Neuropsychopharmacology (2013) 38, S273–S434

www.neuropsychopharmacology.org

Poster Session II-Tuesday
Tuesday, December 10, 2013

T2. Effects of CFA-induced Chronic Inflammatory Pain on Opioid Self-administration and Accumbal Dopamine Release in Heroin Dependent Rats

Jose Moron-Concepcion*, Lucia Hipolito-Cubedo

Columbia University, New York, New York

Background: The use of opioid-based therapies in patients with chronic pain is a challenge in the medical practice because of drug abuse liability. This problem is magnified in the case of patients with a previous history of opioid abuse leading to reduced treatment of pain conditions in this population. Surprisingly, few studies have investigated how chronic pain could alter opioid intake patterns and none of them have focused in an opioid dependent population. In addition, although it is known that opioid reinforcement is mediated through the activation of the mesolimbic dopamine (DA) neurons projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), there is uncertainty about the effects of pain on DA transmission within these brain areas and whether pain-induced effects on DA transmission within the VTA-NAc pathway may impact the reinforcing properties of opioids.

Methods: In the present study, we investigated the effects of chronic inflammatory pain on i.v. heroin self-administration under fixed ratio (FR) and progressive ratio (PR) schedules of reinforcement. We selected the complete Freund's adjuvant (CFA) rat model of inflammation to assess the effect of chronic inflammatory pain on opioid abuse. In addition, we investigated the neurochemical changes induced by chronic inflammatory pain on DA transmission within the mesolimbic pathway by conducting *in vivo* microdialysis studies. All experimental protocols in animal studies were approved by the Institutional Animal Care and Use Committee at Columbia University.

Results: First, to examine whether the injection of CFA could affect the correct performance of the self-administration task we analyzed the effect of CFA-induced chronic inflammation on sucrose intake under a FR2 schedule, 2 and 7 days after saline or CFA injection. Neither CFA nor saline injection altered the number of correct responses *vs* the number of incorrect responses during sucrose FR2 self-administration sessions, indicating that CFA injection does not affect the correct performance of this task. Next, we studied motivated behavior for heroin self-administration. Seven days following iv cannulation, animals were placed in the operant chamber and trained to self administer sucrose until they could complete three consecutive sessions by self administering 60 sucrose pellets per session. Once the animals acquired the self administration behavior, they were given 2 h access to heroin (50 μg/kg/infusion) under FR1 schedule. After stable drug intake was obtained (defined as 5 consecutive sessions in which the number of infusions did not vary by more than 15% of the mean value obtained across those sessions), the number of responses required for an infusion was increased to 2 and then to 5 for a total of 6 sessions. Then, all animals underwent an initial PR session, where the number of correct responses increases exponentially with each administration, to measure their basal motivation for heroin (50 μg/kg/infusion). Two additional PR sessions at two different time points (2 and 7 days after CFA or saline injection, respectively) were conducted. Pain decreased the breakpoint (maximum number of responses that the animal completes in order to receive a reward) at the two time points tested after CFA injection for heroin at a dose of 50 μg/kg/infusion. These data indicate that CFA-induced pain impacts motivated behavior for self-administration at this lower dose of heroin. Next, we investigated whether this decrease in the break point induced by pain was dependent on the dose of heroin. Animals were trained under FR1 and FR2 schedules (50 μg/kg/infusion heroin) and 2 days after CFA or saline injection they were placed again in the operant box to generate a 'within-session' dose response curve. One of three doses of heroin (50, 100, 200 μg/kg/infusion) was made available for a 1-h time period during the session, with a 15-min resting period between doses. Doses were altered by varying the infusion duration. The presentation of the doses was made in an ascending or descending manner. Both dose-response curves showed a deviation from the expected linear dose-response for heroin, suggesting a possible change in the sensitivity (ie of the reinforcing properties) to heroin in the presence of chronic inflammatory pain. Finally, in order to determine whether these pain-induced altered patterns of opioid self-administration were related to the effects of chronic inflammatory pain on DA transmission within the VTA-NAc pathway we conducted *in vivo* microdialysis studies in CFA- and saline-injected rats. We found that chronic inflammatory pain blunted the effects of a 50 μg i.v. heroin challenge on DA release in the NAc.

Conclusions: These data suggest that chronic inflammatory pain may produce a rightward shift in the rewarding properties of heroin in opioid dependent rats, such that higher doses of heroin are needed for reliable rates of self-administration. In addition, our findings indicate that these effects may be related to an attenuation of DA transmission within the VTA-NAc pathway triggered by the presence of chronic inflammatory pain. Therefore, these data suggest that chronic inflammatory pain induces changes in the VTA-NAc pathway which in turn may facilitate opioid dose escalation in order to maintain the rewarding properties of the drug.

Keywords: pain, opioid abuse, self-administration, Nucleus accumbens, dopamine.

Disclosures: J. Moron-Concepcion, Nothing to Disclose; L. Hipolito-Cubedo, Nothing to Disclose.

T3. Contributions of Glial Glutamate Transport and NMDA Receptors in Nicotine Relapse

Cassandra Gipson*, Yonatan M Kupchik, Neringa Stankeviciute, Peter W Kalivas

Medical University of South Carolina, Charleston, South Carolina

Background: Addiction to nicotine produces long-lasting, stable changes in brain synaptic physiology that might contribute to the vulnerability to relapse. However, it is not known if synaptic changes are initiated by and contribute to nicotine relapse. Cues associated with nicotine use can precipitate relapse, and using a rat model of cue-induced reinstatement of nicotine seeking, we quantified relapse-associated nucleus accumbens core (NAcore) extracellular glutamate as well as synaptic plasticity via morphological changes in dendritic spine head diameter and electrophysiological estimates of excitatory synaptic transmission (AMPA:NMDA ratio). As well, we quantified changes in proteins relevant to glutamatergic transmission following extinction from nicotine self-administration, including the AMPA receptor subunit GluA1, the NMDA receptor subunits GluN2A and GluN2B, and the glial glutamate transporter GLT-1. Finally, we examined inhibition of GluN2A and GluN2B receptors as well as restoration of GLT-1 on cue-induced nicotine seeking.
Methods: Male Sprague-Dawley rats were trained to press an active lever for delivery of chow pellets prior to self-administration of nicotine (0.02 mg/kg/infusion) on an FR1 schedule during 2 h sessions each day, for approximately two weeks. A lever press resulted in a nicotine infusion paired with presentation of light and tone cues. Following self-administration, rats were placed in an extinction training phase in which a lever press no longer resulted in drug or drug-paired cues. Rats were given contingent cues in which lever press responding led to contingent presentations of the compound cue stimulus previously paired with nicotine infusions, but no nicotine was delivered. In some experiments, rats were injected prior to reinstatement with either ifenprodil (0, 1, or 3 mg/kg, i.p.), TCN-201 (0, 0.01, or 0.1 nmole, intra-NAcore), or were injected chronically with N-Acetylcysteine (100 mg/kg, i.p.). Responding to the lever previously associated with drug delivery was the measure of nicotine-seeking behavior. After the appropriate timepoint during reinstatement (0 or 15 min), rats were sacrificed for western blots, spine morphology, or electrophysiological measures. Nucleus accumbens core (NAcore) tissue for western blots was dissected, and membrane fractionation was prepared. For spine morphology, lipophilic dye was diolistically delivered onto NA slices, and dendritic spines were then imaged and analyzed. NA slices were also taken from animals for electrophysiology, and AMPA and NMDA currents were recorded in whole cell patch-clamp configuration. As well, NA tissue was taken from separate nicotine-extinguished animals and processed for appropriate western blotting for GluA1, GluN2A, GluN2B, and GLT-1. Separate animals were also used to examine nicotine conditioned cue-induced glutamate release in the NAcore via *in vivo* microdialysis.
Results: Withdrawal from nicotine self-administration caused a basal increase in NAcore spine head diameter and ratio compared to yoked saline animals, and cue-induced reinstated nicotine seeking elicited further increases in head diameter and AMPA:NMDA ratio within 15 min of the priming stimulus. Enlargement of dendritic spines has been associated with increased synaptic strength, as well as an upregulation in surface expression of GluA subunits of AMPA and GluN2B-containing NMDA receptors. Importantly, we found that GluA1, GluN2A, and GluN2B were upregulated after extinction from nicotine self-administration in parallel with an increase in spine head diameter and AMPA:NMDA ratio. Additionally, a decrease in the glial glutamate transporter GLT-1 was found. When nicotine seeking was reinstated by presentation of conditioned cues, there were parallel increases in behavioral responding and NAcore extracellular glutamate. These findings suggest that targeting glutamate transmission might inhibit cue-induced nicotine seeking. In support of this hypothesis, we found that pharmacological inhibition of GluN2A with TCN-201 or GluN2B with ifenprodil, as well as upregulation of GLT-1 with N-acetylcysteine, abolished reinstated nicotine seeking in nicotine-extinguished animals.
Conclusions: These results indicate that up-regulated GluN2A, GluN2B, down-regulated GLT-1, and rapid synaptic potentiation in the accumbens contribute to cue-induced relapse to nicotine use, and may be important pharmacotherapeutic avenues in reducing nicotine relapse.
Keywords: nicotine, glutamate, relapse, addiction, nucleus accumbens.
Disclosures: C. Gipson, Nothing to Disclose; Y. Kupchik, Nothing to Disclose; N. Stankeviciute, Nothing to Disclose; P. Kalivas, Nothing to Disclose.

