells in the VTA, ie increased firing rate and burst firing. NS1738 also enhanced the antipsychotic-like effect of a low dose of risperidone that by itself could not generate sufficient antipsychotic activity. These data suggest that in combination with an α7 PAM, a dose reduction of risperidone with maintained antipsychotic effect may be achieved. Moreover, a combination of NS1738 and risperidone in concentrations that were ineffective when given alone, enhanced cortical glutamatergic NMDA receptor-mediated transmission, an effect which in principle may contribute to improve working memory. In conclusion, the present results suggest that, in similarity with directly acting agonists, α7 PAMs may be used to augment the therapeutic effect of antipsychotic drugs, such as risperidone, and in addition to improve cognitive impairment and, by inference, also negative symptoms in schizophrenia.
Keywords: alpha7 nicotinic receptor PAM antipsychotic drug schizophrenia cognition dopamine NMDA prefrontal cortex.
Disclosures: M. Marcus, Nothing to Disclose; □. Konradsson-Geuken, Nothing to Disclose; K. Feltmann, Nothing to Disclose; V. Ivanov, Nothing to Disclose; B. Schilström, Nothing to Disclose; K. Jardemark, Nothing to Disclose; T. Svensson, Nothing to Disclose.

W202. Acute Vilazodone Administration Induces Hypothermia in Mice Through a 5-HT1A Mechanism

Alvaro Garcia-Garcia*, Pradeep Banerjee, E David Leonardo

Columbia University, New York, New York

Background: The serotonin 1A receptor is an inhibitory G-protein coupled receptor that exists in two major forms in the nervous system. It functions as an autoreceptor on serotonergic neurons in the raphe nuclei, where it regulates serotonergic tone, and it exists as a heteroreceptor in non-serotonergic neurons where it mediates a hyperpolarizing response to released serotonin. Vilazodone is a potent selective serotonin reuptake inhibitor as well as a high affinity 5-HT1A partial agonist,.
Methods: Here we examined the effects of acute Vilazodone administration on body temperature. Previous studies in rats demonstrated that 8-OH-DPAT but not Vilazodone administration resulted in a hypothermic response. This response is thought to be mediated by non-raphe 5-HT1A heteroreceptors in the rat, suggesting that vilazodone did not act at these receptors. In this study, we performed a similar experiment in mice, where the hypothermic response to 5-HT1A agonists is known to be mediated through 5-HT1A autoreceptors.
Results: Our results demonstrate a robust hypothermic response to Vilazodone administration. Such a response was not observed with a pure SSRI like Citalopram, and the response was blocked when the 5-HT1A antagonist WAY 100,635 was co-administered. We further demonstrate that Vilazodone but not Citalopram is able to block the hyperthermic response to stress, and that this effect is blocked by co-administration of WAY 100,635.
Conclusions: These data strongly suggest that Vilazodone has significant effects at 5-HT1A autoreceptors in the raphe. In addition, coupled with the previous experiments conducted in rats, these data confirm an interesting species difference between rats and mice in 5-HT1A mediated thermoregulation.
Keywords: 5-HT1A, vilazadone, citalopram, depression, anxiety.
Disclosures: A. Garcia-Garcia, Nothing to Disclose; P. Banerjee, **Part 1:** I am a full time employee of Forest Laboratoires and I hold company stocks. **Part 3:** I am a full time employee of Forest Laboratoires and I hold company stocks. **Part 5:** Forest Laboratories Inc.; E. Leonardo, **Part 4:** Received a two year grant from Forest Research Laboratories to investigate the role of 5-HT1A agonism in the efficacy of Vilazodone.

W203. Discovery of Metabotropic Glutamate Receptor Subtype 5 PAMs that Display Stimulus Bias Reveals that *in Vivo* Efficacy in Animal Models can be Achieved Without Direct Potentiation of NMDAR Currents

Jerri M Rook, Paige N Vinson, Thomas M Bridges, Shaun R Stauffer, Ayan Ghoshal, J Scott Daniels, Colleen M Niswender, Hilde Lavreysen, Claire Mackie, Jose Manuel Bartolome, Gregor J Macdonald, Thomas Steckler, Carrie K Jones, Craig W Lindsley, P Jeffrey Conn*

Vanderbilt University Medical Center, Nashville, Tennessee

Background: Multiple psychiatric and neurological disorders, such as schizophrenia, are associated with disruptions in excitatory synaptic signaling primarily mediated through the *N*-methyl-*D*-aspartate subtype of glutamate receptors (NMDAR). Accumulating evidence indicates that the metabotropic glutamate receptor subtype 5 (mGlu5) is a closely associated signaling partner with the NMDAR and may be important in regulating NMDAR function in forebrain regions implicated in the pathology of schizophrenia. Our group and others have shown that selective potentiation of mGlu5 responses using positive allosteric modulators

(PAMs) potentiates NMDAR function and produces efficacy in animal models of cognition and psychosis. Recently, we have identified different mGlu5 PAMs that can exhibit distinct stimulus bias and can selectively modulate coupling to some, but not all signaling pathways. We now report the discovery and characterization of the selective mGlu5 PAM VU0409551 that does not enhance NMDAR signaling, but displays robust efficacy in preclinical models of antipsychotic-like activity and enhancement of cognition.

Methods: HTS, cheminformatics and medicinal chemistry approaches were used to develop the new, highly selective series of mGlu5 PAMs as represented by VU0409551. An iterative analog approach was used in order to generate chemical libraries around initial hits for each receptor subtype. Calcium mobilization assays were used in parallel to generate functional potency and efficacy data for all compounds, and potential off target activity was assessed in Panlabs lead profiling screens by percent radioligand displacement at 10 μM. Pharmacokinetic parameters were determined in a rat plasma-brain-level study using LC/MS detection of drug levels. Whole cell patching was used to measure NMDAR current following drug administration. Finally, *in vivo* efficacy was determined across several preclinical rodent models predictive of antipsychotic-like activity or cognitive enhancement in both mice and rats.

Results: Here we report discovery of the selective mGlu5 PAM VU0409551, which exhibits EC50 and Glumax values of 235 nM and 75%, respectively, for potentiation of mGlu5 with no activity at the other mGlu subtypes or off target activity at other GPCRs. Unlike previously reported mGlu5 PAM, VU0409551 does not enhance NMDAR currents in hippocampal neurons or potentiate threshold long term potentiation (LTP) in the SC-CA1 synapse at a 10 mM concentration in slice-based physiology experiments. Interestingly, VU0409551 produced robust dose-dependent effects in animal models of antipsychotic-like activity, including reversal of amphetamine-induced hyperlocomotion and disruption of prepulse inhibition. Moreover, VU0409551 was highly efficacious in several rodent models of cognitive function, including enhancement of the acquisition of the hippocampal-dependent contextual fear conditioning task.

Conclusions: Collectively, our studies with VU0409551 demonstrate that allosteric modulation of mGlu5 can exhibit stimulus bias and still show *in vivo* efficacy without potentiating direct coupling of mGlu5 to potentiation of NMDAR currents. These studies provide further support for the development of mGlu5 PAMs for the treatment of symptoms associated with psychiatric disorders including schizophrenia.

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Keywords: mGluR5 schizophrenia animal models physiology allosteric modulators.

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W204. Want It, Need It, and Can't Control It: The Dynamic Relationship between Impulsivity and the Propensity to Binge Eat

Noelle Anastasio*, Kathryn Cunningham

University of Texas Medical Branch, Galveston, Texas

Background: Eating is essential for life, but repeated consumption of large amounts of food in a brief period (ie, bingeing) can alter the reward value of food and food-related cues and fuel binge-eating cycles. Binge eating disorder (BED) is the most prevalent eating disorder in the U.S., and is linked to severe obesity as well as psychological and medical consequences. Maladaptive patterns of intake may be encoded by a preexisting vulnerability coupled with the influence of environmental variables. Impulsivity, a predisposition toward rapid unplanned reactions to stimuli, is one of the multifaceted determinants underlying the

etiology of dysregulated eating, its pathogenesis, and treatment outcomes. Impulsivity and dysregulated eating converge mechanistically at the level of serotonin (5-HT) neurotransmission at the 5-HT2A receptor (5-HT2AR) and 5-HT2CR within an integrated neural network that orchestrates a balance between stimulus-driven and goal-driven behaviors. Disturbances in this system may engender maladaptive eating behaviors [esp., binge eating on palatable high fat/sugar ('sweet-fat') foods] and the response to food stimuli. Yet, our understanding of the relationships linking impulsive behavior to binge eating and/or relapse in the presence of food stimuli, and their shared mechanisms, is very limited.

Methods: Three assays in male Sprague-Dawley rats were established to explore this hypothesis: (1) rats were identified as high (HI) or low impulsive action (LI) phenotypes in the 1-choice serial reaction time (1-CSRT) task, (2) the propensity for binge eating was assessed upon 2-h access to sweet-fat chow (17% sucrose and 45% fat by kCal), and (3) the motivational efficacy of sweet-fat pellets and associated cues were assessed in a self-administration/forced abstinence paradigm. All experiments were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals (2011) and with the University of Texas Medical Branch Institutional Animal Care and Use Committee approval.

Results: The upper and lower 25% of rats, respectively, were identified as high impulsive (HI) or low impulsive (LI) phenotypes based on premature responses in the 1-CSRT task. Levels of impulsive action positively correlated with the ratio of the 5-HT2AR to 5-HT2CR protein expression in the medial prefrontal cortex ($r = 0.558$, $p < 0.01$), suggesting a potentially-causal role for a 5-HT2AR:5-HT2CR imbalance in this phenotype. HI rats consumed significantly more kcal during the 2-h binge *vs* LI rats ($p < 0.05$), experimentally linking the propensity for impulsive action to the magnitude of binge eating. Acquisition of sweet-fat chow self-administration was identical in HI and LI rats. However, HI rats exhibited a higher breakpoint and higher sweet-fat chow-seeking behavior *vs* LI rats ($p < 0.05$), indicating a greater motivation for sweet-fat chow and reactivity to sweet-fat chow stimuli. Viral-mediated genetic elimination of the 5-HT2CR in the nucleus accumbens, a subregion of the corticostriatal circuitry important in reward and motivation, resulted in high binge eating, high impulsivity, and a shift in functional tone of the homologous 5-HT2AR.

Conclusions: We propose that the high sugar/fat food is more 'wanted' by high *vs* low impulsive rats which promotes impulsive behavior and binge eating more so in high impulsive rats. Further, these data support the hypothesis that inherent impulsivity and binge eating reciprocally interact at the level of an imbalance in 5-HT2AR:5-HT2CR homeostasis within the corticostriatal circuit. Through addressing a fundamental gap in our knowledge of how the neural and behavioral aspects of impulsive action relate to binge eating, we hope to develop pharmacological strategies to minimize binge eating and enhance clinical practice for disorders of overeating.

Keywords: impulsivity, binge-eating, serotonin, corticostriatal circuit.

Disclosures: N. Anastasio, Nothing to Disclose; K. Cunningham, Nothing to Disclose.

W206. Electrophysiological Investigation of the Effects of a Subanesthetic Dose of Ketamine on Monoamine Systems

Pierre Blier*, Karim El Iskandarani, Chris Oosterhof, Mostafa El Mansari

University of Ottawa, Ottawa, Ontario, Canada

Background: Considerable attention has been devoted to the glutamatergic system as a possible contributor to the antidepressant response in treatment-resistant patients. The most striking breakthrough is the rapid antidepressant action of low doses of ketamine administered intravenously. Ketamine, a derivative of phencyclidine (PCP), is a non-competitive NMDA antagonistthat acts primarily by blocking the NMDA receptor at the PCP site [1]. The antidepressant qualities of ketamine were also demonstrated preclinically, where a low dose (10 mg/kg) resulted in a decrease in latency of escapes in the learned helplessness test and a decrease in immobility times in the forced swim test, both behavioral models of depression in rodents [2]. Evidence demonstrates tight interactions between the glutamatergic and monoaminergic systems. It is thus hypothesized that the rapid antidepressant effects of ketamine are due to, at least in part, its effects on the monoaminergic systems.

Methods: Experiments were carried out on male Sprague Dawley rats weighing 275–300 g at the time of the experiments. Rats were anesthetized with chloral hydrate (400 mg/kg; ip) and mounted on a stereotaxic frame. Extracellular unitary recordings of monoamine neurons were performed using single-barrel glass micropipettes filled with 2 M NaCl solution, with impedances ranging from 2 to 4 MΩ. Recordings were carried out in the locus coeruleus (LC) and the ventral tegmental area (VTA). Presumed NE and DA neurons were then identified using the criteria previously described. Extracellular Recordings were carried out 30 min following intraperitoneal ketamine administration (10 mg/kg) for acute experiments. Extracellular recording and microiontophoresis of CA3 pyramidal neurons in the hippocampus were carried out with five-barreled glass micropipettes. The central barrel used for the unitary recording was filled with a 2 M NaCl solution, and the impedance of these electrodes ranged from 2 to 4 MΩ. The CA3 region of the hippocampus was identified using the criteria previously described, and the responsiveness of CA3 pyramidal neurons to ionitophoretically applied AMPA and NMDA was assessed prior to, and up to 30 min following administration of ketamine.

Results: Followingacute ketamine administration, an immediate and significant increase in the firing rate of NE neurons was observed in the LC (control: 1.4 ± 0.06 Hz, ketamine: 1.8 ± 0.08 Hz, $p < 0.05$). Additionally, there was an enhancement in the number neurons exhibiting bursting activity (control: $17\% \pm 1.7$, ketamine: $30\% \pm 3.8$, $p < 0.01$). A doubling in the number of spontaneously firing DA neurons was also observed in the VTA (control: 1.2 ± 0.08 neurons/track, ketamine: 2.6 ± 0.4 neurons/track, $p < 0.01$). The effect of ketamine on these electrophysiological parameters was prevented by pre-administration of the AMPA receptor antagonist NBQX 10 min prior to ketamine administration. Despite CA3 pyramidal neurons displaying no change in their responsiveness to iontophoretically applied NMDA, an increase in the responsiveness to iontophoretically applied

AMPA was observed 30 min following ketamine administration at a dose of 10 mg/kg (control: 119 ± 12 spikes/nA, ketamine: 191 ± 28 spikes/nA, $p < 0.05$). However following administration of a higher, yet subanesthetic dose of ketamine (25 mg/kg), pyramidal neurons exhibited a decreased responsiveness to NMDA (45% ± 21, $p < 0.05$), as well as an enhanced responsiveness to AMPA (45% ± 9, $P < 0.05$). The latter was more rapid at the higher dose (15 mins).

Conclusions: The present results suggest that acute ketamine administration produces an increase in NE and DA activity. This enhancement of firing and bursting activity of NE neurons in the LC, as well as the increase in number of spontaneously firing DA neurons in the VTA following acute ketamine administration were blocked by NBQX, indicating that these effects are mediated, at least in part, *via* AMPA receptors. Furthermore, ketamine administration caused an increase in the responsiveness of pyramidal neurons to iontophoretically applied AMPA, suggesting an increased responsiveness of these receptors. Collectively, these results suggest that a potentiation of AMPA receptors responses might play a vital role in the rapid, ketamine-induced antidepressant response. Further investigations are underway in order to determine the actions of ketamine on glutamate and monoaminergic projection areas. 1. Strayer R.J., Nelson L.S. 2008. Adverse events associated with ketamine for procedural sedation in adults. Am J Emerg Med 26, 985–1028. 2. Chung C.H. 2012. New perspectives on glutamate receptor antagonists as antidepressants. Arch Pharmacal Res 35, 573–577.

Keywords: ketamine; norepinephrine, dopamine; electrophysiology, antidepressant.

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W207. The Effects of Methylphenidate and Atomoxetine on Glutamate in the Prefrontal Cortex of the Awake Spontaneously Hypertensive Rat Model of ADHD

Paul Glaser, Erin Miller, Greg Gerhardt*

University of Kentucky, Lexington, Kentucky

Background: Recent clinical research has implicated glutamate in the etiology of attention-deficit/hyperactivity disorder (ADHD). Using proton magnetic resonance spectroscopy, it was discovered that children and adults with ADHD exhibited increased levels of a marker for glutamine/glutamate in the prefrontal cortex (PFC). Methylphenidate (MPH), a dopamine reuptake inhibitor, and atomoxetine (ATX), a selective norepinephrine transporter inhibitor, were both found to lower the levels of the glutamate marker in both children and adults. Previous evaluations of the spontaneously hypertensive rat (SHR/NCrl) model of ADHD found that the SHR have increased AMPA receptor activity and elevated calcium levels in the PFC, suggesting that altered glutamatergic neurotransmission may exist in the PFC of the SHR. Thus, we sought to investigate direct glutamate signaling in the SHR and the effects of MPH and ATX on these glutamate levels.

Methods: All studies were performed using constant potential amperometry with the FAST16mkIII system (Quanteon LLC). Glutamate oxidase-coated S2 MEAs (4 Pt sites measuring 15 × 333 μm arranged vertically in dual pairs) were used as previously described (see Hinzman *et al*, 2010). A size exclusion layer of 1,3–phenylenediamine (mPD) was electroplated onto the Pt sites to block large molecule interferents, such as ascorbic acid and dopamine, from reaching the recording sites. Immediately prior to implantation, an *in vitro* calibration was performed to ensure that the MEA was sensitive and selective for glutamate. Seven week old male SHR and WKYs were anesthetized with isoflurane (2–3%) and the MEA was inserted into the prelimbic prefrontal cortex (from bregma, AP: +3.2 mm; ML: −0.8 mm; from surface of brain, DV: −3.5 mm). A Ag/AgCl reference electrode was inserted into the contralateral hemisphere, posterior to bregma. Finally, the modified MEA and Ag/AgCl reference were secured with dental cement. After a recovery period of 2 days, the animals were connected to a miniaturized pre-amplifier that allowed for unrestricted movement around an open-field activity box. Glutamate recordings were obtained at 40 Hz on alternate days for 11 consecutive treatment days. Saline was administered s.c. after an hour of baseline recording, followed by MPH (2 mg/kg s.c.) or ATX (1 mg/kg s.c.) administered an hour post-saline injection. A final 3 h period of glutamate and behavior or behavior alone was recorded. Behavior was recorded using Digiscan Animal Monitoring activity boxes (Omnitech Inc.). Resting glutamate levels pre- and post-treatment were calculated, as well as phasic glutamate during each recording period. The animals' activity was determined on tethered (glutamate recording) and non-tethered (no glutamate) days, as were the effects of acute and chronic MPH and ATX treatment between strains. Data were analyzed using two-way (strain x day) repeated-measures ANOVAs followed by Bonferroni *post-hoc* comparisons. All experiments 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: Locomotion data was consistent with previous reports using MPH and ATX in these strains. MPH increased behavior acutely in the SHR and chronically in both the SHR and WKY and ATX reduced locomotion acutely as well as chronically in the SHR only. On the first day of the glutamate recordings, the SHR was found to have higher tonic levels than the WKY in the prelimbic cortex (100 *vs* 50 μM). Chronic treatment with both MPH and ATX reduced tonic levels to that of control in the prelimbic cortex. Phasic release of glutamate in the PFC was found to be similar between the SHR and WKY (average amplitude of 10 μM and frequency of 1000 phasic events). Interestingly, both chronic MPH and ATX treatment increased the amplitude while decreasing the frequency of these phasic glutamate events in the SHR (average amplitude of 15 μM and frequency of 750 phasic events) but not the WKY.

Conclusions: We have demonstrated elevated tonic and phasic glutamate in the prelimbic prefrontal cortical region of the SHR. These data provide evidence that drugs acting on the catecholaminergic systems have a profound effect on glutamate in the SHR model of ADHD, suggesting that novel therapeutics targeting glutamate may have a beneficial impact on individuals with ADHD.

Keywords: glutamate ADHD attention atomoxetine methylphenidate.
Disclosures: P. Glaser, Nothing to Disclose; E. Miller, Nothing to Disclose; G. Gerhardt, **Part 1:** Owner of Quanteon, LLC, **Part 2:** Owner of Quanteon, LLC, **Part 4:** Contracts from Eli Lilly and Medtronic.

W208. Characterization of the Novel M4 Muscarinic Acetylcholine Receptor Positive Allosteric Modulator Vu0467154 in Animal Models of Antipsychotic-like Activity, Cognitive Enhancement and Changes in Sleep-wake Architecture

Carrie K Jones*, Thomas M Bridges, Michael Bubser, Robert W Gould, Ditte Dencker Thorbek, Michael D Grannan, J Scott Daniels, Meredith J Noetzel, Colleen M Niswender, Mark E Duggan, Nicholas J Brandon, John Dunlop, Michael W Wood, Craig W Lindsley, P Jeffrey Conn

Vanderbilt Center of Neuroscience Drug Discovery, Nashville, Tennessee

Background: Accumulating evidence indicates that selective positive allosteric modulators (PAMs) of the M4 muscarinic acetylcholine receptor may provide a novel therapeutic strategy for the treatment of psychotic symptoms and behavioral disturbances observed in various psychiatric and neurologic disorders. Previously, our group reported the discovery of an early generation of systemically active M4 PAMs, represented by VU0152100, which displayed central effects in reversing amphetamine-challenge models in rodents. While VU0152100 served as an important tool compound for initial proof of concept studies on the effects of M4 PAMs *in vivo*, limitations in the physiochemical and pharmacokinetic properties of the ligand restricted its broader use for extensive characterization in preclinical models of psychosis and cognitive disruptions. We now report the identification of a highly optimized series of selective M4 PAMs, represented by VU0467154 that exhibits low nanmolar potency, enhanced pharmacokinetic properties in mice and rats for *in vivo* dosing and robust efficacy across several preclinical models of antipsychotic-like activity and enhancement of cognition.
Methods: HTS and medicinal chemistry approaches were utilized to identify this highly optimized series of selective M4 PAMs, represented by VU0467154. Through an iterative analog approach, chemical libraries were generated around initial hits for the M4 receptor subtype. Calcium mobilization assays were used in parallel to generate functional potency and efficacy data for all compounds, and potential off target activity was assessed in Recerca lead profiling screens by percent radioligand displacement at 10 μM. Disposition was determined in mouse and rat pharmacokinetic studies using LC/MS/MS quantitation. *In vivo* efficacy was determined across several mouse and rat models of antipsychotic-like activity and in a touchscreen based pairwise discrimination task alone and after psychostimulant challenge. We also assessed the effects of VU0467154 on sleep-wake architecture using electroencephalography (EEG) studies in rats.
Results: *In vitro*, VU0467154 is a highly selective and low nanomolar potency M4 PAM with no activity at the M1,2,3,5 up to 30 μM or significant off target activity at other GPRCs at 10 μM. VU0467154 is a low clearance (CLp; 7.8 ml/min/kg), long half-life (t1/2; 5.7 h), and highly brain penetrant ligand with excellent pharmacokinetic properties, including high oral bioavailability (%F; 61) following administration of 3 mg/kg to rats with a maximum concentration in plasma (Cmax) of 700 nM and a time to reach Cmax (Tmax) of 3 h. VU0467154 produced robust dose-dependent reversals of amphetamine-induced hyperlocomotion in rats and mice within a dose range that produced no adverse effects or motor impairments as measured using a modified Irwin neurological test battery and rotarod test. In addition, VU0467154 produced reversals of the *N*-methyl-*D*-aspartate subtype of glutamate receptor (NMDAR) antagonist MK-801-induced hyperlocomotion and disruptions of contextual fear conditioning and pairwise discrimination, two hippocampal-mediated memory tasks. Finally, VU0467154 selectively increased the latency to paradoxical sleep (a correlate of REM sleep) and reduced paradoxical sleep without affecting sleep onset or total sleep duration similar to clinically available antipsychotics. However, in contrast to some antipsychotic medications, VU0467154 did not decrease delta power during slow wave sleep, a critical measure of sleep quality.
Conclusions: Taken together, VU0467154 represents a highly optimized M4 PAM tool compound for the evaluation of the role of M4 *in vivo* and provides further validation for the development of selective M4 PAMs as a novel approach for the psychotic symptoms and behavioral disturbances observed in psychiatric and neurologic disorders including schizophrenia and Alzheimer's disease. The work is funded by NIMH RO1 grants# 2R01 MH73676-06, 1R01 MH086601-04, AstraZeneca Vanderbilt is a Specialized Chemistry Center within the Molecular Libraries Probe Centers.
Keywords: M4 muscarinic cholinergic receptor schizophrenia cognition animal models EEG.
Disclosures: C. Jones, **Part 1:** research support from Bristol Myers Squibb, Johnson & Johnson, and AstraZeneca; royalties from Bristol Myers Squibb, Johnson & Johnson, and AstraZeneca, **Part 2:** licensing and milestone payments from AZ, BMS and JNJ in excess of 10 000 during 2011–2013, but no equity stake, **Part 4:** Sponsored research with AZ, Johnson & Johnson, BMS, and funding through the Michael J. Fox Foundation, Barrus Foundation and the National Institues of Health; T. Bridges, **Part 1:** research support from Johnson & Johnson and AstraZeneca, **Part 2:** licensing and milestone payments from AZ, but no equity stake, **Part 4:** Sponsored research with AZ and Johnson & Johnson; M. Bubser, **Part 4:** Sponsored research with AZ, BMS, and funding through the Michael J. Fox Foundation; R. Gould, Nothing to Disclose; D. Dencker Thorbek, Nothing to Disclose; M. Grannan, Nothing to Disclose; J. Daniels, **Part 1:** research support from Bristol Myers Squibb, Johnson & Johnson, and AstraZeneca; royalties fromBristol Myers Squibb, Johnson & Johnson, and AstraZeneca, **Part 2:** licensing and milestone payments from AZ, BMS and JNJ in excess of 10 000 during 2011–2013, but no equity stake, **Part 4:** Sponsored research with AZ, Johnson & Johnson, BMS, and funding through the

Michael J. Fox Foundation and the National Institues of Health; M. Noetzel, **Part 4:** Sponsored research with AZ and Johnson & Johnson; C. Niswender, **Part 1:** research support from Bristol Myers Squibb, Johnson & Johnson, and AstraZeneca; royalties from Bristol Myers Squibb, Johnson & Johnson, and AstraZeneca, **Part 2:** licensing and milestone payments from AZ, BMS and JNJ in excess of 10 000 during 2011–2013, but no equity stake, **Part 4:** Sponsored research with AZ, Johnson & Johnson, BMS, and funding through the Michael J. Fox Foundation and the National Institues of Health; M. Duggan, **Part 5:** AstraZeneca; N. Brandon, **Part 5:** AstraZeneca; J. Dunlop, **Part 5:** AstraZeneca; M. Wood, **Part 5:** AstraZeneca; C. Lindsley, **Part 1:** research funding from AstraZeneca, Johnson & Johnson, and BMS for the development of CNS therapeutics. Inventor of patents licensed to AstraZeneca, Johnson & Johnson, and BMS. Dr Lindsley also consults for AbbVie Pharmaceuticals, **Part 4:** Sponsored research with AZ, Johnson & Johnson, BMS, and funding through the Michael J. Fox Foundation and the National Institues of Health; P. Conn, **Part 1** research support from Bristol Myers Squibb, Johnson & Johnson, and Astrazeneca and is an inventor on composition patents, licensed to Bristol Myers Squibb, Johnson & Johnson, and AstraZeneca. Jeff Conn is a consultant for Karuna Pharmaceutical Company, **Part 4:** Sponsored research with AZ, Johnson & Johnson, BMS, and funding through the Michael J. Fox Foundation and the National Institues of Health.

W209. Hypnotic and Anxiolytic Properties of the Selective Melatonin MT2 Receptor Partial Agonist UCM765

Stefano Comai*, Rafael Ochoa-Sanchez, Quentin Rainer, Gabriella Gobbi

McGIll University Health Center and McGill University, Montreal, Quebec, Canada

Background: Sleep disorders represent a public health problem with a prevalence ranging from 11 to 16% and they are often in co-morbidity with anxiety and depression. The neurobiology of sleep disorders is still matter of research and currently available drugs such as benzodiazepines and non-benzodiazepines are often associated with unwanted side effects such as sedation, tolerance, dependence, next-day impairments, and abuse liability. Melatonin (MLT) is a neuromodulator derived from serotonin and synthesized mainly in the pineal gland. MLT controls numerous physiological processes including circadian rhythms, mood, sleep, and pain. MLT mostly acts through the activation of two high-affinity G-protein coupled receptors, MT1 and MT2. At present, it is unknown which MLT receptor subtype mediates hypnotic and anxiolytic properties of MLT. The goal of the present study was to examine whether the selective MT2 receptor partial agonist UCM765 *N*-{2-[(3-methoxyphenyl) phenylamino]ethyl}acetamide possess anxiolytic and hypnotic pharmacological properties in rodents.
Methods: Experiments were conducted in adult Sprague-Dawley male rats. *Anxiety:* Rats were acutely injected with UCM765 (5–10–20 mg/kg) and anxiety-related behaviours were assessed in the elevated plus maze test (EPMT), novelty suppressed feeding test (NSFT) and open field test (OFT). *Sleep:* Electroencephalographic (EEG) and electromyographic (EMG) sleep-wake patterns were registered across the 24-h light-dark cycle. For EEG and EEG monitoring, three stainless-steel epidural electrodes were positioned on the skull over the parietal cortex and three flexible stainless-steel wire electrodes were implanted into the neck muscles, respectively. UCM765 was tested at the different doses of 20, 40, and 60 mg/kg and was injected subcutaneously every 4 h due to its short half-life. The MT2 antagonist 4-PPDOT was used to antagonize the effects of UCM765 on anxiety and sleep and MLT was also test to compare its effects with UCM765.
Results: UCM765, at the dose of 10 mg/kg, displayed anxiolytic properties by increasing the time spent in the open arms (+208%) of the EPMT and by decreasing the latency to eat in a new environment (−46%) without affecting the latency to eat, as well as the amount of food intake per animal body weight, in the home cage. UCM765 did not alter measures of anxiety behavior and did not reduce locomotor activity in the OFT. These effects were blocked by the pre-treatment of 4P-PDOT. In addition, it did not increase the number of falls in the rotoroad test, a measure related to the sedative properties of a putative psychotropic compound. UCM765 at the dose of 40 mg/kg significantly reduced the latency to non rapid eye movement sleep (NREMS) by 59% while increasing the amount of NREMS during the 24-h by 38%. The increase in NREMS amount was mainly evident during the light/inactive phase of the 24-h light/dark cycle and was MT2 dependent since was nullified by the pre-treatment with 4P-PDOT. Rapid eye movement sleep (REMS) amount and latency were not altered by UCM765. The amount of wakefulness was instead significantly decreased (−22%) due to the increase in NREMS amount paralleled by no changes in REMS amount. In comparison, MLT slighly reduced the latency to the first episode of NREM, without changing the total duration of NREMS and REMS. In addition, UCM765 significantly increased the number of sleep spindles per min of NREMS (+16%), enhanced the power of the delta band of NREMS (+14%), and did not alter REMS theta power.
Conclusions: We have provided the first evidence that depending on the dosage, the melatonin MT2 receptor partial agonist UCM765 has anxiolytic and hypnotic pharmacological properties. In particular, at the dose of 10 mg/kg UCM765 produces anxiolytic effects with no sedation. At the higher dose of 40 mg/kg it increases the amount of NREMS without affecting REMS. UCM765 is more potent than MLT in sleep induction and total sleep increase. Currently available treatments for anxiety and insomnia (benzodiazepines and non-benzodiazepine) usually produce a similar dose-dependent effect on the central nervous system but are endowed with several side effects such as sedation. These pharmacological studies have proven that targeting MT2 receptors may become a novel pharmacological strategy in anxiety and sleep research and therapy, representing a valid alternative to benzodiazepines and benzodiazepine-derivatives.
Keywords: insomnia, anxiety, drug-discovery, melatonin, sleep, REM, NREM, EEG.
Disclosures: S. Comai, Nothing to Disclose; R. Ochoa-Sanchez, Nothing to Disclose; Q. Rainer, Nothing to Disclose; G. Gobbi, **Part 2:** I am the inventor of the patent Pub. No. WO/2007/079593.