T4. Behavioral and Molecular Consequences of GAD1 Downregulation in Cannabinoid Receptor 1 Expressing Interneurons

Jacquelyn Brown*, Szatmar Horvath, Krassimira Garbett, Monica Everheart, Karoly Mirnics

Vanderbilt University, Nashville, Tennessee

Background: Schizophrenia is a devastating neurodevelopmental disorder that affects approximately 1% of the population. Reduced expression of the gene GAD1 encoding the 67-kDa isoform of glutamic acid decarboxylase (GAD67) is a hallmark of the disease. In schizophrenia, GAD67 downregulation occurs in multiple interneuronal subpopulations, including the cannabinoid receptor type 1 positive (CB1R +) cells, but the behavioral consequences of these disturbances are not well understood.
Methods: To determine empirically whether GAD67 downregulation is sufficient to induce alterations in the brain and behavior and whether these alterations are dependent on the interneuron subtype affected, we have developed a novel method for silencing GAD67 in distinct subpopulations of interneurons in transgenic mice. We created bacterial artificial chromosome (BAC) constructs containing the CB1R promoter-enhancer elements, an eGFP reporter, and a synthetic microRNA (miRNA) targeted to silence GAD67 mRNA. Construct efficacy and validity was assessed using immunohistochemistry. Male transgenic mice ($n = 12$) and

their wildtype littermates ($n = 12$) were subjected to a broad behavioral testing battery to assess general neurological function (Irwin Screen), learning, memory, anxiety, social behavior, sensorimotor gating, and locomotor activity. Monoamine levels in the striatum were assessed using HPLC.
Results: Our construct effectively suppressed GAD67 expression in CB1R+ interneurons. While eGFP was detected in CBR1+ cells, GAD67 expression could not be detected in the targeted interneurnal subpopulation. Behavioral characterization of this mouse model showed elevated persistent conditioned fear cue based memory attenuation and significantly altered response to amphetamine (3 mg/kg) challenge. These deficits could not be attributed to sensorymotor perturbations or changes in baseline learning and memory. Furthermore, these mice, when challenged by amphetamine and CB1 agonist CP55940, showed significantly elevated levels of serotonin in the striatum.
Conclusions: We conclude that GAD67 downregulation in a single interneuron cell type is sufficient to induce behavioral changes, and suggest that adolescent cannabionoid abuse might have a significant effect on interneurons, playing a critical role in predisposing to schizophrenia. Furthermore, our results suggest that GABA system dysfunction in the CB1R+ interneurons has a profound effect on the dopaminergic/serotoninergic system, and this physiological interdependence warrants further examination.
Keywords: schizophrenia, cannabinoid, CNR1, GABA, GAD67, GAD1, transgenic, serotonin, dopamine.
Disclosures: J. Brown, Nothing to Disclose; S. Horvath, Nothing to Disclose; K. Garbett, Nothing to Disclose; M. Everheart, Nothing to Disclose; K. Mirnics, Nothing to Disclose.

T5. Modeling Fall Propensity in Parkinson's Disease: Deficits in the Attentional Control of Complex Movements in Rats with Cortical-Cholinergic and Striatal-Dopaminergic Deafferentation

Martin Sarter*, Aaron Kucinski

University of Michigan, Ann Arbor, Michigan

Background: Cognitive symptoms, complex movement deficits and increased propensity for falls are interrelated and levodopa-unresponsive symptoms in patients with Parkinson's Disease (PD). We developed a test system for the assessment of fall propensity in rats and tested the hypothesis that interactions between loss of cortical cholinergic and striatal dopaminergic afferents increase fall propensity.
Methods: We developed a new behavioral test system (Michigan Complex Motor Control Task, MCMCT) that is designed to tax the ability to rapidly correct movement errors when traversing complex rotating surfaces (square rods). Traversing rotating rods requires exquisite gait control, carefully timed and precisely placed weight-shifting steps, and nearly perfect limb coordination. Rats were trained to traverse stationary and rotating rods, placed horizontally or at inclines, and while exposed to distractors. Rats also performed an operant Sustained Attention Task (SAT). Partial cortical cholinergic and/or caudate dopaminergic deafferentation were produced by bilateral infusions of 192 IgG-saporin (SAP) into the basal forebrain and/or 6-hydroxydopamine (6-OHDA) into the caudate nucleus, respectively, modeling the lesions seen in early PD.
Results: Rats with dual cholinergic-dopaminergic lesions (DL) fell more frequently than SAP or 6-OHDA rats. Falls in DL rats were associated with incomplete rebalancing after slips and low traversal speed. Ladder rung walking and pasta handling performance did not indicate sensorimotor deficits. SAT performance was impaired in DL and SAP rats; however, SAT performance and falls were correlated only in DL rats. Furthermore, in DL rats, but not in rats with only dopaminergic lesions, the placement and size of dopaminergic lesion correlated significantly with fall rates.
Conclusions: The results support the hypothesis that after dual cholinergic-dopaminergic lesions, attentional resources can no longer be recruited to compensate for diminished striatal control of complex movement, thereby 'unmasking' impaired striatal control of complex movements and yielding falls. The new test system is suitable for assessing the potential of new treatments for falls and related movement errors, including the freezing of gait. These levodopa-unresponsive symptoms often are disabling and currently remain untreated. Nicotinic drugs have been demonstrated to benefit SAT performance (Howe *et al*, 2010) and thus, in interaction with potentially relatively low doses of L-DOPA, may benefit the non-motor symptoms of PD. Preliminary evidence treating DL rats with such an alpha4beta2* agonist and a low dose of L-DOPA support the promise of such a treatment combination.
Keywords: attention, aging, Parkinson's disease, nicotine, animal model.
Disclosures: M. Sarter, Nothing to Disclose; A. Kucinski, Nothing to Disclose.

T6. Different Adolescent Traumatic Stress Pre-exposures Differentially Modify Adulthood Predator Stress Responses in Rats

Nicole LT Moore*, Daniel E Altman, Sangeeta Gauchan, Raymond F Genovese

Walter Reed Army Institute of Research, Silver Spring, Maryland

Background: Studies in human subjects have shown that childhood adversity may affect response to traumatic stress in adulthood. Early-life exposure to traumatic stress may alter key developmental outcomes related to stress response, influencing physiological and behavioral response to subsequent stressful stimuli in adulthood. To characterize the nature of these developmental changes, we set out to model traumatic stress exposure during the adolescent developmental phase and evaluate adulthood response to a subsequent traumatic stress.
Methods: Adolescent rats (postnatal day 37–40) underwent a 20-s submersion underwater trauma (UWT), multiple predator exposure (PE), or sham stress experience. Behavioral evaluations were conducted at baseline and immediately after adolescent stress. Upon reaching adulthood, all rats (postnatal day 73–75) underwent PE followed by predator scent exposure trials one week later. Behavioral

tests and blood sampling for corticosterone analyses were conducted before and after PE, and before and after scent reminder.

Results: Within-groups analyses showed that UWT and PE during adolescence caused a significant decrease in EPM exploratory behavior. Adult baseline testing revealed similar behavioral profiles across adolescent treatment groups. Adulthood behavioral responses to PE stress were differentiated by (1) the presence or absence of adolescent stress history, and (2) whether the adolescent stressor was the same or different than the adulthood stressor. Lack of adolescent stress led to significantly decreased exploratory behavior after adulthood PE. Adolescent UWT stress reduced behavioral change after adult PE, and adolescent PE exposure led to increased exploratory behavior following the adulthood PE.

Conclusions: Not only did adolescent stress exposure produce a potential stress resilience effect in adulthood, the type of traumatic stress event used during adolescence produced different adulthood stress response profiles. These results begin to unravel the details of the lasting effects of developmental stress exposure upon adulthood stress responses.

Supported by the Military Operational Medicine Research Program, US Army Medical Research and Materiel Command. NLTM is a National Research Council Fellow. Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 2011 edition. All procedures were reviewed and approved by the WRAIR/NMRC Institutional Animal Care and Use Committee, and performed in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Keywords: stress, underwater, predator, adolescence, behavior.

Disclosures: N. Moore, Nothing to Disclose; D. Altman, Nothing to Disclose; S. Gauchan, Nothing to Disclose; R. Genovese, Nothing to Disclose.

T7. Genetic Interaction Between Integrin β3 (Itgb3) and Serotonin Transporter (Slc6a4) Modifies Depressive-like Behaviors in the Mouse

Seth Varney, Alonzo Whyte, Tammy Jessen, Ana Carneiro*

Vanderbilt University, Nashville, Tennessee

Background: Dysfunctions in serotonergic systems have been associated with a plethora of psychiatric illnesses, including major depression, anxiety, obsessive-compulsive disorder, post-traumatic stress disorder, autism, and substance abuse disorders, amongst others. The serotonin transporter gene *SLC6A4*, has been associated with risk for anxiety and depression, mostly in interaction with environmental or genetic factors. Understanding how these interactions influence risk for mood and anxiety disorders is essential for the development of preventive measures and efficient, patient-specific treatment. Converging evidence from genetic analysis of human subjects and platelet physiology has implicated the integrin beta3 subunit, encoded by the *ITGB3* gene as a potential modulator of serotonergic systems via genetic and functional interactions with *SLC6A4*. In the brain, integrin beta3 interacts with the integrin alphav subunit to form the vitronectin receptor and modulate synaptic function. Here, we used a double heterozygous *Itgb3* and *Slc6a4* (IS) mouse model to examine the behavioral, neurochemical, and biochemical consequences of integrin beta3 haploinsufficiency in the context of *SLC6A4* haploinsufficiency.

Methods: To investigate the *in vivo* behavioral and physiological consequences of the genetic interaction between SERT and the integrin beta3 in the brain, we employed a mutant mouse model heterozygous for both the *Slc6a4* and *Itgb3* genes. We analyzed wild type, double heterozygous and single heterozygous mice for *Itgb3* or *Slc6a4* to identify significant contributions of each gene to behavioral and neurochemical measurements. Western blot analysis was used to measure changes in the expression of synaptic proteins. Overall locomotor and anxiety behaviors were obtained through total distance, stereotypy and thigmotaxis analysis in the open field test. Anxiety and depressive related phenotypes were evaluated in wild type and *Itgb3/Slc6a4* double heterozygous mice (IS) using the elevated zero maze, novelty suppressed feeding (NSF) and Porsolt forced swim tests (FST). To evaluate the integrity of brain monoaminergic neurotransmitter systems in the presence of double *Itgb3/Slc6a4* heterozygosity, we measured tissue levels of monoamines and their metabolites using high performance liquid chromatography. We determined the predictive validity of our model by measuring neurobehaviors and serotonin homeostasis following chronic dosing with the selective serotonin reuptake inhibitor citalopram at 5 mg/kg for 14 days.

Results: Western blot analysis confirmed that SERT expression is reduced in midbrains of IS mice, in comparable levels to *Slc6a4* heterozygous mice. Integrin beta3 and integrin alphav expression levels were not significantly altered in IS mice, where *Slc6a4* haploinsufficiency rescued integrin beta3 expression. Whether previous studies have found tissue monoamine levels to be unaffected by *Slc6a4* heterozygosity, we observed significantly increased tissue serotonin levels in the midbrain and cortex of IS mice. *Slc6a4* also significantly influenced turnover rates in midbrain, hippocampus and cortex, with no effects arising from *Itgb3* contributions. Behavioral analysis of IS mice revealed that, while *Slc6a4* haploinsufficiency significantly enhances anxiety, *Itgb3* haploinsufficiency induces alterations in depressive indices beyond those seen with *Slc6a4* haploinsufficiency alone. Chronic dosing with the SSRI citalopram normalized tissue levels of serotonin in the midbrains, but not cortices or hippocampi of IS mice. We also observed no citalopram-mediated effects on hippocampal neurogenesis or immobility time in the FST. Linear regression analysis of our citalopram cohort reveals that

variations in integrin alphav expression is strongly correlated with immobility time in the FST.
Conclusions: Taken together, our data reveals a significant *Slc6a4* x *Itgb3* genetic interaction in the modulation of serotonin homeostasis in the central nervous system. Altered 5-HT homeostasis likely arises from the functional interaction between integrin alphavbeta3 and SERT, which may modulate 5-HT transport capacity in serotonergic synapses. Our data also suggests separate, but related, mechanisms modifying anxiety and depressive-like behaviors. Both anxiety- and depressive behaviors depend on *Slc6a4* haploinsufficiency, but we observed significant alterations in depression tests only in the context of *Itgb3* haploinsufficiency. We suspect that these behaviors are associated with integrin alphavbeta3-dependent changes in neuronal structure and function, likely through the modulation of other neurotransmitter systems, as neurogenesis or immobility times did not follow the normalization of serotonin homeostasis by chronic citalopram treatment. Finally, linear regression revealed that immobility time is correlated with integrin alphav expression, a measure modified by Slc6a4 haploinsufficiency. Therefore, integrin alphavbeta3 is a novel modulator of neurogenesis and depression in the central nervous system. As *ITGB3* is a highly polymorphic gene, it may significantly contribute to risk for mood and anxiety disorders, especially in the context of *SLC6A4* haploinsufficiency.
Keywords: depression, genetic interaction, integrin, serotonin, transporter.
Disclosures: S. Varney, Nothing to Disclose; A. Whyte, Nothing to Disclose; T. Jessen, Nothing to Disclose; A. Carneiro, Nothing to Disclose.

T8. Dopamine-Independent Motor Control and Hyperactivity Involving Acetylcholine Systems