W210. Forebrain-specific CRF Overexpression During Early Life Increases Vulnerability for PTSD-like Symptoms in Adulthood

Mate Toth*, Maya Gross, Isabelle Mansuy, Emilio Merlo-Pich, Victoria Risbrough

University of California San Diego, La Jolla, California

Background: Compared to the fairly high incidence of lifetime trauma, the prevalence to develop posttraumatic stress disorder (PTSD) is significantly lower suggesting individual vulnerability factors originating from candidate genes and previous stressful life events. Accordingly, early life stress was repeatedly shown to increase the risk to develop PTSD, however, the underlying neurobiological mechanisms need to be clarified. Corticotropin releasing factor (CRF) is a key regulator of the stress response, showing elevated concentration in the cerebrospinal fluid of individuals with PTSD or childhood trauma history. Hence, CRF may mediate some impacts of early life stress on stress vulnerability later in life. Additionally, differences in CRFergic signaling may also explain the higher risk of women for stress-related psychiatric disorders.
Methods: We induced transient CRF over-expression (CRFOE) in double mutant male and female mice during prepuberty (P2-P23) and tested their susceptibility for subsequent traumatic stress in adulthood using the predator stress model of PTSD. Focusing on PTSD-like symptoms, we tested startle reactivity (hyperarousal) and avoidance behaviors. Using a forebrain-restricted reverse-tetracycline system, CRFOE was limited to forebrain regions to exclude direct alterations of the hypothalamic-pituitary-adrenal axis. Effects on arousal (acoustic startle), generalized avoidance (open field, light dark box, social preference) and trauma-specific memory (avoidance of predator odor) were assessed between 1 and 5 weeks after predator exposure.
Results: Prepubertal CRFOE resulted in lasting enhancement of startle reactivity compared to mice with no CRFOE history as indexed by increased startle magnitude, reduced prepulse inhibition and reduced habituation of startle assessed in adulthood. The impact of prepubertal CRFOE on generalized and trauma-specific avoidance was highly sex-dependent. Whereas females exhibited increased avoidance following traumatic stress independently from CRFOE pre-exposure, traumatic stress induced heightened avoidance behaviors only in male subjects pre-exposed to prepubertal CRFOE.
Conclusions: Our findings suggest that enhanced CRF neurotransmission during early life leads to enduring hyperarousal and increased vulnerability to traumatic stress. Our data suggest that predator stress alone is sufficient to induce robust trauma-induced anxiety in females regardless of developmental CRF signaling status, while in males, resiliency is markedly reduced by CRF hypersignaling during early life supporting the 'double-hit' hypothesis in the development of heightened anxiety.
Keywords: CRF, anxiety, PTSD, development, sex difference.

Disclosures: M. Toth, Nothing to Disclose; M. Gross, Nothing to Disclose; I. Mansuy, Nothing to Disclose; E. Merlo-Pich, **Part 5:** Dr Merlo-Pich declares that during the past three year he was full-time employee of GlaxoSmithKline in 2010–11 and since 2012 he has been a full-time employee of F. Hoffmann-La Roche, Basel; V. Risbrough, **Part 1:** In the last three years Dr Risbrough has received funding from Omeros Pharmaceuticals, Johnson and Johnson, and Sunovion Pharmaceuticals and has consulted for Clear View Healthcare Partners.

W211. Transdermal Cannabidiol: Long-lasting Beneficial Actions in Animal Models of Drug Seeking, Anxiety, and Impulsivity

Friedbert Weiss*, Remi Martin-Fardon, Dana Hammell, Stan Banks, Rajita Sinha, Audra Stinchcomb, Gustavo Gonzalez-Cuevas

The Scripps Research Institute, La Jolla, California

Background: A major challenge for the successful treatment of drug and alcohol (EtOH) addiction is long-lasting susceptibility to relapse. While various medications are currently in use for relapse prevention, success rates achieved with these agents are typically unimpressive. Considering that drug addicts enter vulnerability states for several reasons, treatment drugs that concurrently target multiple precipitating factors are likely to provide greatly improved therapeutic efficacy. Our findings suggest that cannabidiol (CBD), the main non-psychoactive and non-addictive component of the cannabis sativa plant, may provide such a profile of actions.
Methods: We examined the effects of a transdermal CBD preparation (tCBD) on both EtOH- and cocaine seeking in reinstatement models of cue- and stress-induced relapse. Additionally, we evaluated tCBD actions on two vulnerability states for relapse: negative affect as measured by anxiety-like behavior (elevated plus maze test) and impulsivity associated with a history of EtOH dependence, as measured by a delay discounting task. Male Wistar rats with a history of EtOH or cocaine self-administration were treated at 24 h intervals with tCBD (15 mg/kg) or vehicle for seven days. Reinstatement tests were conducted during treatment as well as a 5-month post-treatment phase.
Results: tCBD reduced cocaine and EtOH-seeking behavior induced by drug-related environmental stimuli and stress in animal models of relapse, attenuated anxiety-like behavior following cocaine and EtOH withdrawal, and reversed impulsive behavior following chronic EtOH intoxication. tCBD produced these actions without non-specific behavioral suppression or tolerance to the drug's 'therapeutic' effects with repeated treatment. Of particular significance was the finding that the reduction of drug seeking in animal models of relapse outlasted a brief 7-day cannabidiol treatment period and remained undiminished after nearly five months of testing.

Conclusions: Together, these observations suggest that tCBD may restore normal function to neural mechanisms regulating incentive motivation, impulsivity, stress, and anxiety and, thus, may have unique treatment drug potential beyond mere transient pharmacotherapeutic amelioration of vulnerability states.
Keywords: cannabidiol; relapse, impulsivity, anxiety; drug addiction.
Disclosures: F. Weiss, Nothing to Disclose; R. Martin-Fardon, Nothing to Disclose; D. Hammell, Nothing to Disclose; S. Banks, Nothing to Disclose; R. Sinha, Nothing to Disclose; A. Stinchcomb, Nothing to Disclose; G. Gonzalez-Cuevas, Nothing to Disclose.

W212. Intranasal Delivery of an Interfering Peptide with Antidepressant-like Effect

Fang Liu*, Virginia Brown

Centre for Addiction and Mental Health, Toronto, Ontario, Canada

Background: Major depressive disorder is an illness associated with significant morbidity that may lead to substantial impairment in functioning. With current clinical antidepressant treatments, only 1/3 of patients achieve full remission of their symptoms after a single trial of antidepressant medications. Even with multiple antidepressant trials, 10–15% of patients continue to experience persistent depressive symptoms and few alternatives have been available for the treatment of resistant symptoms.
Methods: Forced swimming test, intranasal delivery.
Results: We previously reported that we developed a small interfering peptide that is able to disrupt a pathological receptor-receptor interaction between the D1 and D2 dopamine receptors present in Major Depressive Disorder and exert anti-depressant-like effects in animal models of depression (Pei *et al*, 2010). We report here that our interfering peptide can be delivered to relevant brain areas using a novel intra-nasal delivery system developed by Impel Neuro Pharma. We validate this delivery method by demonstrating that, at sufficient doses, our interfering peptide has an anti-depressant effect comparable to that of imipramine in the forced swimming test, a common test for antidepressant efficacy.
Conclusions: This study provides further support for the D1-D2 interfering peptide as a potential new treatment option for patients suffering from major depression disorder.
Keywords: antidepressant, intranasal delivery, dopamine D1-D2 receptor complex, interfering peptide.
Disclosures: F. Liu, Nothing to Disclose; V. Brown, Nothing to Disclose.

W213. Adenosine Receptor Involvement in Methamphetamine Conditioned Place Preference, Self-administration and Reinstatement

Ryan Bachtell*, Kevin Kavanagh, Sophia Levis, Casey O'Neill

University of Colorado, Boulder, Colorado

Background: Methamphetamine is an incredibly addictive and destructive drug regularly abused by over 35 million people worldwide. Despite the growing popularity and increasingly destructive impact of methamphetamine (MA) use on society, the neurobiological alterations resulting from MA abuse remain largely unknown. The present study explored the alterations in adenosine receptors following MA self-administration in rats and tested the ability of adenosine receptor agonists to reduce MA reward, reinforcement and relapse.
Methods: Male Sprague-Dawley rats were implanted with jugular catheters and were trained to self-administer MA (0.05 mg/kg per infusion) or saline for 14 days. Tissue from the nucleus accumbens core and shell was collected 24 h following the last self-administration session and immunoblotting was used to detect changes in A1 and A2A adenosine receptor subtypes in dopamine terminal areas such as the nucleus accumbens, amygdala and prefrontal cortex. In separate sets of rats, we tested whether stimulating A1 and A2A adenosine receptor subtypes with CPA (0.1 mg/kg, ip) and CGS 21680 (0.03 mg/kg, ip), respectively, would alter the development and expression of MA-induced place preference using an unbiased design. Finally, we tested the effects of stimulating A1 and A2A adenosine receptors on MA self-administration on fixed and progressive ratio schedules and MA-induced reinstatement to MA seeking.
Results: MA self-administration significantly decreased both A1 and A2A adenosine receptor expression in the nucleus accumbens shell, but not the nucleus accumbens core. MA self-administration significantly increased A2A adenosine receptor expression in the amygdala. Stimulating either A1 or A2A adenosine receptors significantly reduced MA-induced place preference. Interestingly, stimulating A1, but not A2A adenosine receptors reduced MA self-administration responding and MA-induced reinstatement.
Conclusions: These findings demonstrate that MA self-administration produces brain region specific alterations in adenosine receptor expression and that stimulation of adenosine receptors reduces several behavioral indices of MA addiction. Together, these studies help to shed light onto the neurobiological alterations in the adenosine receptor systems incurred through chronic MA abuse that may aid in the development of novel treatments for MA addiction.
Keywords: psychostimulant, dopamine, relapse, reward, reinforcement.
Disclosures: R. Bachtell, Nothing to Disclose; K. Kavanagh, Nothing to Disclose; S. Levis, Nothing to Disclose; C. O'Neill, Nothing to Disclose.

W214. Evaluating a Novel Brain-penetrant HDAC Inhibitor in Rat Behavioral Models in Relation to Target Occupancy Assessed by Pet Imaging

Frederick A Schroeder*, Changning Wang, Misha M Riley, Surya Reis, Yan-Ling Zhang, Stephen J Haggarty, Jacob M Hooker

Center for Human Genetic Research, Boston, Massachusetts

Background: Evidence increasingly supports that targeting epigenetic mechanisms and chromatin-mediated neuroplasticity may improve treatments for neuropsychiatric

diseases. Progress toward the goal of identifying such a treatment is currently impeded by: (i) the lack of highly brain-penetrant compounds; (ii) limited tools to evaluate epigenetic target binding in the living brain; and (iii) a poor understanding of the link between HDAC target occupancy and the pharmacological effects of an inhibitor. Given the growing interest in targeting histone deacetylases in the brain, we have recently characterized a hydroxamic acid that we term 'Martinostat'as a highly potent inhibitor of class I HDAC isoforms 1, 2, and 3, and the class II isoform, HDAC6. Carbon-11 radiolabeling and positron emission tomography (PET) imaging of this compound revealed robust brain uptake in rat and non-human primate models which was dose-dependently blocked by pretreatment with the unlabeled compound. These unique features of Martinostat and its [11C]-labeled version provide highly useful tool compounds to advance understanding of the molecular and behavioral effects of CNS-penetrant HDAC inhibitors. Our previous results in rodent behavioral models have shown that sufficient levels of HDAC inhibition in brain can result in mood-stabilizing and cognition-enhancing responses. However, little is known about the *in vivo* level and duration of HDAC target occupancy required to induce these changes in behavior. As a number of HDAC inhibitors have been shown to result in antidepressant-like effects in rodents, here we report on the assessment of Martinostat using the forced swim test (FST) to begin to investigate effects following treatment in rats. Additionally we used open field (OF) activity tests to assess treatment effects on motor activity.

Methods: Adult, male Sprague-Dawley rats (250–300 g, $n = 4$–6/group) were tested in the FST or OF tests 24 h after intraperitoneal administration of HDAC inhibitor or vehicle control. FST testing: Rats were exposed to a 15 min session of forced swimming in 25°C water (20 cm diameter cylinder, 30 cm water depth). Based on our PET imaging experiments, we chose to investigate doses of Martinostat representing a range of HDAC occupancy in the brain. Immediately after FST exposure, rats were treated via intraperitoneal injection with one of three treatments: (i) vehicle (10% DMSO, 10% Tween80, 80% saline), (ii) Martinostat at 1 μg/kg (15% HDAC occupancy) or (iii) Martinostat at 1 mg/kg (80% HDAC occupancy). One day later, rats were tested for immobility time during 5-min FST sessions which were videotaped and scored by a trained observer blinded to treatment groups. OF testing: equivalent rats and treatment groups described above were used to test for motoric effects using OF test chambers (an 60×60 cm box in which infrared beams track relative position, activity and distance traveled). OF was conducted 24 h after drug administration. An independent cohort of rats ($n = 3$/treatment group) was used to measure relative histone acetylation levels in brain tissue *ex vivo* via western blotting with antibodies against acetylated histone H4Lys12, acetylated histone H3Lys9, and total histone H3 or H4 levels as a loading control. These experiments served as a pharmacodynamics measure of HDAC inhibition in brain induced by Martinostat treatment. Analysis of behavioral and biochemical results were relative to response in vehicle-treated controls with effects averaged over treatment groups.

Results: We found that HDAC inhibitor treatment significantly altered forced swimming behavior without an effect in the open field test indicating a lack of general motoric effects. Histone acetylation in the brain was increased by Martinostat treatment but had limited sensitivity to dose. Comparing the behavioral results to the level of *in vivo* HDAC target binding indicated by [11C]-CN133 PET experiments, we determined that occupancy of as little as 15% of HDAC subtypes 1,2,3 and 6 in the brain—a subset of class I/II HDAC enzymes—is sufficient to reduce immobility in the FST, an antidepressant-like effect.

Conclusions: These results represent the first study to evaluate behavioral changes induced by the potent, highly brain penetrant HDAC inhibitor, Martinostat. Furthermore, this work provides a clear path for using [11C]-CN133 to measure HDAC target occupancy in the brain following treatment with other HDAC inhibitors across doses and treatment paradigms. Through this mechanism, it is therefore feasible to clarify understanding of HDAC target occupancy in the brain as a function of blocked [11C]-CN133 uptake which can then be interpreted with respect to diverse effects of these compounds on rodent behavior already in the literature base. The immediate impact of this study is the provision of new tool compounds that will advance preclinical HDAC inhibitor drug screening efforts. With the long-term goal of imaging [11C]-CN133 in humans, further optimization these tool compounds could provide a *non-invasive* means to quantify HDAC protein targets in the brain of patients as well as to monitor treatment effects mediated by binding and inhibiting HDAC enzymes in the CNS.

Keywords: HDAC, rodent, depression, mood, epigenetic.

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W215. β-arrestin Dependence of the Putative Antipsychotics M100907 and LY379268, in Animal Models of Psychosis

Caitlin E McOmish*, James Hanks, Elizabeth LaMarca, Molly Belkin, Elena Y Demireva, Jay A Gingrich

Columbia University Medical Center, New York, New York

Background: NMDAR antagonists such as PCP, MK801 and ketamine induce or worsen schizophrenia-like symptomatology. These compounds are considered the foremost pharmacological model for psychosis in animals, and are used as preclinical screens for novel antipsychotic compounds. Nonetheless, the mechanism by which non-competitive NMDAR antagonists mediate their effects, and the manner in which current and putative antipsychotic drugs attenuate this response has not been elucidated. This lack of understanding is further compounded by the complexity of factoring in both traditional G protein signaling as well as non-canonical β-arrestin

dependent signaling. A better understanding of how these models work may help us to unravel why the translation from animal models to human efficacy has not achieved high success rates to date. Two classes of putative antipsychotic compounds that have not lived up to expectation in clinical trials, are serotonin 2A receptor (5-HT2AR) antagonists, and group II metabotropic glutamate receptor (mGlu) agonists. 5-HT2AR and mGlu2, both 7TM GPCRs, have been demonstrated to modulate phenotypes induced by MK801 (hyperlocomotion and disrupted prepulse inhibition). Understanding how these compounds mediate their effects and how they differ from compounds that we know to be effective in humans may prove to be a useful strategy for improving success rates in translational neuropharmacology. Here, we investigate the involvement of non-canonical β-arrestin dependent signaling in the response to clozapine, M100907 (a 5HT2AR antagonist) and LY379268 (a mGlu2/3 agonist).

Methods: Wild-type (WT) and β-arrestin 2 knockout (β-arrKO) mice were tested in behavioral assays from 12 wks postnatal. Prepulse inhibition was performed in Kinder Scientific Startle Chambers. Baseline noise was set at 70 dB and startle response was induced by a 115 dB pulses with prepulses of 2,4,8,12 and 16 dB to measure sensorimotor gating. Locomotor behavior was performed in Kinder Scientific arenas and was recorded for 60–150 min. Mice were treated with 0.3 mg/kg MK801, 2 mg/kg M100907, 3 mg/kg LY379 268, clozapine 1.5 mg/kg (low dose), or clozapine 5 mg/kg (high dose). Mice were euthanized 2 h following pharmacological treatment and brains dissected. Striatum was processed and run on 10% reducing gels, transferred to PVDF membrane, and probed for pGSK and GSK, with GAPDH used as a housekeeping gene. A common internal control was used to allow for normalization across membranes.

Results: MK801 produced significant disruption in open field and PPI behavior of WT mice. β-arrKO mice demonstrated comparably disrupted PPI, but showed a significantly reduced locomotor hyperactivity in response to psychotomimetic doses of MK-801, relative to WT mice. High doses of clozapine produced sedative effects in both WT and KO mice. The onset of this locomotor suppression was significantly faster in β-arrKO, suggesting a role for β-arrestin in protecting against the negative effects of clozapine. Low dose clozapine was effective in reducing the effects of MK-801 in both WT and β-arrKO mice, however M100907 and LY 379268, while effective in WT mice, did not reduce the hyperactivity produced by MK801 in the open field in β-arrKO mice. Western blotting revealed increased levels of pGSKβ in the β-arrKO mice, as has been previously described. Interestingly, M100907 and LY379268 produced a similar increase in pGSKβ in WT mice, but did not affect expression in KO mice. The ability of clozapine to regulate these signaling pathways is currently under investigation.

Conclusions: This study reveals a complex involvement of β-arrestin in animal models of psychosis. In the absence of β-arrestin2, mice remain responsive to MK801—albeit at a greatly reduced level. These findings align with the levels of pGSKβ observed in the striatum, a region strongly targeted by the dopaminergic activity of many antipsychotic compounds. This study shows that β-arrKO mice show levels of pGSKβ comparable to those observed in WT following AP treatment, and thus, that increasing pGSKβ in the striatum confers reduced risk of displaying the hyperactivity induced by psychotomimetics. However, in the absence of β-arrestin, the ability to attenuate the residual hyperactivity with LY379268 or M100907 is lacking. Clozapine retained it's antipsychotic efficacy. This can be explained either by the broad binding profile of clozapine (it includes D2 antagonism in it's repertoire, consistent with findings showing that haloperidol was also effective in the absence of these signaling components), or by differential abilities of clozapine, and M100907, to stimulate and internalize the receptor, in the absence of β-arrestin. Ongoing studies will be required to unravel these possibilities.

Keywords: mGlu2, 5-HT2A, clozapine, animal model, schizophrenia.

Disclosures: C. McOmish, Nothing to Disclose; J. Hanks, Nothing to Disclose; E. LaMarca, Nothing to Disclose; M. Belkin, Nothing to Disclose; E. Demireva, Nothing to Disclose; J. Gingrich, Nothing to Disclose.

W216. Development of a Novel Class of Antipsychotics: Multifunctional PAT Compounds (5HT2A Antagonists/ 5HT2C Agonists) Ameliorate the Positive and Cognitive Disrupting Symptoms Associated with Psychosis

Drake Morgan*, Clinton Canal, Krishnakanth Kondabolu, Myong Kim, Kimberly Robertson, Neil E Rowland, Glen M Sizemore, Raymond G Booth

University of Florida, Gainesville, Florida

Background: There are 3 core classes of symptoms associated with schizophrenia and drug-induced (ie amphetamine) psychotic disorders: positive symptoms including hallucinations, negative symptoms such as social withdrawal, and cognitive disruption including inattention. A primary problem with currently used antipsychotic drugs is their lack of efficacy for treating the cognitive-disrupting symptoms. The goals of the current studies were to delineate the potential of novel phenyldimethylaminotetralin (PAT) compounds to attenuate the positive symptoms and the cognitive-disruptive symptoms associated with schizophrenia and drug-induced psychoses. Parallel studies assessed the potential for weight gain—a troubling side effect associated with essentially every currently used antipsychotic.

Methods: The (−) and (+) enantiomers of *trans*-4-phenyl-2-*N,N*-dimethylaminotetralin were synthesized and characterized *in vitro* for binding and function at human 5-HT2A, 2B, and 2C receptors. Subsequently, the PAT enantiomers were assessed *in vivo* for efficacy after peripheral administration in mouse models related to schizophrenia, drug-induced psychosis, and weight gain: attenuation of the head-twitch response elicited by the 5HT2 agonist (−)-2,5-dimethoxy-4-iodoamphetamine (DOI), hyperactivity induced by the psychostimulant amphetamine, and a model of binge, compulsive eating. Also, (−)-trans-PAT and amphetamine were assessed for efficacy afer peripheral

administration in a rat model of the cognitive disruption associated with psychoses, the observing response procedure.
Results: *In vitro*, the (+)- and (−)-PAT enantiomers functioned as 5HT-2A antagonists and 5HT-2C agonists, and did not activate 5HT-2B receptors. Regarding both binding and function, the (−)-enantiomer was far more potent and efficacious. In the mouse models of positive symptoms of psychoses, the PATs attenuated the number of DOI-induced head twitches and also negatively modulated amphetamine-induced hyperactivity, with the (−) enantiomer being more potent and effective than the (+) enantiomer. In the compulsive (binge) eating model, (−)-trans-PAT decreased consumption of palatable food, suggesting that weight gain will not be a side effect of this class of compounds. In the rat observing response procedure, attention was operationalized as responding on one lever to gain access to stimulus conditions that indicate to the rat whether reinforcers are available via responding on another lever—if rats 'pay attention', they can respond more effectively to receive reinforcers. Amphetamine, the most commonly used drug for inattention-related disorders, increased responding on the observing or attending lever. Similarly, (−)-trans-PAT alone and/or in combination with amphetamine produced significant increases in attention in every rat tested.
Conclusions: These and other data indicate the multifunctional serotonin 5HT2 receptor pharmacology of certain PAT analogs translates in rodent models to alleviate cognitive dysfunction as well as putative 5HT2A-mediated positive symptoms characteristic of the complex and multifaceted symptomology observed in schizophrenia and drug-induced (eg amphetamine) psychotic disorders.
Keywords: serotonin-2 schizophrenia animal models head-twitch response functional selectivity.
Disclosures: D. Morgan, Nothing to Disclose; C. Canal, Nothing to Disclose; K. Kondabolu, Nothing to Disclose; M. Kim, Nothing to Disclose; K. Robertson, Nothing to Disclose; N. Rowland, Nothing to Disclose; G. Sizemore, Nothing to Disclose; R. Booth, Nothing to Disclose.

W217. Chronic Ethanol Increases Excitability in the Ventral Bed Nucleus of Stria Terminalis via Postsynaptic Serotonin2c Receptor Signaling

Catherine Marcinkiewcz*, Cayce Dorrier, Thomas L Kash

Bowles Center for Alcohol Studies, Chapel Hill, North Carolina

Background: Chronic alcohol use can induce persistent changes in circuit function in the extended amygala which may provoke stress responses and subjective feelings of anxiety during abstinence. Previous results from our lab have shown that chronic intermittent ethanol vapor exposure can elicit robust changes in anxiety-like behavior in the social interaction test in mice which is at least partially mediated by 5HT2c receptors. This was confirmed by the presence of increased FOS-like immunoreactivity (FOS-IR) in the bed nucleus of stria terminalis after 5 days of ethanol vapor, which was normalized in mice that were pretreated with a 5HT2c-R antagonist. These results suggest that the BNST may be an important neural substrate in the behavioral adaptations elicited by alcohol. In this study, we used slice electrophysiology to examine post-ethanol modifications in neuronal excitability and 5HT2c-R signaling within the BNST.
Methods: Male DBA/2J mice injected with pyrazole (1 mmol/kg, i.p.) were exposed to ethanol vapor or room air for 5 days using a 16 h on, 8 h off schedule. Following a 24 h withdrawal period, coronal slices of the BNST were prepared for electrophysiology. All experiments were conducted in current clamp mode. Membrane excitability in air- and ethanol-exposed mice was measured using the rheobase (current required to inducing firing) and the voltage at first spike from resting membrane potential and from a holding potential of −70 mV. We also analyzed the number of spikes elicited by discrete current steps from 0 to 150 pA (VI plots). These experiments were repeated in the presence of the 5HT2c-R antagonist RS102221 (10 μM). Postsynaptic effects of the 5HT2-R agonist mCPP (20 μM) and RS102221 on membrane potential were also measured in the presence of TTX. The expression of 5HT2c receptors in the BNST was also examined by Western blot in air- and ethanol-exposed animals.
Results: Ethanol vapor exposure increased spiking frequency, but not threshold, when current was injected starting at rest and at −70 mV, which was normalized by preapplication of the 5HT2c-R antagonist RS102221. The net change in membrane potential induced by both RS102221 (hyperpolarization) and mCPP (depolarization) in the presence of TTX was also increased following ethanol vapor, suggesting that ethanol upregulates 5HT2c receptor signaling in the BNST. This was confirmed by Western blot analysis 5HT2c receptor expression in the BNST following ethanol vapor, which indicated an increase in 5HT2c receptor protein.
Conclusions: Taken together, these results suggest that ethanol modulates neuronal excitability in the BNST via modifications in 5HT2c receptor signaling, possibly by upregulating expression of postsynaptic 5HT2c receptors.
Keywords: alcohol, serotonin, BNST, electrophysiology, 5HT2c.
Disclosures: C. Marcinkiewcz, Nothing to Disclose; C. Dorrier, Nothing to Disclose; T. Kash, Nothing to Disclose.

W218. Role of Central Amygdala PACAP in the Stress Response

Valentina Sabino*, Attilio Iemolo, Riccardo Dore, Xiaofan Wang, Pietro Cottone

Boston University School of Medicine, Boston, Massachusetts

Background: Anxiety disorders are psychiatric conditions characterized by feelings of excessive and uncontrollable apprehension and/or fear in the absence of any specific external stimuli. They are the most common form of mental disorders in the United States, affecting nearly 40 million adults. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a 38-amino acid peptide belonging to the GHRH/secretin/glucagon/VIP superfamily. PACAP neurons and fibers are located in the paraventricular (PVN),

ventromedial and supraoptic nuclei of the hypothalamus, as well as in various extra-hypothalamic regions. PACAP and its receptor PAC1 have been proposed to play a key role in mediating the endocrine and behavioral responses to stress. While PACAP activity in the hypothalamus and the pituitary has been reasonably well-studied, little attention has been paid thus far to the investigation of the potential role of the extrahypothalamic PACAP system in the response to stress; in particular, the central nucleus of the amygdala (CeA), where PACAP and its receptor PAC1 are highly expressed, may represent a key brain site for the actions of PACAP. The aim of this work was to elucidate the role of the PACAP/PAC1 system of the CeA and the BlA in the context of anxiety-like behavior and hypothalamus pituitary adrenal (HPA) axis activation.
Methods: PACAP was bilaterally administered into the CeA and the BlA of male, adult Wistar rats, and its effects on anxiety-like behavior using the elevated plus maze test were evaluated; corticosterone levels were also measured in a different set of experiments.
Results: PACAP administered bilaterally into the CeA and BlA of male, adult Wistar rats (0.03–1 μg/rat) significantly reduced the percent of time spent in the open arms of an elevated plus maze, demonstrating an anxiogenic profile. Conversely, bilateral injection of PACAP into the BlA had no effect on anxiety-like behavior in the same test. In addition, bilateral injection of PACAP (0.1–1 μg/rat) into the CeA increased the levels of circulating corticosterone, indicating that PACAP, acting locally in the CeA, can activate the HPA axis. Finally, a single restraint stress significantly elevates the levels of PAC1 receptor mRNA and protein expression in the CeA, and not in the BlA.
Conclusions: The present data demonstrate an anxiogenic and HPA activating role for the endogenous PACAP/PAC1 system of the CeA, and suggest that this is system is recruited following exposure to stress.
Keywords: anxiety, amygdala, PAC1, CRF, HPA.
Disclosures: V. Sabino, Nothing to Disclose; A. Iemolo, Nothing to Disclose; R. Dore, Nothing to Disclose; X. Wang, Nothing to Disclose; P. Cottone, Nothing to Disclose.

W219. NMDA Receptors in the Nucleus Accumbens Shell Mediate Compulsive Eating of Palatable Food

Pietro Cottone*, Karen Smith, Rahul Rao, Marta Valenza, Clara Velazquez-Sanchez, Valentina Sabino

Boston University School of Medicine, Boston, Massachusetts

Background: Binge-eating disorder affects nearly 15 million people in the U.S.A. and is characterized by excessive consumption of palatable food within brief periods of time, and loss of control over eating. The cyclic binge/restriction pattern of consumption of highly palatable foods has raised the question of whether binge eating disorder can be considered an addiction-'like' disorder. Theglutamatergic NMDA receptor system is highly involved in reward-related processes, but its role in hedonic food intake and binge eating is poorly understood. This study is aimed at investigating the effects of uncompetitive NMDA receptor blockade in a rat model of binge-like eating.
Methods: We trained male Wistar rats to consume either a highly palatable diet (Palatable group) or a standard chow diet (Chow group) in operant sessions (1 h/day) on a fixed ratio 1 (FR1) schedule of reinforcement. We tested the effects of the uncompetitive NMDA receptor antagonists, ketamine and memantine on binge-like eating. Moreover, we tested the effects of memantine on palatablefood seeking behavior using a second order schedule of reinforcement. In addition, using a light/dark conflict test, we also investigated whether memantine could block the time spent and the food eaten in an aversive, open compartment, where the palatable diet was offered. Furthermore, we examined the effects of memantine on excessive chow intake induced by food restriction. Finally, we investigated the role of NMDA receptors in binge-like eating by microinfusing memantine into either the Nucleus Accumbens Shell (NAccShell) and Core (NAccCore).
Results: The palatable-fed rats quickly developed binge-like eating behavior and exhibited compulsive eating and risk-taking behavior when palatable food was presented in an aversive environment. Palatable fed rats also showed heightened food seeking behavior compared to control rats. Memantine dose-dependently decreased binge-like eating without affecting water intake, and blocked compulsive eating of palatable food. Conversely, ketamine did not affect food and water responding in neither Chow nor Palatable rats. Furthermore, memantine treatment reduced food seeking behavior, compulsive eating and risk-taking behavior without affecting restriction-induced excessive chow intake. Memantine treatment did not affect any of the variables in control chow-fed rats. Intra-NAccShell injections of memantine dose-dependently decreased binge-like eating selectively in the bingeing rats, without affecting water responding. Intra-NAccShell injections of memantine did not affect food or water intake in control Chow rats. No effects were observed when memantine was microinfused into the NAccCore.
Conclusions: Overall, these findings confirm the hypothesis that dyregulation of glutamatergic NMDA receptor system within the NAccShell is a key component of neuroadaptive mechanisms that lead to the development of binge-like eating. Our findings propose a novel potential pharmacological strategy to combat binge eating disorder.
Keywords: binge eating, palatability, memantine, ketamine, addiction.
Disclosures: P. Cottone, Nothing to Disclose; K. Smith, Nothing to Disclose; R. Rao, Nothing to Disclose; M. Valenza, Nothing to Disclose; C. Velazquez-Sanchez, Nothing to Disclose; V. Sabino, Nothing to Disclose.