Kazutaka Ikeda*, Yoko Hagino, Shinya Kasai

Addictive Substance Project, Tokyo, Japan

Background: The disruption of dopamine (DA) systems has been implicated in major neurological and psychiatric disorders, including Parkinson's disease (PD) and schizophrenia. However, effective movement in patients with Parkinson's disease in certain situations, kinesia paradoxa, and typical antipsychotic-resistant and atypical antipsychotic-sensitive positive symptoms of schizophrenia and their underlying mechanisms remain to be elucidated.
Methods: The experimental procedures and housing conditions were approved by the Institutional Animal Care and Use Committee (Animal Experimentation Ethics Committee of Tokyo Metropolitan Institute of Medical Science, Approval ID: 12–43), and all of the animals were cared for and treated humanely in accordance with our institutional animal experimentation guidelines. Dopamine-deficient (DD) mice were generated using a transgenic rescue approach, in which tyrosine hydroxylase (TH) expression in noradrenergic and adrenergic cells in mice that lack TH expression was complemented by a specific DA β-hydroxylase gene promoter. These mice exhibited a restoration of norepinephrine (NE) and epinephrine synthesis and prevention of the usual perinatal lethality and cardiac dysfunction observed in TH knockout mice. Dopamine, serotonin, NE, and acetylcholine (ACh) concentrations in brain microdialysates were determined by high-performance liquid chromatography with electrochemical detection (HTEC-500, Eicom, Kyoto, Japan). Locomotor activity was measured with Supermex (Muromachi Kikai, Tokyo, Japan) and a sensor monitor mounted above the chamber. After a 180 min habituation period, the drugs were administered subcutaneously, and locomotor activity was monitored continuously for 180 min. The mice walked freely on the runway, and their locomotor movements were recorded at 200 frames per second using a high-speed digital image camera system (HAS-220, DITECT, Tokyo, Japan). Movement analyses were limited to the sagittal plane parallel to the direction of walking. Custom-designed image analysis software (DIPP-Motion 2D, DITECT, Tokyo, Japan) was used to extract the two-dimensional coordinates of the various joint markers and reconstruct a stick diagram representation of the right hindlimb. The expression profiles of the genes related to ACh metabolism and signaling transduction pathways were analyzed using the Illumina iScan system with MouseRef-8 Expression BeadChips (Illumina, San Diego, CA, USA), which contain probes that detect over 24 000 transcripts. Immunoblot analysis and immunohistochemistry were also performed.
Results: The present study showed that DD mice can move effectively and are rather hyperactive. DD mice require daily administration of L-dihydroxyphenylalanine (L-DOPA), the precursor of DA, to maintain feeding. After 3 days withdrawal from L-DOPA, their striatal extracellular DA levels fell to less than 0.2% of wildtype mice, and they were hyperactive with a slight movement disorder in a novel environment. The atypical antipsychotic drug clozapine markedly ameliorated this hyperactivity, with no effect of the typical antipsychotic drug haloperidol. Furthermore, the nonselective muscarinic ACh receptor agonist oxotremorine-M and ACh esterase inhibitor donepezil blocked hyperactivity in DD mice. These mice exhibited a reduction of choline acetyltransferase (ChAT) gene and protein expression in the basal ganglia and a reduction of extracellular ACh levels in the striatum, suggesting that reduced cholinergic function may underlie hyperactivity in DD mice.
Conclusions: The DD mice in the present study revealed a novel motor-control and hyperactivity mechanism that is independent of DA and involves ACh systems. The activity of ChAT has been found to be normal or reduced in the striatum in PD patients. Furthermore, the basal ganglia are interconnected with the pedunculopontine nucleus (PPN). The PPN is thought to be involved in the initiation and modulation of gait and other stereotyped movements. In PD patients, the loss of cholinergic neurons in the PPN has been reported. The decrease in dopaminergic neuronal transmission in the basal ganglia might result in the inactivation of the PPN in PD patients and DD mice. The present results, together with previous findings, suggest that the ACh systems may be involved in the mechanisms that underlie DA-independent motor control, and motor impairment in PD patients may be ameliorated by DA-independent mechanisms. Although DA plays a prominent role in the pathogenesis and treatment of schizophrenia, several lines of evidence suggest an important role for the

cholinergic system in the pathophysiology of schizophrenia. Hyperactivity induced by muscarinic receptor antagonists has been suggested to model antimuscarinic psychosis and cholinergic-related psychosis in schizophrenia. Hyperactivity in DD mice was ameliorated by a muscarinic receptor agonist in the present study, suggesting that such hyperactivity may be induced by similar mechanisms that underlie cholinergic-related psychosis in schizophrenia. DD mice might be useful for the identification of the neuronal mechanisms involved in cholinergic-related psychosis in schizophrenia.

Keywords: dopamine, atypical antipsychotic drug, clozapine, motor control, acetylcholine.

Disclosures: K. Ikeda, Nothing to Disclose; Y. Hagino, Nothing to Disclose; S. Kasai, Nothing to Disclose.

T9. A New Model for Studying Effects of Witnessing Traumatic Events in Rats

Samina Salim*, Gaurav Patki, Naimesh Solanki, Farida Allam, Amber Ansari

University of Houston, Houston, Texas

Background: Post-traumatic stress disorder (PTSD) can result from virtually any type of trauma. Most research is focused in victims who have directly experienced a traumatic event, however, witnessing a traumatic event and not physically experiencing it, can also lead to PTSD. This aspect has received only marginal attention. And, isolation stress can worsen PTSD whether a traumatic event is experienced directly or indirectly. Our laboratory is the first to develop a rat model that depicts effects of witnessing a traumatic event and also compares effect of isolation *vs* bonding on PTSD-like effects in rats. We describe our model as the 'Trauma Witness Model' (TWM).

Methods: Two sets of experiments were conducted. Set I: Sprague Dawley (SD) rats were housed two in a cage, allowed to bond and acclimatize. Then, one of the SD rats was subjected to social defeat stress for 7 consecutive days. During each exposure of social defeat, an SD rat (intruder) was placed into the home cage of an unfamiliar Long-Evans rat (resident). The intruder was defeated assuming supine position. After defeat, a wire mesh enclosure was placed in the cage to prevent physical contact for 5-min. The defeat was observed by the cage mate of the intruder designated as the trauma witnessing (TW) rat, which was present outside the home cage in a clear plexiglass enclosure allowing auditory, sensory and visual stimuli. Two more bouts of social defeat were performed with 5-min separation, to reinforce the visual stress in the TW rat. After social defeat, TW and SD rats were housed together and 24 h later, behavioral experiments examining depression-like, anxiety-like and cognitive functions were conducted. Set II: Similar procedures as above were conducted in this set, except that the TW and SD rats were housed separately after each social defeat exposure.

Results: Our results suggest that witnessing traumatic events leads to severe behavioral and biochemical impairments in rats, which become worse when rats are housed separately. Set I results: A significant decrease in body weight, increased water-intake, increased corticosterone levels, elevated whole body and brain indices of oxidative stress, increased inflammation in the brain, heightened anxiety-like behavior (open-field, elevated-plus maze and light-dark test), increased anhedonia and depression-like behavior (sucrose preference test and forced swim test) as well as learning-memory deficits (Radial arm-water maze test) were observed in TW and SD rats both, as compared to the controls (no resident/trauma). Molecular targets considered critical for anxiety, depression and cognition including Glyoxalse-1, calmodulin-dependent protein kinase (CAMK) IV, cAMP response element-binding protein (CREB) and brain derived neurotrophic factor (BDNF) showed dramatic reductions in specific brain areas including hippocampus and amygdala of TW rats. These levels remained unchanged in the frontal cortex. These effects were comparable to the effects observed in rats that were directly subjected to social defeat trauma and later housed together with cage mate. Set II results: Decrease in body weight, increased water-intake, increased corticosterone levels, elevated whole body and brain indices of oxidative stress, heightened anxiety-like behavior, increased anhedonia and depression-like behavior as well as learning-memory deficits observed in TW and SD rats in set I experiments, were further deteriorated in rats that were housed separately after social defeat exposures. Hippocampus and amygdala exhibited a further decline in the levels of Glyoxalse-1, CAMKIV, CREB and BDNF in these sets of animals, suggesting that perhaps a trajectory involving the above molecules, play an important role in regulation of stress-response.

Conclusions: Data from our rat TWM suggest that witnessing repeated traumatic events are as stressful as experiencing the events first hand, causing severe behavioral and biochemical impairments. Post-trauma conditions such as that of isolation potentially contribute to severity of PTSD-like symptoms in rats. It is of immediate importance to understand the mechanisms by which these effects occur in order to identify suitable treatment strategies. A pathway involving activation of oxidative stress mechanisms, elevation of inflammatory mediators and diminished neuroprotection, all seem to modulate stress response in this model. Finally, our results have direct relevance not only with combat related PTSD but also with increased incidence of day-to-day gun violence related traumatic events, witnessed by people of all ages, in different settings, including our schools, public gatherings and cinema halls.

Keywords: PTSD, trauma, anxiety, oxidative stress, cognition.

Disclosures: S. Salim, Nothing to Disclose; G. Patki, Nothing to Disclose; N. Solanki, Nothing to Disclose; F. Allam, Nothing to Disclose; A. Ansari, Nothing to Disclose.