W220. Panic Disorder and Agoraphobia: Novel Glutamate Mechanisms and Therapeutic Approaches from Preclinical Model

Anantha Shekhar*, Philip L Johnson, Andrei Molosh, Stephanie D Fitz, Amy Dietrich, William Truitt, Cris Barnaby, Luc Ver Donck

Indiana University School of Medicine, Indinapolis, Indiana

Background: Panic disorder is a severe anxiety syndrome that is characterized by recurrent spontaneous panic attacks and often leads to rapid development of avoidance

behaviors that are hard to extinguish (phobias). A majority of these patients also demonstrate panic attacks following sodium lactate infusions. We have established a preclinical model which demonstrates acute panic-like responses characterized by increased acute anxiety, 'flight'-like locomotion, respiration, and heart rate responses following lactate infusions. Here we report on a series of experiments that test to see if such panic prone animals: (a) develop conditioned fear responses much more readily and/or show delayed fear-extinction; (b) demonstrate disrupted neural network properties in the amygdala; (c) show specific gene changes in key brain areas implicated in panic and phobias such as the amygdala. compared to controls; and (d) if such gene changes would be supported by testing novel therapeutics to ameliorate panic and phobia vulnerability in these animals.

Methods: Panic-prone animals were prepared by chronically inhibiting GABA synthesis in the perfornical/dorsomedial hypothalamus (PeF/DMH), a key panic generating region of rats, with local infusions of active l-allylglycine (l-AG) and inactive d-AG. We tested such panic vulnerable rats in series of experiments: (a) for the induction of conditioned fear in the standard auditory-cue fear-conditioning test; (b) whole-cell patch clamp recordings from basolateral amygdala projection neurons; (c) for GABA and glutamate related gene expression changes (mRNA levels) in key brain regions; and (d) therapeutic benefits of treating them with JNJ-40411813, a positive allosteric modulator of the metabotropic glutamate receptor 2 (mGluR2), a target supported by the gene change studies. It also displays weak 5-HT2A-antagonism.

Results: The panic prone rats compared to their inactive controls demonstrated normal acquisition of fear conditioning. However, during the extinction trials, the panic prone animals showed significant delay in extinction. Next, using whole-cell patch clamp recordings from pyramidal neurons in the basolateral region of amygdala, we demonstrated that the panic prone state induced a significant increase in excitability compared to control rats. Gene array results demonstrated unique gene expression changes in the amygdala of panic-prone rats that result in enhanced synaptic excitation, specifically a down regulation of the mRNA for mGluR2. Finally pretreatment of these animals with the mGluR2 positive allosteric modulator JNJ-40411813 dose-dependently blocked the panic prone state by blocking lactate-induced panic-like responses. Studies are underway to assess the efficacy of this mechanism in phobia amelioration.

Conclusions: These data suggest that panic-prone state results in unique gene and electrophysiological changes in the limbic circuits that are, at least in part, the result of reduced mGluR2 modulation and enhanced excitability. This appears to result in acute panic-like responses to lactate and other panicogenic challenges, as well as poor ability to extinguish conditioned fears, resulting in vulnerability to phobias. Treatment with mGluR2 allosteric modulators such as JNJ-40411813, a compound that is currently in clinical trials for neuropsychiatric disorders, could be an effective approach to treating panic and agoraphobia.

Keywords: panic disorder agoraphobia metabotropic glutamate receptor 2 gene conditioned fear LTP.

Disclosures: A. Shekhar, Nothing to Disclose; P. Johnson, Nothing to Disclose; A. Molosh, Nothing to Disclose; S. Fitz, Nothing to Disclose; A. Dietrich, Nothing to Disclose; W. Truitt, Nothing to Disclose; C. Barnaby, Nothing to Disclose; L. Ver Donck, **Part 1:** I am an employee of JnJ.

W221. Subgrouping Central Serotonin Neurons by Their Networks

Yue Ping Guo, Kathryn Commons*

Boston Children's Hospital, Boston, Massachusetts

Background: A current hypothesis is that serotonin neurons are organized into functional subgroups and as a consequence, dysfunction of particular subgroups could yield different neuropsychiatric disorders. However, meaningful functional subgroups remain poorly defined, even though many unique morphological features of groups of serotonin neurons have been identified. In order to be functionally distinct, neurons must be embedded in different circuits. Therefore in this study we sought to define subregions of the largest group of serotonin neurons, those located within the dorsal raphe nucleus (DRN), by differential afferent input.

Methods: Results from anterograde tract tracing experiments in mouse available from the Allen Brain Connectively Atlas were searched using the DRN as the target structure (Website: ©2012 Allen Institute for Brain Science. Allen Mouse Brain Connectivity Atlas [Internet]. Available from: http://connectivity.brain-map.org/). This was an informatics analysis that did not involve the new use of mice or protocol approval. Twenty-three tract-tracing cases were selected that represented projections from multiple areas. From each case, six image planes spanning the rostrocaudal extent of the DRN were identified. These images also included areas of the median raphe nucleus (MRN). Images were manually aligned to cognate reference sections of serotonin neurons, also taken from the Allen Brain Atlas. Using the reference images of serotonin neurons, areas of interest were identified that either included a cluster of serotonin neurons in the DRN or MRN or areas lateral to these nuclei. With NIH's Image J software, the density of the projections to the regions of interest was measured for each projection case. Principal component analysis and unsupervised hierarchical clustering using Ward's method and z-score standardization were used.

Results: Two major subdivisions of the DRN were found. Specifically, there were major differences in afferent input to the rostral two thirds of the DRN in comparison to the caudal one third. Brain regions that innervated the rostral two thirds of the DRN (in preference to the caudal third of the DRN) included zones within the hypothalamus, medial and lateral preoptic areas. The caudal third of the DRN was more similar in afferent innervation to the MRN than it was to the rostral two thirds of the DRN. Afferent projection sites that targeted the caudal DRN (in preference to the rostral DRN) included the anterior cingulate cortex, medial septum, mammillary nuclei, lateral habenula and interpeduncular nucleus. While a major division between rostral and caudal DRN was identified, there were additional minor

subdivisions noted as well. For example, the rostral two thirds of the DRN appeared to have distinctive midline and lateral subcomponents. Afferents that provided substantive innervation to the lateral DRN arose from the central nucleus of the amygdala, agranular insular cortex and medulla.
Conclusions: These results indicate the DRN is composed of two distinct parts organized along the rostrocaudal axis. While this is the clear primary division, it is possible that additional subregions within these areas also exist such as the rostral-midline and lateral components. Considering afferent projection patterns together with known patterns of output, the rostral-midline DRN may play a role in motor and motivated behaviors. The lateral DRN may have a greater association with intero- and exteroreceptive functions. The role of the caudal DRN may be more closely aligned with arousal state relevant to mood, particularly under aversive conditions.
Keywords: depression, serotonin, raphe, informatics, tract tracing.
Disclosures: Y. Guo, Nothing to Disclose; K. Commons, Nothing to Disclose.

W222. Kappa Opioid Receptors Inhibit Glutamatergic Transmission to the Extended Amygdala in an Input Specific Manner

Thomas L Kash*, Nicole Capik, Michael R Bruchas

University of North Carolina School of Medicine, Chapel Hill, North Carolina

Background: The Bed Nucleus of the Stria Terminalis (BNST) is a component of the extended amygdala, and plays a key role in both stress and addiction. While the BNST receives multiple glutamatergic inputs, and expresses both dynorphin and Kappa Opioid Receptor (KOR), to date there have been no studies examining the impact of KOR signaling on glutamatergic transmission.
Methods: Slice electrophysiology coupled with optogenetic approaches was used to probe input specific modulation of kappa opioid receptors in the BNST. *In vivo* optogenetic approaches were used to determine the functional interaction on kappa opioid receptors and amygdala inputs to the BNST.
Results: We found that application of KOR agonists caused a decrease in frequency, but not amplitude of miniature EPSCs, suggesting the locus of action for this inhibition was presynaptic. We next explored the signaling involved in this inhibition. While a previous study found that ERK signaling was required for KOR inhibition of GABA release in the BNST, we found that KOR inhibition of glutamate release was p38map kinase dependent. We then dissected the circuitry involved in KOR inhibition in the BNST. We targeted the prefrontal cortex (PFC) and basolateral amygdala (BLA) with AAV5 CamKiia ChR2 eYFP to demonstrate pathway specific KOR inhibition. KORs inhibited light evoked EPSCs at BLA but not PFC.
Conclusions: In combination with previous studies conducted by our lab, these results suggest that KORs inhibit GABA and glutamate transmission in the BNST via different signaling pathways. Ongoing studies are exploring how exposure to stress or drugs of abuse can selectively alter specific pathways and their modulation by KOR signaling.
Keywords: amygdala, opioid, stress, anxiety, fear.
Disclosures: T. Kash, Nothing to Disclose; N. Capik, Nothing to Disclose; M. Bruchas, Nothing to Disclose.

W223. Suicidal Ideation in Depressed New Mothers: Relationship with Childhood Trauma and Sleep Disturbance

Dorothy Sit*, James Luther, Jesse Dills, Heather Eng, Dan Buysse, Michele Okun, Stephen Wisniewski, Katherine L Wisner

University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Background: Of new mothers with a positive depression screening (Edinburgh Postnatal Depression Scale—EPDS > 10) 19.3 percent ($n = 1396$) had thoughts of self-harm (Wisner *et al*, JAMA Psychiatry 2013). The high rate of suicidal ideation (SI) in new mothers is a major concern. Without proper treatment, mothers with SI are at increased risk for suicide. From the 1997–1999 UK Confidential Enquiries into Maternal Death suicide was the leading cause of maternal death from 42 days to 1 year postpartum; 28 percent of maternal deaths ($n = 242$) resulted from suicide (Oates, 2003). In this cross-sectional study, the objective was to characterize potential risk factors for postpartum women with SI who were enrolled in the primary study to screen for postpartum depression (PPD)(Wisner *et al*, 2013). The aims were to examine the relationship between SI and childhood or adult history of trauma (physical abuse or sexual abuse) and sleep disturbances in new mothers within 4–6 weeks after delivery. The hypothesis was childhood trauma, current sleep disturbance and the interaction between childhood trauma and current sleep disturbance were associated with SI.
Methods: Eligible subjects included new mothers who received an EPDS depression screen within 4–6 weeks after delivery and a completed home visit evaluation. The primary psychiatric diagnosis was confirmed by the Structured Clinical Interview for DSM-IV (SCID). Patients with Bipolar Disorders, primary psychotic disorders, alcohol or substance use disorders were excluded. We compared the groups with an EPDS item 10 ('The thought of harming myself has occurred to me') = 0 ('never'), 1 ('hardly ever'), 2 or 3 ('sometimes' or 'quite often'). We recorded baseline demographic characteristics including age, race, educational level, marital status, health insurance and parity. We examined comorbid disorders, adult and childhood abuse history, onset of the current depressive episode, total depression scores on the Structured Interview Guide for the Hamilton Depression Rating Scale, Atypical Depression Symptoms Version (SIGH-ADS)—a 29-item instrument that incorporates the Hamilton Rating Scale for Depression and a set of questions to assess atypical neurovegetative symptoms and the global assessment of function. For sleep disturbance we examined the SIGH-ADS sleep symptoms (insomnia—items H6, H7, H8 and wake time after sleep onset-WASO greater than 20 min).

Results: Of 648 eligible mothers, 496 (77%) reported that they 'never' had thoughts of self-harm; 98 (15%) had SI 'hardly ever', and 34 (5%) had SI 'sometimes or quite often'. Younger mothers, African Americans and mothers with public health insurance were significantly more likely to have SI. Mothers with the onset of depressive episodes before pregnancy or in the post-partum, a history of physical abuse in childhood, and lowered global functioning were significantly more likely to have SI. Cumulative logistic regression models suggested a main effect for history of childhood physical abuse (odds ratio-OR = 1.681, 95% confidence interval-CI = 1.045, 2.704, $p = 0.032$) and a marginally significant interaction of childhood physical abuse with WASO > 20 min (OR = 1.576, 95%CI 0.950, 2.614, $p = 0.078$).
Conclusions: A high percentage of maternal deaths are from suicide (Oates, 2003). Our data suggested that among mothers with a positive depression screening, having a past history of childhood trauma and possibly having recent sleep disturbance contributed to increased risk for thoughts of suicide. Altered stress responses in patients with early life abuse could increase their susceptibility to suicide (Lupien *et al*, 2009). Patients with childhood abuse who completed suicide expressed reduced levels of glucocorticoid receptor-GR whereas non-abused patients who completed suicide did not express altered GR (McGowan, Meaney *et al*, 2009). New research is imperative to extend our knowledge of the relationship between abnormal responses to stress and the cognitive processes involved with making decisions and experience of reward (Dombrovski *et al*, 2013) which could underlie suicidal thoughts and precede suicide in post-partum mothers.
Keywords: postpartum depression, suicide ideation, childhood trauma, sleep disturbance.
Disclosures: D. Sit, Nothing to Disclose; J. Luther, Nothing to Disclose; J. Dills, Nothing to Disclose; H. Eng, Nothing to Disclose; D. Buysse, Nothing to Disclose; M. Okun, Nothing to Disclose; S. Wisniewski, Nothing to Disclose; K. Wisner, Nothing to Disclose.

W224. Gonadal Hormone Regulation of Stress Circuitry Activity in Healthy Women is Disrupted in Major Depressive Disorder

Emily G Jacobs*, Jill M Goldstein, Laura M Holsen, Katrina Lancaster, Anne Remington, Stephen Buka, Susan Whitfield-Gabrieli, Anne A Klibanski

Harvard Medical School, Cambridge, Massachusetts

Background: As of 2013, major depressive disorder (MDD) is the leading cause of disability worldwide, and women have twice the risk of men. The neurobiological mechanisms that help explain the sex difference in MDD prevalence are not fully understood. While dysregulation of the hypothalamic-pituitary-adrenal (HPA) stress axis in MDD is well-established, recent work has begun to characterize deficits within the hypothalamic-pituitary-gonadal (HPG) axis as well as HPA-HPG interactions. Many of the brain regions that regulate the HPA axis, including the amygdala, hippocampus, hypothalamus and medial prefrontal cortex (mPFC), are densely populated with sex steroid and glucocorticoid receptors. Moreover, in a functional MRI study in healthy women, we found that the activation of stress circuitry regions in response to a stress challenge was modulated across the menstrual cycle, strongly implicating sex steroids in the regulation of stress response circuitry. Examining whether –and how– this process is disrupted in MDD could improve our understanding of why women are at an increased risk of the disorder. The current study was designed to assess the relationship between sex steroid hormones and stress circuitry dysregulation in a sample of women with recurrent MDD and matched healthy controls.
Methods: In a repeated measures design, 11 women with recurrent MDD, in remission, and 13 matched healthy controls underwent behavioral testing, hormonal evaluations and fMRI scanning on two occasions (resulting in a total acquisition of 48 datasets). Cases and controls were comparable on age, ethnicity, handedness, SES of origin and verbal IQ. Participants were seen once in the early follicular phase of their menstrual cycle and once in the late follicular phase, order counter-balanced. Fasting blood samples were acquired at 8 AM, immediately prior to the scan, to evaluate steroid hormone concentrations. The current analyses focused on the impact of 17β-estradiol (E2) given that (i) there are dense populations of estrogen receptor (ER)-α and β within cortical and subcortical regions that regulate the HPA stress axis (eg amygdala, hippocampus, hypothalamus, mPFC) and (ii) E2 has the strongest affinity at these receptors compared with estrone and estriol. Progesterone levels were uniformly low at both visits, but still controlled for in order to isolate the impact of E2 on stress circuitry activation. Subjects performed a mild stress challenge fMRI paradigm in which they viewed negative valence/high arousal and neutral valence/low arousal images, adapted from the *International Affective Picture System*. Validity of this task for activating stress response circuitry was previously demonstrated in healthy and depressed populations. Statistical maps comparing negative > neutral images were generated at the random effects level (FWE $p < 0.001$). Regions of interest (ROIs) were defined as 5 mm (subcortical) and 10 mm (cortical) spheres around peak loci. Mean beta weights were extracted and compared as a function of hormone state (low, high estradiol) and case status.
Results: The two test visits were categorized as 'low E2' or 'high E2' based on the relative change in serum E2 concentrations within an individual. Thus, analyses were based on actual steroid hormone levels and not solely on menstrual cycle history. Mean hormone levels did not differ between cases and controls at the low or high E2 visit ($p = 0.63$). Within healthy controls, under low E2 conditions women showed robust task-related BOLD activity across all subcortical ROIs examined, including bilateral amygdala, hippocampus and hypothalamus. Under high E2 conditions, activity in these regions was significantly diminished [paired *t*-test (low > high E2); right amygdala, $p = 0.003$; left hippocampus, $p = 0.029$; right hypothalamus, $p = 0.011$]. Comparable trend-level differences were observed in corresponding bilateral ROIs. Within MDD women, however, this hormonal regulation was completely absent. There were no significant signal intensity changes in stress circuitry ROIs between low and high estradiol sessions ($p > 0.05$ for all ROIs).
Conclusions: In this study, we found that endogenous fluctuations in 17b-estradiol over the menstrual cycle regulate activity within stress response circuitry (including amygdala, hippocampus and hypothalamus) during a mild visual stress

paradigm. In healthy women, robust task-related BOLD activations observed under low E2 conditions were blunted when E2 levels were naturally elevated. These results confirm and extend our previous work in healthy women showing that BOLD activity in cortical and subcortical stress response regions is attenuated during late follicular compared to early follicular cycle phase. We now pinpoint this regulation to endogenous fluctuations in 17b-estradiol. This hormonal capacity to regulate activity in stress circuitry was largely absent in remitted MDD women, suggesting that gonadal hormone dysregulation is a potential trait characteristic. The case-control difference may be due to interactions between the HPA and HPG axes. Further, a recent study of ours found an association between hypercortisolemia and amygdala activation in response to stress in MDD women. It is possible the for the MDD women in our sample an adrenal response to the stress challenge overrode the ability of gonadal hormones to regulate regions within stress circuitry.

Keywords: depression, sex differences, 17b-estradiol, stress circuitry.

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W225. Linked Sex Differences in Cognition and Functional Connectivity in Youth

Theodore D Satterthwaite*, Daniel Wolf, David Roalf, Kosha Ruparel, Guray Erus, Simon Vandekar, Efstathios Gennatas, Mark Elliott, Alex Smith, Hakon Hakonarson, Ragini Verma, Christos Davatzikos, Raquel E Gur, Ruben C Gur

University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania

Background: Sex differences in human cognition are prominent and well documented. However, it is not known whether sex differences in brain structure translate into sex differences in functional brain networks. While several prior studies have presented evidence for sex differences in functional connectivity, no prior study has attempted to understand how sex differences in patterns of cognition may relate to sex differences in brain connectivity. Here, in a large sample of children, adolescents, and young adults studied as part of the Philadelphia Neurodevelopmental Cohort, we investigated how sex differences in functional connectivity relate to sexually divergent patterns of cognition. Our hypothesis was that the extent to which a given subject demonstrated a stereotypically 'male' or 'female' pattern of brain connectivity would be related to the masculinity or femininity of their cognitive profile.

Methods: Subjects included 312 males and 362 females ages 8–22 who were studied as part of the PNC. Groups were matched on age and in-scanner motion using a greedy matching algorithm. Cognition was assessed across major domains using the Penn Computerized Neurocognitive Battery (CNB). All imaging data were acquired on the same scanner (Siemens Tim Trio 3 Tesla) using the same imaging sequences. Sequences included resting-state functional connectivity (231 volumes, 3 s TR, 3 mm isotropic voxels), T1-weighted MPRAGE (1 mm isotropic voxels), and a B0 field map. Functional images were registered to template space via co-registration to the anatomic image using BBR with integrated distortion correction, followed by normalization to template space using ANTS. Subject-level timeseries processing utilized an improved framework for minimization of motion artifact. Network nodes were defined using the system delineated by Power *et al* (2011), and included 264 individual nodes (35 716 edges) belonging to 13 large-scale brain networks. Graphical measures of network topology including node and network-wise connectivity strength and participation coefficient were calculated using the Brain Connectivity Toolbox. Network statistics and edgewise connectivity was compared between sexes using two-sample *t*-tests. Significance thresholds of all mass-univariate analyses of both cognitive and imaging data were calculated using the false discovery rate ($Q<0.05$). Multivariate patterns of cognition and connectivity were used to predict an individual subject's sex within a 10-fold cross-validated framework using support vector machines (SVM) implemented in LIBSVM. Predictive significance was assessed using permutation testing (1000 permutations). Finally, dimensional summaries of the masculinity/femininity of multivariate patterns of cognition and connectivity were related using Pearson's correlations.

Results: Expected sex differences in cognition were found, including male superiority in spatial and motor tasks, and female superiority on non-verbal reasoning and emotion identification. Males had a significantly higher mean network positive participation coefficient than females ($t(672)=2.21$, $p=0.027$), reflecting a greater balance of between-module connectivity in males and within-module connectivity in females. Similarly, on a node-wise level, males demonstrated a higher positive participation coefficient at several locations in multiple networks. As suggested by the differences in the participation coefficient, edgewise analyses revealed that the proportion of significantly different connections that were within-module *vs* between module varied systematically by sex: connections that were stronger in females were more likely to be within-module, whereas connections that were stronger in males were more likely to be between modules ($\chi 2=10.16$, $p=0.001$). Notably, sex differences in both cognition and brain connectivity were present across the entire age range studied and neither linear nor nonlinear age by sex interactions were present. Multivariate pattern classification using SVM demonstrated that while cross-validated sex prediction using patterns of cognition and connectivity were both highly significant (both $p<0.001$), classification using connectivity was more accurate than classification using cognition (71 *vs* 63%). Critically, the degree of masculinity or femininity in participants' cognitive profile was significantly correlated with the masculinization (or feminization) of their brain connectivity ($p=1.2\times 10–7$).

Conclusions: While sex differences in cognitive ability are well established, prior accounts have not described how differences in brain organization allow such divergent cognitive styles to occur. Our results demonstrate that sex differences in patterns of brain connectivity are present at an early age, with males having greater between-module connectivity and females having more within-module connectivity across multiple scales of analysis. More importantly, our results show that sex differences in patterns of brain connectivity are related to sex-specific profiles of cognitive performance, for the first time

establishing a link between sex differences in cognition and the organization of the brain's functional connectome.
Keywords: MRI, functional connectivity, cognition, sex differences, multivariate methods, adolescence, development.
Disclosures: T. Satterthwaite, Nothing to Disclose; D. Wolf, Nothing to Disclose; D. Roalf, Nothing to Disclose; K. Ruparel, Nothing to Disclose; G. Erus, Nothing to Disclose; S. Vandekar, Nothing to Disclose; E. Gennatas, Nothing to Disclose; M. Elliott, Nothing to Disclose; A. Smith, Nothing to Disclose; H. Hakonarson, Nothing to Disclose; R. Verma, Nothing to Disclose; C. Davatzikos, Nothing to Disclose; R. Gur, Nothing to Disclose; R. Gur, Nothing to Disclose.

W226. Sex Differences in Marijuana's Positive Subjective Effects in Daily Marijuana Smokers

Ziva D Cooper*, Margaret Haney

Columbia University, New York, New York

Background: Marijuana continues to be the most widely used illicit drug worldwide, with recent epidemiological studies reporting parallel increases in rates of abuse and number of people seeking treatment for cannabis-use disorders in the United States. Sex appears to contribute to the development of marijuana-use disorders, with women exhibiting an accelerated progression from first use to a marijuana-use disorder relative to men; however, whether there are sex-dependent differences in marijuana's direct effects that may contribute to these divergent trajectories is unknown. This between-groups analysis directly compared the subjective effects of acute marijuana administration in male and female non-treatment seeking marijuana smokers matched for current patterns of marijuana use in an effort to control for the potential impact of tolerance to cannabinoid effects.
Methods: Select data from four, double-blind, within-subject, outpatient studies carried out at the New York State Psychiatric Institute were used for this analysis ($N = 146$). Each of these studies measured ratings of drug quality, drug effect, mood, and physiological effects of a single strength of active marijuana (3.27–5.50% THC, depending on the study) relative to inactive marijuana (0.00% THC). From each study, data from equal numbers of male ($N = 35$) and female ($N = 35$) participants, matched for frequency of marijuana use (days/week) and amount of marijuana smoked per day (marijuana cigarettes/day), were pooled. Subjective and cardiovascular data were collected before (baseline) marijuana smoking and at a range of time-points after smoking. The time-course for marijuana's subjective and cardiovascular effects was analyzed according to marijuana condition (active and inactive) and sex.
Results: Male and female participants did not differ in marijuana smoking frequency ($M = 6.9 \pm 0.4$, $F = 6.8 \pm 0.5$ days/week), number of marijuana cigarettes smoked per day ($M = 4.9 \pm 2.9$, $F = 6.3 \pm 5.6$), or age ($M = 27 \pm 5$ years, $F = 27 \pm 6$ years). They also did not differ in rates of tobacco cigarette or alcohol use. However, men weighed significantly more than women ($M = 72.3 \pm 8.7$ kg, $F = 65.4 \pm 15.7$ kg). Ratings associated with marijuana quality, 'Liking,' 'Good Effect,' 'Take Again,' and 'Strong,' and subjective drug effect, 'High' and 'Stimulated' were significantly greater under active relative to inactive marijuana conditions ($p \leqslant 0.001$). Women reported significantly higher ratings of 'Good Effect' and 'Take Again' ($p \leqslant 0.05$) relative to men under the active marijuana condition. Active marijuana increased heart rate relative to inactive marijuana; no difference in this effect was observed between men and women.
Conclusions: These results indicate that among daily marijuana smokers, women show higher abuse-related subjective effects than men. These sex-dependent differences in marijuana's direct effects may contribute to marijuana use trajectories and the development of marijuana-use disorders.
Keywords: marijuana, dependence, subjective effects, sex differences, abuse liability.
Disclosures: Z. Cooper, Nothing to Disclose; M. Haney, Nothing to Disclose.

W227. Sex-specific Behavioral and Neuroanatomical Markers of Susceptibility to Failed Fear Suppression

Tina Gruene, Elian Roberts, Rebecca Shansky*

Northeastern University, Boston, Massachusetts

Background: A majority of Americans experience a severe trauma in their lifetime, and yet only a small fraction of those people go on to develop Post-Traumatic Stress Disorder (PTSD) as a result. The identification of biological markers that distinguish resilience *vs* susceptibility in this context has been a major focus in both clinical and basic research in the last decade, but a clear picture has yet to emerge. Information is particularly lacking in female-oriented research, despite reports that women are twice as likely as men to develop PTSD after a trauma. In animal models, fear conditioning and extinction paradigms are used to understand the neural circuits and mechanisms that mediate the processing of a traumatic event. However, less than two percent of this research has been done in female animals, and a consensus on the nature of sex differences in these processes has yet to be reached.
Methods: To address this problem, we conducted a large-scale evaluation of behavioral variability in fear conditioning and extinction in gonadally intact male and female rats. Animals that fail to maintain fear suppression after extinction may be a good model of PTSD susceptibility, and we sought to determine whether the behavioral and neuroanatomical profile of susceptible males differed from that of susceptible females. $N = 55$ male and $n = 60$ female Sprague Dawley rats underwent stereotaxic surgery to inject the retrograde tracer Fluorogold (FG) into the BLA, for later identification of the IL-BLA circuit, known to be critical to fear suppression. After recovery from surgery, all animals were tested on classic 3-day cued fear conditioning, extinction, and extinction retrieval. Animals that reached criterion for fear conditioning were sorted by % freezing to tone during extinction retrieval, and the top and bottom 25% for each sex were classified as high fear (HF) or low fear (LF). To probe potential neurological bases for these behavioral discrepancies, we next asked whether HF and LF animals differed in the morphological characteristics of BLA-projecting IL neurons. FG-labeled IL neurons were filled with Lucifer Yellow and imaged in 3D using confocal microscopy. Neurons were digitally reconstructed and analyzed for dendritic arborization and complexity, as well as spine density and morphology.
Results: HF and LF males did not differ in freezing during fear conditioning, but LF males demonstrated facilitated extinction learning. Conversely, HF females froze significantly more during fear conditioning than LF females, but

the two groups did not differ during extinction learning. Preliminary neuroanatomical data suggest distinct disparities between HF *vs* LF males and females.
Conclusions: Our data suggest that both the behavioral predictors and neuroanatomical markers of failed fear suppression are fundamentally different in males and females, and hold implications for the interpretation of these measures in future sex differences studies, as well as for the understanding of the biological substrates of susceptibility in males and females.
Keywords: PTSD, extinction, sex differences, individual differences, vulnerability.
Disclosures: T. Gruene, Nothing to Disclose; E. Roberts, Nothing to Disclose; R. Shansky, Nothing to Disclose.