T10. Identification of Early Risk for Substance Use: fMRI Responses to Cocaine-associated Cues in Juvenile Rats

Steven Lowen, Michael Rohan, Britta S Thompson, Kai Sonntag, Susan L Andersen*

McLean Hospital, Belmont, Massachusetts

Background: Drug-associated cues play a significant role in relapse of drug-seeking behavior. These effects are mediated

in part by the D1 dopamine receptor on glutamate neurons that project from the prelimbic prefrontal cortex (plPFC) to the nucleus accumbens. We have recently demonstrated that this D1 receptor is selectively overexpressed in adolescent rats, rendering this age group more sensitive to cocaine-associated cues. While elevated risk for drug use is common for adolescents, clinical studies show that early initiation of drug use (eg, 12–14 years of age in humans) is four-times more likely to lead to lifelong addiction. In this study, we asked whether we could recapitulate both the mechanism (eg, D1 over-expression) and risk behavior in juvenile rats and then identify the neural circuitry that is activated to these drug-associated cues in these same subjects using fMRI.

Methods: Sprague-Dawley juvenile male rats ($n = 6$–8) were transduced with a lentiviral vector that was specific for glutamate neurons (eg, the CamKIIa promotor) that expressed either D1 or control GFP (CK.D1 or CK.GFP). These vectors were stereotaxically injected into the plPFC on postnatal day 19 (P19). Five days later, subjects were screened in place conditioning chambers where each side was paired with a discrete odor. After no odor/side bias was detected on this first day, subjects were conditioned for two days (60 min each) to 10 mg/kg cocaine (a dose not preferred by juvenile rats), and tested on the fourth day. The following day on P28, behaviorally characterized subjects were anesthetized and placed into a Varian 9.4T scanner. The same cocaine-paired odors were discretely delivered across 15 s in a block design in a counterbalanced order. Whole brain BOLD data with 0.625 mm isotropic resolution were processed with FSL modified for the rat brain, using their preference score for cocaine-associated cues as a covariate. Functional results were aligned with the matched anatomic scan and again with the averaged anatomic brain. Group results were calculated using a mixed effects model over all subjects. Uncorrected, all voxel-wise significance values were thresholded to a significance level of $p < 0.01$ and clusters of the thresholded voxels were found. Using Gaussian random field theory, achieving a cluster-wide significance level of $p < 0.05$, family-wise error (FWE) corrected for multiple comparisons over the whole brain.

Results: CK.D1 juveniles formed significant place preferences for cocaine-associated environments, whereas no such preference was observed in the CK.GFP juveniles. Neither group had a differential preference for either of the odors. Relative to CK.GFP subjects, CK.D1 subjects had significant changes in BOLD response to the cocaine-paired odor. Significant activation patterns were evident in the PFC, the nucleus accumbens, dorsal striatum, and the dentate gyrus.Comparison of CK.D1 to CK.GFP rats, however, further revealed activation in the amygdala.

Conclusions: The overexpression of D1 dopamine receptors produces increased sensitivity to cocaine-associated cues that can be visualized by distinct changes in BOLD response in juvenile rats using fMRI. Importantly, the results of this study are consistent with previous fMRI findings of cue reactivity in humans as well as neurobiological findings with more invasive approaches. Localized injections of lentiviral vectors provide a mechanistic assessment of at least one facet of the underlying neurobiology of these fMRI results (eg, D1 receptors). The use of odor-cocaine paired cues may eventually lead to the identification of individuals who are at high-risk for substance use by unique patterns of blood flow.

Keywords: risk addiction adolescent fMRI cocaine.

Disclosures: S. Lowen, Nothing to Disclose; M. Rohan, Nothing to Disclose; B. Thompson, Nothing to Disclose; K. Sonntag, Nothing to Disclose; S. Andersen, Nothing to Disclose.

T11. Interaction Between BDNF and Social Environment in Brain Physiology and Behavior

Robert Schloesser*, Dennisse Jimenez, Julia Hill, Keri Martinowich

University of Maryland, Baltimore, Maryland

Background: Social experience affects expression of genes implicated in synaptic function and neural plasticity, influences structural remodeling including dendrite and spine morphology and improves cognitive and behavioral performance. The neurotrophin BDNF is a key regulator of neural and synaptic plasticity, and may mediate some of the beneficial neurobiological changes observed after positive social experiences.

Methods: For these studies we utilized a mutant mouse line in which activity-dependent expression of BDNF is significantly attenuated (BDNF-KIV) coupled with quantification of gene expression levels; immunohistochemical analysis; examination of animal behavior; and *in vivo* measurement of brain electrical activity. Wild-type and BDNF-KIV mice were housed from weaning in either social isolation or a socially enriched environment. At adulthood, we assessed levels of synaptic and interneuron markers, performed a battery of behavioral tests, assessed baseline brain electroencephalogram activity over the circadian cycle, and quantified evoked cortical activity.

Results: We found significant interactions between early social environment and *BDNF* genotype on brain physiology, behavior and levels of interneuron markers. Specifically, social isolation magnified deficits in expression of markers of plasticity and inhibitory interneurons, deficits in sensorimotor gating, expression of abnormal repetitive behaviors, alterations of evoked potentials. These effects were significantly improved or rescued entirely after early social enrichment.

Conclusions: These studies establish gene-environment interactions between BDNF and early social enrichment on brain physiology, inhibitory circuitry and behavioral outcomes, and show that early social experience can significantly influence physiological deficits induced by genetically hard-wired alterations in BDNF signaling.

Keywords: BDNF, hyperactivity, wavelets, evoked potentials, EEG, LFP, matlab, enriched social environment, C57bl/6, behavior, interneuron, neurplasticity.

Disclosures: R. Schloesser, Nothing to Disclose; D. Jimenez, Nothing to Disclose; J. Hill, Nothing to Disclose; K. Martinowich, Nothing to Disclose.

T12. Early Life FGF2 Treatment Alters Vasopressin and Oxytocin Gene Expression in Animals That Differ in Their Response to Novelty

Cortney Turner*, Pamela Maras, Yoav Litvin, Stanley J Watson, Bruce S Mcewen, Huda Akil

Molecular & Behavioral Neuroscience Institute, Ann Arbor, Michigan

Background: Selectively bred high responder rats (bHRs) exhibit high levels oflocomotor activity in response to a novel environment and low levels ofanxiety-like behavior. Conversely, selectively bred low responder rats (bLRs)exhibit low levels of novelty-induced locomotion and high levels ofanxiety-like behavior. Previously, we found that administration of fibroblastgrowth factor-2 (FGF2) early in life can decrease anxiety-related behavior in thehighly anxious bLRs when tested as adults. However, the mechanism for thiseffect has not been fully explored. Activation of the oxytocin receptor (OTR) isknown to have anxiolytic effects, while activation of the vasopressin receptor1A (AVPR1A) has anxiogenic effects.
Methods: Therefore, we wondered whether early life FGF2 treatment (20 ng/g, s.c.) on postnatal day 1 (PND1) would have an effect on gene expression of theoxytocin and vasopressin receptors both early in life and in adulthood. To thisend, we measured gene expression by mRNA *in situ* hybridization.
Results: On PND11, bHRs had more OTR gene expression in multiple brain regionsthan bLRs. Conversely, bLRs had more AVPR1A gene expression the centralamygdala than bHRs. In adulthood, bHRs had more OTR gene expression in theventromedial hypothalamus than bLRs. Conversely, bLRs had more AVPR1A geneexpression in the central amygdala than bHRs. Strikingly, neonatal FGF2 normalizedmRNA levels of both receptors in bLRs, making their gene expression more bHR-like.Receptor autoradiography studies are underway to determine differences inbinding.
Conclusions: In summary, the central amygdala exhibits alterations in both OTR andAVPR1A gene expression in animals that differ in the response to novelenvironments. Taken together, the results suggest that the highly anxious bLRscan benefit from early life FGF2 treatment in terms of their oxytocin andvasopressin gene expression.
Keywords: anxiety, growth factors, *in situ* hybridization.
Disclosures: C. Turner, Nothing to Disclose; P. Maras, Nothing to Disclose; Y. Litvin, Nothing to Disclose; S. Watson, Nothing to Disclose; b. mcewen, Nothing to Disclose; H. Akil, Nothing to Disclose.