W228. Estrogen Influences C-fos Expression in the Fear Extinction Network in Female Rats

Kara K Cover, Lisa Maeng, Aaron Landau, Daria Turner, Mohammed R Milad, Kelimer Lebron-Milad*

Harvard Medical School, Charlestown, Massachusetts

Background: Previous studies suggest that estrogen plays a critical role during the consolidation of extinction memory. However, the mechanisms of action by which estrogen influences fear extinction remain to be investigated. In this study we began to investigate the role of estrogen in the expression of biological markers of learning and memory, such as c-fos, in the fear extinction network. One of the objectives of this initial study is to examine how estrogen may or may not affect baseline functioning of brain regions involved in fear conditioning and extinction.
Methods: Experimentally naïve female rats received exogenous IP injections of estradiol (15 μg/kg) or vehicle during the metestrus phase of the menstrual cycle. Seventy-five min after injection, the rats were killed and their brain tissue was collected. An immunohistochemistry protocol was applied to analyze c-fos expression in the ventromedial prefrontal cortex, amygdala and hippocampus.
Results: C-fos expression was significantly reduced in the central nucleus of the amygdala, but not in the infralimbic region of vmPFC in animals receiving estradiol. Further analyses are being conducted to investigate the influence of estradiol in the hippocampus and the basolateral nuclei of the amygdala. In animals that underwent fear conditioning and failed to extinguish fear, there was further reduction of c-Fos expression with both the amygdala and IL, regardless of estrogen administration.
Conclusions: These findings suggest that estradiol modulates c-fos expression in the amygdala but not in vmPFC even at baseline. Future studies are needed to investigate the influence of estrogen in naturally cycling females rat that do not fail extinction. Overall, these findings are necessary to begin to elucidate the molecular cascade by which estradiol facilitates extinction memory and could lead to new developments in the treatment of anxiety disorders.
Keywords: estrogen, fear extinction, psychiatry disorders, c-fos, PTSD.
Disclosures: K. Cover, Nothing to Disclose; L. Maeng, Nothing to Disclose; A. Landau, Nothing to Disclose; D. Turner, Nothing to Disclose; M. Milad, Nothing to Disclose; K. Lebron-Milad, Nothing to Disclose.

W229. Response to Yohimbine and Cocaine Cues in Cocaine-dependent Individuals

Megan Moran-Santa Maria*, Aimee McRae-Clark, Nate Baker, Viswanathan Ramakrishnan, Kathleen T Brady

Medical University of South Carolina, Charleston, South Carolina

Background: Noradrenergic (NA) transmission has been implicated in the symptoms of cocaine withdrawal. Preclinical studies demonstrate significant differences in NA activity between drug naïve animals and animals exposed to cocaine. However, these findings have not been characterized in clinical populations. The objective of this study was to examine the subjective, endocrine and physiologic responses to the α-2 adrenergic receptor antagonist yohimbine and drug-paired cues between cocaine-dependent individuals and controls.
Methods: In a double-blind placebo controlled cross-over study, cocaine-dependent men ($n=32$), cocaine-dependent women ($n=30$), control men ($n=32$) and control women ($n=25$) received either yohimbine or placebo prior to two cocaine cue reactivity sessions.
Results: Anxiety reported in response to yohimbine as compared with placebo was significantly greater in cocaine-dependent individuals than controls both before ($p<0.001$) and after the cue ($p<0.001$). Anxiety reported in response to yohimbine and the cue as compared with placebo and the cue was significantly greater in cocaine-dependent women than cocaine-dependent men ($p=0.052$). Craving in response to yohimbine and the cue as compared with placebo and the cue was significantly greater in cocaine-dependent women than cocaine-dependent men ($p=0.001$). Cortisol ($p<0.001$) and DHEA ($p=0.003$) levels were significantly higher following yohimbine than placebo. Women had a significantly greater heart rate response to yohimbine than men ($p<0.001$).
Conclusions: These findings suggest a functional link between NA transmission and symptoms of withdrawal in cocaine dependent individuals. Moreover cocaine-dependent women reported greater subjective responses to yohimbine than cocaine-dependent men, suggesting that sex differences in NA tone may be an important neurobiologic mechanism underscoring gender differences in addiction.
Keywords: cocaine-dependence, noradrenergic transmission, stress, cues, sex differences.
Disclosures: M. Moran-Santa Maria, Nothing to Disclose; A. McRae-Clark, **Part 4:** Forest Pharmaceuticals: Medication only provided for NIH grant; N. Baker, Nothing to Disclose; V. Ramakrishnan, Nothing to Disclose; K. Brady, **Part 1:** AstraZeneca Pharmaceuticals, **Part 4:** Teva Pharmaceuticals.

W230. Sex Differences in Progesterone, Allopregnanolone, and ACTH Responses to Metyrapone in Men and Women with PTSD

Sabra Inslicht*, Erin Madden, Anne Richards, Evelyn Rucker, Aoife O'Donovan, Madhu Rao, Lisa Talbot, Thomas Metzler, Richard Hauger, Thomas Neylan

University of California, San Francisco, California

Background: Epidemiological data suggest that women are twice as likely as men to develop Posttraumatic Stress

Disorder (PTSD). The biological mechanisms that may account for increased rates of PTSD in women are not known. The sex hormone, progesterone, through conversion to allopregnanolone (ALLO), has potent anxiolytic effects in the brain via interactions with GABA-A receptors, which may serve to terminate the acute stress response. Several studies using either a direct infusion of CRF, or an indirect CRF stimulation by metyrapone through inhibition of the 11-β-hydroxysteroiddehydrogenase type-1 enzyme, suggest that CRF, produces a potent increase in progesterone and ALLO. However, the potential effect that progesterone and ALLO reactivity may account for sex differences in PTSD has not been previously examined.

Methods: Forty-four medically healthy medication-free male and follicular phase female participants with chronic PTSD (50% mean age 30.63, SD = 6.63) and 44 age- and sex- matched controls (50% female; mean age 30.39, SD = 8.15), participated in a study to examine the effects of sex and PTSD status on ALLO, progesterone, and ACTH responses to a metyrapone challenge. Participants were admitted into a General Clinical Research Center (GCRC) for 3 nights. PTSD status was determined using DSM-IV criteria for PTSD with the Clinician Administered PTSD Scale (CAPS) interview. Women participated during the follicular phase of the menstrual cycle and female groups were blocked on the presence of menopausal symptoms. On the second morning on the GCRC, subjects were given an oral dose of metyrapone 750 mg every 4 h starting at 12 h before habitual sleep onset, for a total of 3 doses, and one dose of 2.5 g at habitual sleep onset along with 30ccs of an antacid. Two hours before habitual sleep onset, a catheter was inserted in an antecubital vein for repeated sampling of blood on nights 2 & 3 (5.5 ccs q 15 min providing 32 samples for ACTH and progesterone (16- pre and 16- post metyrapone) and 6 samples for ALLO (3 pre- and 3 post-metyrapone). Samples were analyzed using radioimmunoassay.

Results: Mixed model analyses of log transformed data revealed a significant sex by PTSD by night interaction in mean progesterone levels, $F(1, 2038) = 11.7$, $F(1, 1073) = 2.83$, $p < 0.05$. Women with PTSD had a greater change in pre- to post-metyrapone progesterone levels than control women, $F(1, 974) = 3.92$, $p < 0.05$. In contrast, males with PTSD had a smaller change in progesterone levels than controls, $F(1, 1064) = 8.24$, $p < 0.005$. We also found a significant sex by night interaction indicating a greater increase in ALLO response to metyrapone in women compared to men, $F(1, 1075) = 12.86$, $p < 0.0001$. While there was no difference between PTSD + women and control women, men with PTSD had a greater ALLO response to metyrapone compared to control men, $F(1, 565) = 7.32$, $p < 0.01$. Additionally, a significant sex by night interaction was found for ACTH levels, indicating that women had a greater ACTH response compared to men, $F(1, 2043) = 80.99$, $p < 0.0001$. Among women, those with PTSD had a greater change in pre- to post- metyrapone ACTH levels compared to female controls, $F(1, 976) = 15.16$, $p < 0.0001$, but there was no significant difference among male groups. A greater hourly change in ALLO and progesterone was associated with a greater hourly change in ACTH, $r(66) = 0.31$, $p < 0.05$ and $r(74) = 0.59$, $p < 0.0001$ respectively.

Conclusions: Our findings suggest sex differences in the rise of ACTH, progesterone and ALLO in response to metyrapone and associations between the hourly changes in progesterone and ALLO with ACTH. Progesterone has multiple actions at regulatory sites throughout the HPA axis, which may account for the association with ACTH seen in our study. Progesterone modulates CRH promoter activity through cAMP response elements. Progesterone can also directly bind to type I and type II GR and interfere with glucocorticoid negative feedback regulation. Progesterone may also augment the HPA axis response by increasing chaperone proteins. Our findings of an associations between ALLO and ACTH are consistent with studies that have found that ALLO increases following injections of CRF and ACTH and in response to various stressors, including swim stress, footshock and CO2 exposure. The function of increased ALLO may be to counter-regulate increasing ACTH and progesterone levels. ALLO binds to GABA receptors and modulates GABAergic inhibition of the HPA axis. Rodent studies have shown that inhibition of GABA directly inhibits ACTH and corticosterone secretion. ALLO administration blunts cortisol responses to stress, decreases CRF-enhanced startle, and counteracts anxiogenic responses to CRF during challenging maze tasks. Given that alterations of the HPA axis have been associated with PTSD, and our findings that among women, those with PTSD have greater progesterone and ACTH responses to metyrapone, but, in contrast to men, no greater increase in the ALLO response, it is possible that a dysregulation of ALLO may be involved in the sex bias of PTSD in women.

Keywords: PTSD, sex differences, progesterone, allopregnanolone, ACTH.

Disclosures: S. Inslicht, Nothing to Disclose; E. Madden, Nothing to Disclose; A. Richards, Nothing to Disclose; E. Rucker, Nothing to Disclose; A. O'Donovan, Nothing to Disclose; M. Rao, Nothing to Disclose; L. Talbot, Nothing to Disclose; T. Metzler, Nothing to Disclose; R. Hauger, Nothing to Disclose; T. Neylan, Nothing to Disclose.

W231. Diet-induced Obesity Alters Drug Reward Differentially in Males and Females

Sari Izenwasser*

University of Miami Miller School of Medicine, Miami, Florida

Background: The prevalence of overweight and obese children and adolescents has been escalating, and the number of overweight adolescents has tripled in the past thirty years. High fat and/or high sugar diets have been shown to overlap in activating the same pathways in the brain as drugs of abuse. Adolescence is a vulnerable period associated with a high incidence of drug abuse initiation and an increased risk for developing dependence and addiction, and drug use during this critical developmental period has been associated with higher incidence of cocaine abuse later during adulthood. It is important to understand factors that modulate the effects of drugs of abuse during this critical period of development, as well as the underlying neurochemical changes mediating these effects.

Methods: In this study, the effects of a high fat diet on cocaine reward in male and female adults and adolescents were examined on cocaine reward and on anxiety using a plus maze.

Results: The data show that a high fat diet produced different effects in males and females, both during adolescence and adulthood. In males, a high fat diet led to greater sensitivity to cocaine reward in males, but not in females. In contrast, removal of a high fat diet greatly diminished cocaine reward in males, but made adult females more sensitive to the rewarding effects of cocaine. The data also show that levels of proteins involved in dopaminergic transmission, as well as hormones involved in feeding were differentially affected by diet in males and females.
Conclusions: These data show that a high fat diet during adolescence differentially affects males and females. Males on a high fat diet may be more vulnerable to the effects of drugs of abuse, whereas females have increased vulnerability subsequent to a change in diet.
Keywords: obesity cocaine conditioned place preference.
Disclosures: S. Izenwasser, Nothing to Disclose.

W232. Reduced Motivation to Self-administer Methamphetamine by Oxytocin in a Behavioral-economics Paradigm Predicts Reinstatement of Methamphetamine Seeking

Brittney M Cox*, Brandon Bentzley, Carmela M Reichel, Ronald E See, Gary Aston-Jones

Medical University of South Carolina, Charleston, South Carolina

Background: Human and animal studies suggest that females differ in their motivation to abuse methamphetamine (meth) and show greater propensity to relapse. However, addiction pharmacotherapies have primarily been tested in males, which may not accurately predict treatment outcomes in females. Evidence suggests that oxytocin, an endogenous peptide well known for its role in social behaviors and childbirth, is a promising addiction pharmacotherapy. We have shown that oxytocin differentially affects meth seeking in males *vs* females on a progressive ratio (PR) schedule of reinforcement, but shows similar attenuation of reinstatement of meth seeking. To further examine the relationship between motivation and relapse, we used a within-session behavioral economics (BE) paradigm, which was modeled after BE procedures commonly used to assess motivation for reward in humans and non-human primates. Our paradigm allows for simultaneous measurement of drug demand at high effort (motivation; α), normalized based on the intake at low effort (baseline consumption; Q0). This approach also allowed us to assess individual variability in meth demand in relation to relapse behaviors, and in response to oxytocin administration.
Methods: Male and female Sprague Dawley rats were trained to self-administer meth for multiple days at each fixed ratio (FR) value used in the BE paradigm (1, 3, 10, 32, 100), followed by a shift to daily BE sessions. During the BE sessions, rats self-administered at each FR value in 5 min bins in descending order, with 20 min timeouts in-between each bin. After responding on the BE paradigm stabilized, rats were tested with oxytocin (1 mg/kg, IP) or saline vehicle in a counterbalanced order with a minimum of 3 stabilization days between each test. We also assessed the effects of oxytocin on cue-induced and meth-primed reinstatement of meth seeking.
Results: As compared to males, females showed greater motivation to seek meth (lower α) and higher meth intake (higher *Q0*). In both sexes, α and *Q0* correlated with both cue-induced and meth-primed reinstatement. Oxytocin decreased motivation to seek meth in both sexes during BE and reinstatement. Further, a correlation was seen between the effect of oxytocin during BE (decreased motivation, higher α) and attenuation of cue-induced reinstatement.
Conclusions: These sex differences in motivation and meth intake are consistent with previous data using similar paradigms. However, these results show that the variables (α and *Q0*) assessed during BE accurately predict reinstatement of meth seeking in both sexes. In addition, the effects of oxytocin during the BE paradigm predicted attenuation of cue-induced reinstatement. Overall, this novel paradigm will help to better delineate sex differences observed in the motivation to seek meth and may predict the efficacy of pharmacotherapies to treat meth addiction.
Keywords: self-administration, reinstatement, behavioral economics, methamphetamine, oxytocin.
Disclosures: B. Cox, Nothing to Disclose; B. Bentzley, Nothing to Disclose; C. Reichel, Nothing to Disclose; R. See, Nothing to Disclose; G. Aston-Jones, Nothing to Disclose.