T13. Attitudes of Children and Adolescents and Their Caregivers Towards Long-acting Injectable Antipsychotics in a Cohort of Youth Initiating Oral Antipsychotic Treatment

Christoph U Correll*, Owen Muir, Aseel Al-Jadiri, Sandeep Kapoor, Morgan Carella, Eva Sheridan, Lisa David, John Kane

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

Background: Long-acting injectable antipsychotics (LAIs) are recommended for patients with adherence problems aiming to improve overall outcomes. To date, LAIs have only been studied in adults, although pediatric onset severe psychiatric disorders are have been associated with a more severe and chronic course. Moreover, non-adherence may be a particular issue in adolescence.
Methods: As part of the ongoing Second-Generation Antipsychotic Treatment Indications, Effectiveness and Tolerability in Youth (SATIETY) study, youth aged 4–19 years old who were newly initiated on antipsychotic treatment and their caregivers were each asked within <7 days of antipsychotic initiation about the acceptability of receiving the prescribed antipsychotic as an LAI (being told that this may not be available and that this was not the plan), as well as reasons for their expressed choice.
Results: 343 patients and/or their caregivers provided data about their attitudes. Predictors of youths' favorable attitude included higher number of past admissions ($p = 0.0081$), older age ($p = 0.013$), a primary mood disorder diagnosis ($p = 0.025$), family history of depression ($p = 0.035$), and anxiety disorder ($p = 0.048$). For caregivers, higher number of past admissions ($p = 0.018$) and >1 previous antipsychotic trials ($p = 0.029$) were associated with favorable views. Needle phobia and pain were the reasons most commonly given against LAIs, and ease and compliance were the most common reasons favoring LAIs.
Conclusions: A significant number of youth and caregivers are open to LAI treatment. Caregivers are more in favor of LAIs, citing ease and compliance as reasons. Needle phobia and pain are the primary concerns of both groups, and several factors predict favorable attitude, notably prior admissions.
Keywords: antipsychotics, youth, attitudes, predictors, long-acting injectable.
Disclosures: C. Correll, **Part 1:** Actelion, Alexza, AstraZeneca, Bristol-Myers Squibb, Cephalon, Eli Lilly, Gerson Lehrman Group, IntraCellular Therapies, Lundbeck, Medavante, Medscape, Merck, Novartis, Ortho-McNeill/Janssen/J&J, Otsuka, Pfizer, Roche, ProPhase, Sunovion, Takeda, Teva, and Vanda. **Part 2:** Bristol-Myers Squibb, Cephalon, Janssen, Lundbeck, Merck, Otsuka, ProPhase., **Part 3:** Bristol-Myers Squibb, Lundbeck, Merck, Otsuka, ProPhase., **Part 4:** BMS, Janssen/J&J, Otsuka, **Part 5:** N/A; O. Muir, Nothing to Disclose; A. Al-Jadiri, Nothing to Disclose; S. Kapoor, Nothing to Disclose; M. Carella, Nothing to Disclose; E. Sheridan, Nothing to Disclose; L. David, Nothing to Disclose; J. Kane, **Part 1:** Alkermes. Amgen, BMS, Eli LIlly, Forrest, Genentech, IntraCellular Therapies, Janssen, Lundbeck, Merck, Novartis, Otsuka, MedaVante, Roche, Sunovion, Reviva, **Part 2:** BMS, Eli LIlly, Merck, Genentech, Otsuka, **Part 3:** N/A, **Part 4:** N/A, **Part 5:** N/A.

T14. Comorbidity of PTSD and Alcoholism: A Rat Model of PTSD Leads to Escalated Ethanol Consumption

Jenica Tapocik*, Jesse R Schank, Cheryl Mayo, Courtney King, Jim Koenig, Markus Heilig, Greg I Elmer

NIH, Bethesda, Maryland

Background: High rates of comorbidity exist between alcoholism and posttraumatic stress disorder. However, it

is unclear how exposure to traumatic experience increases the risk for alcoholism in adulthood. Here we developed a novel rat model of PTSD with subsequent alcohol-drinking assessments. The model is designed to determine if animals exposed to a traumatic experience are more vulnerable to develop high rates of alcohol drinking in adulthood.
Methods: Wistar rats were exposed to either a predator scent (PSE, cat odor) or a live predator (LPE, rat snake) for 10 min at post-natal day (PND) 31 and PND 61. At PND 75 anxiety-like behavioral responses to the PSE or LPE were assessed in the elevated plus-maze and acoustic startle boxes. At PND 76 rats underwent a standard alcohol two-bottle choice paradigm assessing intake at 3, 6 and 8% ethanol for 23 days. At PND 106, the LPE rats were exposed to a learned-helplessness paradigm to determine depression-like symptoms.
Results: PSE decreased habituation to the startle response, indicative of hyperarousal. Subsequently, PSE increased alcohol consumption compared to controls at an 8% concentration; however only trend significance was detected. To have a more robust alcohol drinking phenotype, rats were exposed to a live predator. LPE did not produce a classic anxiety or hyperarousal phenotype however the exposure significantly increased alcohol consumption at 3, 6, and 8% concentrations (main effect of treatment $p<0.04$ and concentration $p<0.0009$). Comparison between LPE and PSE indicated that LPE was more efficacious in increasing alcohol consumption (5 *vs* 4 g/kg/day at 8% concentration). LPE significantly increased the percentage of animals showing learned helplessness (80% in stress group compared to 40% in controls).
Conclusions: LPE evoked a more pronounced drinking phenotype than PSE even though it did not produce anxiety or hyperarousal-like phenotypes. LPE not only elicited increased alcohol consumption but also a depressive-like phenotype as suggested by the learned helplessness results. Overall, exposure to a live predator significantly increases the risk for comorbidity of PTSD and alcoholism in adulthood.
Keywords: PTSD, alcoholism, predator exposure, two bottle choice, learned helplessness.
Disclosures: J. Tapocik, Nothing to Disclose; J. Schank, Nothing to Disclose; C. Mayo, Nothing to Disclose; C. King, Nothing to Disclose; J. Koenig, Nothing to Disclose; M. Heilig, Nothing to Disclose; G. Elmer, Nothing to Disclose.

T15. Mouse Model of Chromosome 15q13.3 Microdeletion Syndrome Demonstrates Features of Autism Spectrum Disorder

Jeffrey Kogan*, Adam Gross, Rick Shin, Qian Chen, Noah Walton, Carrie Heusner, Amy Lin, Sosuke Miyoshi, Shintaro Nishimura, Shinichi Miyake, Katsunori Tajinda, Kouichi Tamura, Mickey Matsumoto