W233. Sex Differences in Corticotropin Release Factor-evoked Anxiety-related Behavior

Debra Bangasser*, Hannah Simko, Adam Hawkins, Brittany Wicks, Rob Cole, Jeremy Schmidt, Michelle Lerner

Temple University, Philadelphia, Pennsylvania

Background: Stress-related psychiatric disorders, such as anxiety and depression, occur twice as frequently in women as in men. However, the neurobiological basis for this disparity remains largely unknown. Corticotropin-releasing factor (CRF) orchestrates the stress response and is dysregulated in stress-related disorders. Previously, we identified sex differences in the CRF1 receptor coupling, signaling, and trafficking that increase sensitivity of locus coeruleus neurons to CRF in female compared to male rats. Although these CRF-induced physiological sex differences likely translate into sex differences in behavior, this possibility has not been systematically explored. Additionally, it is unclear whether sex differences in CRF sensitivity are limited to the locus coeruleus, or whether they occur in other brain regions. In the present study, we explore these issues by examining whether CRF induces greater anxiety-related behaviors in female compared to male rats.
Methods: The CRF-evoked behavior task developed by Howard *et al* (2008) was used here to examine, for the first time, behavioral sex differences in response to CRF. To this end, adult (>60 day old) male and female Sprague-Dawley rats were surgically implanted with a cannula aimed at the lateral ventricle for CRF administration. Following recovery (1 week), rats were habituated to a behavior chamber that contained bedding (7.5 cm deep). The next day following a 30 min baseline recording, rats were given the first of three varying doses of ovine CRF (0.1, 0.3, or 3.0 μg in 3 μl intracerebroventricular infusion) or vehicle (artificial cerebral spinal fluid). After the infusions, rats were returned to

the chamber and their behavior was videotaped (1 h) for later evaluation of the following behaviors: burying, a defensive coping strategy indicative of high anxiety; grooming, a displacement behavior associated with arousal reduction; and headshakes, an increased motor response related to high arousal. The rats were then tested with the remaining doses using a counterbalanced, repeated-measures design, with 1 week separating each dose.
Results: A mixed factor ANOVA revealed a significant interaction between sex and dose for grooming [$F(3,48)=4.95$, $p<0.05$]. *Post-hoc* tests showed that the lowest dose of CRF (0.1 μg) increased grooming similarly in males and females ($p>0.05$). However, females groomed significantly more than males at the higher doses (0.3 μg and 3.0 μg) ($ps<0.05$). CRF increased burying behavior in a dose-dependent manner [$F(3,48)=5.19$, $p<0.05$]. However, there was neither a sex difference in burying [$F(1,16)<1.0$] nor a sex by dose interaction [$F(3,48)<1.0$]. Similarly, there was a dose-dependent increase in headshakes [$F(3,48)=6.39$, $p<0.05$], but no effect of sex [$F(1,16)<1.0$] nor an interaction [$F(3,48)<1.0$].
Conclusions: These studies revealed that high doses of CRF administered centrally increased grooming in females more than in males. However, not all anxiety-related behaviors showed a greater response to CRF in females, as sex differences were not observed in burying or headshakes. Because grooming, burying, and headshaking are mediated by different circuits, these results suggest sex differences in CRF sensitivity in the circuits that mediate grooming. A previous study demonstrated that CRF-evoked grooming was primarily mediated by serotonin, at least in males (Howard *et al*, 2008). Thus, the present results suggest that the serotonin system may be more sensitive to CRF in females than in males. Further investigation of sex differences in CRF-serotonin interactions may reveal mechanisms underlying female vulnerability to stress-related disorders.
Keywords: corticotropin releasing hormone, sex difference, anxiety, stress, depression.
Disclosures: D. Bangasser, Nothing to Disclose; H. Simko, Nothing to Disclose; A. Hawkins, Nothing to Disclose; B. Wicks, Nothing to Disclose; R. Cole, Nothing to Disclose; J. Schmidt, Nothing to Disclose; M. Lerner, Nothing to Disclose.

W234. Contributions of Estrogen and Oral Contraceptive Use to Sex Differences in Functional Responding to Conditioned Cues During Fear Conditioning

Moon Jung Hwang, Huijin Song, Rachel Zsido, Edward F Pace-Schott, Karen Klahr K Miller, Mohammed R Milad*

Harvard Medical School and Massachusetts General Hospital, Charlestown, Massachusetts

Background: Recent data from rodents and human imaging studies have shown that sex hormones, especially estrogen, modulate the functional reactivity of the fear extinction network. In parallel, imaging studies in healthy subjects and in PTSD patients have revealed sex differences in the acquisition of conditioned fear. However, the potential contributions of estrogens and oral contraceptive use to sex differences during fear learning have not been specifically examined.
Methods: We conducted an fMRI study with simultaneous recording of skin conductance response (SCR) using a randomized, between-subjects design, coupled with a standard Pavlovian fear conditioning paradigm and a partial reinforcement schedule. Previously acquired data from a healthy, naturally cycling group of women were divided into two subgroups: high estrogen (HE, $n=15$) and low estrogen (LE, $n=12$) groups. An additional group of women using oral contraceptives (OC, $n=13$) and a group of men (M, $n=33$) were included in the analyses. Functional MRI data during the fear conditioning phase were collapsed across all subjects. Using a threshold of $p<0.01$ FDR corrected, functional regions of interest (ROI) were generated from this activation map (across all subjects). The beta values of each ROI that satisfied this threshold were extracted and analyzed for each group. The total number of subjects used for the generation of the functional ROIs was 73.
Results: SCR showed no sex difference between men and all women combined during fear conditioning. Other than in the dorsal anterior cingulate cortex (dACC), sex differences also did not emerge from the functional MRI data when comparing men with all women combined. However, sex differences, as well as differences between the different sub-groups of women, in functional brain responses appeared when women were separated into the three distinct groups (ie, HE, LE and OC). Although group differences in SCR continued not to be seen, sex differences emerged between the M and LE groups within the dorsal anterior cingulate cortex (dACC). Differences in brain reactivity to conditioned cues were noted between the M and OC groups in the following brain regions: thalamus, midbrain, amygdala and hippocampus.
Conclusions: After establishing that there were no sex differences in fear conditioning when men were compared to women as a whole, this study is the first to show that fear conditioning differences in the brain emerged when women were divided into subgroups based on their hormonal status. These differences in hormone levels may influence the reactivity of various neural nodes important for fear conditioning. The specific influence of oral contraceptives on the fear network is important to further our understanding of how oral contraceptive use may interact with natural estrogens during fear learning.
Keywords: estrogen, oral contraceptive, sex difference, fear conditioning, fMRI.
Disclosures: M. Hwang, Nothing to Disclose; H. Song, Nothing to Disclose; R. Zsido, Nothing to Disclose; E. Pace-Schott, Nothing to Disclose; K. Miller, Nothing to Disclose; M. Milad, Nothing to Disclose.

W235. Sex Differences in the Neural Processing of Emotions within the Theoretical Framework of the Circumplex Model of Affect

Jarod Peterson*, James Russell, Yuankai Huo, Angela Tseng, Bradley S Peterson, Zhishun Wang

Columbia University College of Physicians and Surgeons, New York, New York

Background: Although males and females are thought to differ in their experience and processing of emotions, little research supports this claim. We used functional MRI to assess sex-specific effects on neural activity when processing the valence and arousal dimensions of facial emotions within the theoretical framework of the Circumplex Model of Affect.

Methods: We presented monochromatic Ekman faces expressing a wide range of emotions to 56 healthy child and young adult participants (28 males and 28 individually age-matched females, ages 7–34 years, mean 19.5 ± 6.9 years) and asked them to rate the valence and arousal content of each face during fMRI. We correlated BOLD signal, an index of neural activity, with ratings of valence and arousal for each participant, as well as with the absolute value of those ratings (transformational measures intended to identify neural responses that occur in response to the extremes of valence and arousal). We compared those correlations across sex while covarying for age using Bayesian inference. We also assessed how the sex of the stimulus moderated group differences in the neural processing of valence and arousal.
Results: Significant effects, independent of sex of the participant or stimulus, included: (a) valence ratings correlated inversely with neural activity in inferior temporal, superior temporal, supplemental motor, and dorsal parietal cortices; (b) arousal ratings correlated inversely with neural activity in dorsolateral prefrontal, inferior parietal, posterior parietal, and dorsal anterior cingulate cortices, and caudate nucleus. Findings for differences in correlations across males and females included: (a) Valence—inverse correlations were generally stronger in males, particularly in middle frontal, medial temporal, lingual, and precuneate cortices; (b) Arousal—sex differences were not significant; (c) Absolute Valence—correlations with neural activity were inverse in males but positive in females in superior temporal, middle temporal, and posterior cingulate cortices; (d) Absolute Arousal—correlations with neural activity were positive in males but inverse in females in middle frontal, superior temporal, middle temporal, inferior parietal, precuneus, and parahippocampal cortices. Differences in neural activity across sexes varied significantly according to the sex of the stimulus for ratings of (a) Arousal—inverse correlations with neural activity were stronger for females viewing male faces and males viewing female faces in the insula, dorsolateral prefrontal, superior temporal, inferior parietal, and superior parietal cortices; (b) Absolute Valence—inverse correlations were stronger for males viewing male faces and positive correlations were stronger for males viewing female faces in inferior frontal, dorsolateral prefrontal, posterior cingulate, and superior parietal cortices. None of the correlations for valence, arousal, or the absolute values correlated significantly with age of the participants. No interactions of age with sex of the participant were significant.
Conclusions: Temporal and parietal cortices supported the processing of valence independent of sex of the participant. These regions likely support processing of the social content of emotional faces, activating more with progressively more negative valence. Stronger inverse correlations in males suggested that neural response to more negatively valenced faces is stronger in males than in females. Correlations of the absolute value of valence with neural activity also were inverse in males, significantly more so when viewing male faces, indicating that greater neural activity with more negatively valenced faces is nonlinear in males, especially so when viewing male faces. These stimulus-dependent effects may derive from the subjective alarm males experience when viewing the most negatively valenced emotions, which include anger, fear, and sadness, especially when viewing them in male faces. Regions supporting the processing of arousal were dorsolateral prefrontal, parietal, and anterior cingulate cortices, regions that likely support attentional processes. These regions activated progressively more with decreasing levels of arousal, which indicates greater neural activity in arousal circuits when processing progressively more ambiguous facial emotions, which likely requires a greater allocation of attention than when identifying other emotions, especially as ambiguous stimuli are thought to represent an unidentified threat. Correlations of neural activity with absolute measures of arousal differed across sexes, in a direction indicating greater activity in males for the extremes of arousal, suggesting that neural responses to emotionally ambiguous and highly arousing faces is greater in males than in females. Inverse correlations of arousal ratings with neural activity were stronger for males viewing female faces and females viewing male faces, suggesting that the neural effects of ambiguity are greater for both sexes when viewing faces of the opposite sex.
Keywords: circumplex, affect, sex differences, valence, arousal.
Disclosures: J. Peterson, Nothing to Disclose; J. Russell, Nothing to Disclose; Y. Huo, Nothing to Disclose; A. Tseng, Nothing to Disclose; B. Peterson, Nothing to Disclose; Z. Wang, Nothing to Disclose.

W236. Adolescent Sex Differences in Fronto-limbic Activity During Selective Attention and Emotion Processing

Crystal E Schiller*, Joshua Bizzell, Sarah Hart, Ayse Belger

University of North Carolina, Chapel Hill, North Carolina

Background: Sex differences in brain maturation emerge during adolescence, a critical period for the development of executive functioning and emotional processing. Most prior studies of sex differences in brain maturation have focused on structural development and have compared adolescents (ages 9–17) to adults without accounting for the onset of puberty. The purpose of this investigation is to examine sex differences in brain function before and after age 12, which is used as a proxy for pubertal development, in asymptomatic adolescents with and without first-degree family members with schizophrenia. If puberty influences brain maturation, then we would expect older participants show greater sex differences in selective attention and emotion processing. We also explored resting state connectivity in frontal and limbic regions.
Methods: Functional magnetic resonance images (fMRI) were collected from 30 boys and 38 girls ages 9–18. The majority of participants ($n = 49$) were older than 12, and although asymptomatic, 22 participants had a first-degree family member with schizophrenia ('genetic high risk;' GHR). Of the GHR participants, 8 were male and 14 were female. Participants performed an emotional oddball task requiring both selective attention and suppression of task-irrelevant emotional information. Whole brain analyses examined sex × age interactions in peak hemodynamic responses ('brain activation') during the presentation of target and emotional stimuli for the whole sample. Significant results were followed by *t*-tests to examine sex, age, and group (GHR *vs* control) differences. Analyses were conducted using FMIRB's Local Analysis of Mixed Effects

(FLAME) within the fMRI Expert Analysis Tool (FEAT). Group-level activation maps were thresholded using a z score of 2.3 ($p<0.01$) to define contiguous clusters of activation. Resting state fMRI data was acquired in 26 subjects, and an intrinsic network connectivity analysis was run to examine correlations among frontal, striatal, and limbic regions.
Results: During selective attention, there was a significant age × sex interaction in activation of the posterior cingulate. In subjects older than 12, males showed greater activation of the lateral occipital cortex and middle temporal gyrus, whereas females showed greater activation of the paracingulate gyrus and frontal pole. There was not a significant group (GHR *vs* control) by sex interaction effect in the subjects older than 12, and when GHR subjects were excluded from analyses, control subjects showed the same pattern of significant sex differences. During emotion processing, there was a significant age × sex interaction in activation of the hippocampus and lingual gyrus. In subjects older than 12, males showed greater activation of the supramarginal gyrus, hippocampus, precentral gyrus, superior frontal gyrus, and thalamus. Females did not show greater activation than males in any area. Again, there was not a significant group (GHR *vs* control) by sex interaction effect in the subjects older than 12. However, when GHR subjects were excluded from analyses, additional sex differences emerged. In the control group, males showed greater activation of the precuneous cortex, hippocampus, anterior cingulate, precentral gyrus, and inferior frontal gyrus, whereas females showed greater activation of the central opercular cortex during emotional processing. There were no significant sex differences in subjects younger than 12 during selective attention or emotion processing. Resting state analyses revealed that males showed greater connectivity than females between the amydala, hippocampus, cingulate and frontal regions.
Conclusions: Results suggest that sex differences in activation of the neural circuitry engaged during selective attention and emotional processing emerge during puberty. During selective attention, adolescent males showed increased activity in areas associated with object perception and recognition, whereas females showed increased activity in areas associated with conflict monitoring and error detection, which may signify the emergence of different approaches to task performance at puberty. In contrast to our hypothesis, males showed greater activity than females in several subcortical regions during emotion processing. An important limitation of this study is the relatively small number of subjects younger than 12 ($n=19$). Thus, the presence of sex differences in brain activation in older participants and not in younger participants may reflect either the activational effects of puberty on brain maturation or limited power. Resting state results suggest that greater fronto-limbic activity in older males during executive and emotion processing are not task-specific and may reflect gender differences in functional connectivity that emerge at puberty. Further research is needed to examine the mechanisms contributing to neural development at puberty, and subsequent studies should directly examine the role of pubertal development and sex steroids in the maturation of the neural circuitry engaged during selective attention and emotional processing.

Keywords: sex, gender, neuroimaging, puberty, adolescents.
Disclosures: C. Schiller, Nothing to Disclose; J. Bizzell, Nothing to Disclose; S. Hart, Nothing to Disclose; A. Belger, Nothing to Disclose.

W237. Is Alzheimer Disease a Different Disease in Men and Women? Observations from Autopsied Brains and Transgenic Studies

Bradley Chaharyn, Paul Pennington, Kelsey Fehr, Zelan Wei, Jennifer Chlan, Darrell Mousseau*

University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Background: A history of depression can increase the risk of developing Alzheimer disease (AD) in later life, yet how the one leads to the other is unclear. The enzyme monoamine oxidase-A (MAO-A) has been historically associated with depression, but has also been implicated in the early stages of AD. The brains of AD patients often have a higher density of 'plaques' that are composed primarily of the b-amyloid peptide, which is a product of secretase-mediated cleavage of the Amyloid Precursor Protein (APP). Research into causes of AD have focussed primarily on this peptide and its removal from the brain, with very limited clinical success.
Methods: Autopsied brain samples from control and AD patients as well as transgenic mice expressing an AD-related APP were used to compare secretase expression patterns, APP processing, MAO activities and neurochemical correlates in males and females.
Results: While screening autopsied brain samples from control and AD patients, we observed a mismatch between MAO-A activies and MAO-A protein expression in corresponding samples. This mismatch was sex-dependent. In addition, we also observed patterns of APP fragments and secretases that were inconsistent between the male and female samples. These inconsistencies were particularly evident in the female AD samples. Using transgenic mice expressing an AD-related APP (TgAPP), we observed that older TgAPP mice were significantly more 'depressed' (using a test for behavioural 'despair') than younger TgAPP mice. This 'depression-like' phenotype paralleled a shift from a- to b-secretase processing of APP. Overexpression of naturally occurring N- and C-terminal APP fragments in neuronal cell cultures revealed distinct effects on MAO-A activity that could account for a shift in susceptibility for co-morbid depression in AD.
Conclusions: Our data support a novel role for APP that could contribute directly to a [prodromal] depressed phenotype as APP is progressively and invariably cleaved by distinct secretases (such as in the aging brain and more so in the brains of patients with AD). Our data suggest that APP might first and foremost be a depression-related protein and that this unexamined aspect of APP function could contribute to the higher prevalence of depression as well as AD observed in women.
Keywords: secretases; depression; cortex; monoamine; pharmacotherapy.
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