Astellas Research Institute of America LLC, Skokie, Illinois

Background: Chromosome 15q13.3 microdeletion is a pathogenic copy number variation (CNV) conferring epilepsy, intellectual disability, schizophrenia and autism spectrum disorder (ASD). Gene-manipulated mutant mice mirroring the condition of the human 15q13.3 microdeletion syndrome could be a useful model to understand the underlying mechanism(s) of these often comorbid neurodevelopmental/psychiatric disorders.
Methods: We generated C57BL/6 background mutant mice harboring a 1.2 Mb deletion syntenic to the human 15q13.3 microdeletion region. To mirror the human pathological conditions, we studied mutant mice harboring only one chromosome deletion and that retained one intact chromosomal region (D/+ mice). We investigated their brain size, brain gross morphology and behavioral phenotypes to confirm their face validity as a neurodevelopmental disorder model.
Results: Levels of mRNA expression from genes within the deleted region were half down in various brain regions of D/+ mice. Although their body weight was normal, D/+ mice displayed enlarged/heavier brains (macrocephaly) with enlarged lateral ventricles measured using magnetic resonance imaging methods. In male D/+ mice behavioral characteristics relevant to the symptoms of ASD, ie decreased social interactions and ultrasonic vocalizations, and increased self-grooming and risk-taking behaviors, were observed. Locomotor activity levels and cognitive abilities were normal. Female D/+ mice also had increased self-grooming but normal social interaction. Female mice were hypoactive and had deficient working memory. No signs of epilepsy/seizure were detected in either sex.
Conclusions: These results demonstrate that the male 15q13.3 microdeletion mouse model on a C57BL/6 background has strong construct and face validity as an animal model for ASD. Further investigations using the 15q13.3 microdeletion mouse model may uncover the common mechanism(s) underlying ASD and other neurodevelopmental/psychiatric disorders representing the 15q13.3 microdeletion syndrome, such as epilepsy, intellectual disability, and schizophrenia.
Keywords: copy number variation, autism spectrum disorder, schizophrenia, epilepsy, intellectual disability.
Disclosures: J. Kogan, **Part 5:** Astellas Research Institute of America LLC, a subsidiary of Astellas Pharma Inc.; A. Gross, **Part 5:** Astellas Research Institute of America LLC, a subsidiary of Astellas Pharma Inc.; R. Shin, **Part 5:** Astellas Research Institute of America LLC, a subsidiary of Astellas Pharma Inc.; Q. Chen, **Part 4:** Astellas Research Institute of America LLC, a subsidiary of Astellas Pharma Inc.; N. Walton, **Part 5:** Astellas Research Institute of America LLC, a subsidiary of Astellas Pharma Inc.; C. Heusner, **Part 5:** Astellas Research Institute of America LLC, a subsidiary of Astellas Pharma Inc.; A. Lin, Nothing to Disclose; S. Miyoshi, **Part 5:** Astellas Pharma Inc.; S. Nishimura, **Part 5:** Astellas Pharma Inc.; S. Miyake, **Part 5:** Astellas Research Institute of America LLC, a subsidiary of Astellas Pharma Inc.; K. Tajinda, **Part 5:** Astellas Research Institute of America LLC, a subsidiary of Astellas Pharma Inc.; K. Tamura, **Part 5:** Astellas Research Institute of America LLC, a subsidiary of Astellas Pharma Inc.; M. Matsumoto, **Part 5:** Astellas Research Institute of America LLC, a subsidiary of Astellas Pharma Inc.

T16. Distinct Roles of PKC Signaling at Direct and Indirect Pathway Medium Spiny Neurons During Reinstatement of Cocaine-seeking

Pavel I Ortinski*, Lisa A Briand, R Christopher Pierce, Heath D Schmidt

University of Pennsylvania, Philadelphia, Pennsylvania

Background: Cocaine abuse in humans is frequently characterized by withdrawal from and relapse to chronic

drug use. The relapse to cocaine-seeking in rodents can be modeled as enhanced behavioral responding following exposure to a pharmacological trigger.
Methods: In particular, reinstatement of cocaine-seeking can be triggered by stimulation of dopamine receptors in the nucleus accumbens, a component of the mesolimbic dopamine reward pathway.
Results: Here, we show that microinjection of either D1-like or D2-like dopamine receptor agonists into the rat nucleus accumbens shell leads to reinstatement of cocaine-seeking. Administration of the protein kinase C antagonist, chelerythrine, attenuates reinstatement induced by the D2-like, but not the D1-like dopamine receptor agonists. We explore the possible neuronal mechanisms for this phenomenon by evaluating the effects of chelerythrine at medium spiny neurons of the direct (D1-expressing) and indirect (D2-expressing) pathways. We find that exposure of brain slices to chelerythrine modulates excitatory and inhibitory synaptic signaling differently at D1- and D2-expressing neurons. Furthermore, sensitivity of evoked synaptic activity to chelerythrine is dependent upon the frequency of stimulation of synaptic afferents.
Conclusions: These results suggest the possibility that distinct signaling patterns at neurons of the direct and indirect pathways in the nucleus accumbens shell underlie distinct roles of protein kinase C during reinstatement of cocaine-seeking by agonists at D1-like and D2-like dopamine receptors.
Keywords: addiction, pkc, patch-clamp, EPSC, IPSC.
Disclosures: P. Ortinski, Nothing to Disclose; L. Briand, Nothing to Disclose; R. Pierce, Nothing to Disclose; H. Schmidt, Nothing to Disclose.

T17. Amygdala-Ventral Pallidum Pathway Decreases Dopamine Activity Following Chronic Mild Stress in Rats

Chun-hui Chang*, Anthony A Grace

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: Major depressive disorder (MDD) affects more than 15% of the population across their lifespan. In this study, we used the well-characterized unpredictable chronic mild stress (CMS) model of depression to examine this condition.
Methods: Sprague-Dawley rats were presented randomly with mild stressors for four weeks, with body weight and sucrose intake monitored weekly. Locomotor activity and elevated plus maze test/forced swim test were conducted on week 5; Ventral tegmental area (VTA) dopamine (DA) neuron activity was assessed within a week after the behavioral test using three indices: DA neuron population activity (defined as the number of spontaneously firing DA neurons), mean firing rate, and percent burst firing (ie, the proportion of action potentials occurring in bursts).
Results: Consistent with previous studies, we found that, compared to controls, rats that underwent the CMS procedure were slower in gaining body weight, and developed anxiety- and despair-like behavior. We now report a significant decrease in DA neuron population activity of CMS rats, and this decrease is restored by pharmacologically attenuating the activity of either the basolateral nucleus of the amygdala (BLA), or the ventral pallidum (VP). Moreover, pharmacological activation of the amygdala in non-stressed rats decreases DA neuron population activity, mimics CMS-induced attenuation in DA activity, and is reversed by blocking the BLA-VP pathway.
Conclusions: Our results suggest that the CMS rat depression model is associated with a BLA-VP-VTA inhibition of DA neuron activity. This information can provide insight into the circuitry underlying MDD and serve as a template for refining therapeutic approaches to this disorder.
Keywords: unpredictable chronic mild stress; rat; ventral tegmental area; amygdala; ventral pallidum; dopamine.
Disclosures: C. Chang, Nothing to Disclose; A. Grace, Nothing to Disclose.

T18. Roles of Glucocorticoids in a Trajectory from Adolescent Social Stress to Adult Behavior

Minae Niwa*, Akira Sawa

Johns Hopkins University, Baltimore, Maryland

Background: Stress as environmental factors underlies psychiatric disorders. Our recent study showed adolescent stress-induced epigenetic control of dopaminergic neurons via glucocorticoids. Nevertheless, it has not yet been fully demonstrated at which specific time points in adolescence glucocorticoids elicits such dopaminergic disturbance and subsequent behavioral deficits. Thus, it is important to establish the critical period in order to understand the underlying mechanism.
Methods: A genetic model with isolation stress was examined by neurochemical and behavioral assays. The extracellular levels of dopamine and glutamate were measured by *in vivo* microdialysis. The expression levels of tyrosine hydroxylase and glutamate transporters were determined by Western blotting. Two cohorts (the first cohort was used for prepulse inhibition test followed by forced swim test, and the second cohort for testing locomotor activity). Plasma corticosterone was measured by enzyme immune assay. Mice were treated with the glucocorticoid receptor (GR) antagonist RU38486 (mifepristone) from 5 to 8, from 5 to 7, and from 8 to 10 weeks of age.
Results: We exposed DISC1 mutant mice (genetic factor) to 3-week isolation stress during adolescence (from 5 to 8 weeks). At 8 weeks, this gene-environment mouse model (GXE mice) showed a decrease in levels of extracellular dopamine and glutamate at baseline in the frontal cortex. In accordance with the changes in dopamine and glutamate in the GXE model, a decrease in the expression levels of tyrosine hydroxylase and glutamate transporters (GLT-1 and GLAST) was observed in the frontal cortex. These dopaminergic and glutamatergic changes would underlie behavioral deficits in the GXE model. GXE mice also showed significantly elevated levels of plasma corticosterone. We did not observe any molecular, neurochemical, and behavioral changes among wild-type mice without/with isolation stress and DISC1 mutant mice without isolation. Blocking the GR with RU38486 during the 3-week isolation

period successfully normalized such neurochemical and behavioral abnormalities studied in GXE mice. These prophylactic effects were also observed by the administration of the GR antagonist from 5 to 7 weeks, which is first 2-week of the isolation period. In contrast, the antagonist did not have the treatment effects from 8 to 10 weeks after neurochemical and behavioral changes in the GXE model were established.
Conclusions: The present study show that prophylactic administrations of the GR antagonist were more effective than the administrations after the full onset of aberrant behaviors. This may provide a model of how and when subjects with genetic risk(s) are to be intervened under the risk of adolescent stressors.
Keywords: glucocorticoids, adolescence, stress, behavior, neurotransmitter.
Disclosures: M. Niwa, Nothing to Disclose; A. Sawa, **Part 1:** Astellas, Takeda, Tanabe-Mitsubishi, Dainippon-Sumitomo, Johnson and Johnson, Sucampo, Pfizer, Asubio, Eli Lilly, Taisho, Amgen, Afraxis, Sanofi-Aventis, and Astrazeneca, **Part 4:** Astellas, Takeda, Tanabe-Mitsubishi, Dainippon-Sumitomo, Johnson and Johnson, and Sucampo.

T19. High Traumatic Stress Reactivity Alters Behavior and Corticotropin-releasing Factor-1 (CRF1Rs) in Prefrontal Cortex-Amygdala Circuitry

Nicholas W Gilpin*

LSU Health Sciences Center, New Orleans, Louisiana

Background: A portion of humans exposed to traumatic stress develops anxiety disorders that are defined by or co-morbid with long-term increases in alcohol drinking, arousal, and pain processing. Our lab has developed a predator odor stress model in which animals that exhibit high traumatic stress reactivity also exhibit persistent increases in alcohol drinking and altered prefrontal cortex (PFC)-amygdala neuronal activation profiles in response to stress-related stimuli.
Methods: In this study, we utilized a predator odor stress model to examine stress effects on *ex vivo* corticotropin-releasing factor (CRF)-positive cell counts in medial prefrontal cortex (mPFC) and amygdala, as well as stress effects on *in vivo* nociceptive processing in male Wistar rats. We also utilized systemic and brain site-specific pharmacological approaches to determine the role of CRF-1 receptors (CRF1Rs) in mediating stress-induced hyperalgesia. In all experiments, odor-exposed rats were indexed for their avoidance of a predator odor-paired context and divided into 'Avoiders' (ie, high stress reactivity) and 'Non-Avoiders' (ie, low stress reactivity), as previously published by our lab. All experiments also included unstressed controls.
Results: Avoider rats exhibit significant increases in CRF-positive cell counts in ventromedial PFC 9 days following exposure to predator odor. Stressed rats exhibit a collective trend toward fewer CRF-positive cells in the central amygdala (CeA) at the same time point. Avoider rats exhibit thermal hyperalgesia (ie, increased nociception) 48 h post-stress relative to Non-Avoiders, unstressed controls, and their own baseline. Systemic antagonism of CRF1Rs by R121919 reverses stress-induced hyperalgesia in Avoiders, and neuronal signaling in the CeA appears to be critical for this effect.
Conclusions: Rats exposed to stress and especially those that exhibit high stress reactivity (as indexed by persistent avoidance of a predator odor-paired context) exhibit hyperalgesia, lasting changes in CRF cell counts in prefrontal cortex-amygdala circuitry, and altered sensitivity to drugs that antagonize CRF1 receptors, perhaps suggesting stress-induced neuroadaptations. Collectively, our results suggest that dysregulation of brain and behavior by stress is not uniform across animals, but can be predicted based on stress reactivity profiles. Our data also suggest that CRF1 receptors mediate hyperalgesia and perhaps other behavioral changes (eg, increased arousal and increased alcohol drinking) produced by stress, and may represent a promising pharmacotherapeutic target for treatment of traumatic stress-related anxiety disorders.
Keywords: PTSD, amygdala, CRF, nociception, hyperalgesia.
Disclosures: N. Gilpin, Nothing to Disclose.

T20. High-throughput Behavior-based Neuroactive Drug Discovery in Zebrafish

David Kokel*

Massachusetts General Hospital, Charlestown, Massachusetts

Background: Behavioral phenotyping is an effective way to discover novel neuroactive drugs. However, it has been difficult to develop efficient behavioral phenotyping assays for large-scale chemical screens. New technologies and behavioral phenotyping assays in the zebrafish are opening new opportunities to understand the central nervous system (CNS) and discover neuroactive drugs. These technologies are ushering in a new phase of discovery-based research in behavioral pharmacology. Neuroactive compounds with new structures, targets, mechanisms, and functions are being discovered. Given the fundamental differences between the human and zebrafish nervous systems it will be difficult to translate zebrafish discoveries to clinical medicine. However, given the molecular genetic similarities between humans and zebrafish, it is likely that some of the compounds being identified in the zebrafish will find translational utility in humans. The greatest new successes in CNS drug discovery will likely leverage the advantages of many model systems, including *in vitro*, cellular and rodent models, in addition to zebrafish.
Methods: To determine how small molecules affect zebrafish behavior, we have built a fully automated phenotyping system capable of tracking and quantifying zebrafish behaviors in HT, 96-well format. The platform is a high content imaging system that combines robotic stimulus presentation with high-quality digital video capture and image processing algorithms.
Results: Preliminary screening of 30 000 compounds in one behavioral assay has identified 44 'hit' compounds and zero false positives among 5155 DMSO-treated negative control wells. Of these 44 hits, 9 are known bioactive compounds. All 9 of the known hits are annotated as GABA receptor agonists. These data suggest that some of the 35 other novel

hit compounds from this screen may also target GABAergic and other anxiety-related pathways.
Conclusions: The compounds identified in this screen may have utility beyond anxiety-related research. It is difficult to predict exactly how behavior-modifying compounds will work, or what their translational utility will be. However, history suggests that psychiatric medicines are often discovered based on phenotypic observations. Given the efficacy of behavior-based neuroactive drug discovery, and the historical lack of high-throughput phenotyping technologies, it is likely that new large-scale behavior-based screening efforts in the zebrafish will successfully identify new neuroactive compounds.
Keywords: drug-discovery zebrafish phenotype-based screening HTS.
Disclosures: D. Kokel, Nothing to Disclose.

T21. Cortical Synaptic Alterations and Pharmacologic Rescue of Behavioral Changes in a Mouse Model of Bipolar Disorder with Conditional Forebrain Knockout of Ankyrin-G

Shanshan Zhu, Solange P Brown, Vann Bennett, Mikhail V Pletnikov, Christopher A Ross*

Johns Hopkins University, Baltimore, Maryland

Background: The *ANK3* (Ankyrin-G) locus is a risk factor for bipolar disorder and schizophrenia. In CNS neurons, Ankyrin-G localizes to the axon initial segment and node of Ranvier, and tethers sodium and potassium channels. In local cortical circuits that are believed to be altered in psychiatric disorders, chandelier cells are fast spiking interneurons that form axo-axonic inhibitory synapses on pyramidal cells, forming GAT-1 and GAD67 positive cartridge-like structures wrapping around their axon initial segments. Previous studies from David Lewis's lab have found decreased Ankyrin-G label in postmortem schizophrenia cerebral cortex, suggesting loss-of-function effects.
Methods: We established a forebrain-specific Ankyrin-G conditional knockout mouse model (CamKII-Cre X Flox-AnkG exon 23), which deletes all major forms of Ankyrin-G (*vs* a previous model which deleted one isoform, highly expressed in cerebellum, resulting in ataxia). In our current model, CaMKII-Cre expression, and thus Ankyrin-G knockdown, begins in adulthood. We characterized behavior, examined the effects of psychiatric medications, and explored the cellular and molecular mechanisms, using electrophysiology and double label immunofluorescence.
Results: Ankyrin-G knockout mice displayed increased motor activity in the open field apparatus, increased exploratory activity and less anxiety-like behavior in the elevated plus maze, and only mild cognitive deficit in the Y-maze, reminiscent of affective disorder. Treatment with several anti-mania agents, such as Clozapine, Lithium and Valproic Acid, ameliorated the hyperactivity. Pyramidal neuron excitability from injected current was altered in slices from the knockouts. Chandelier GABA interneuron cartridge structures on pyramidal neuron axon initial segments were strikingly diminished in the Ankyrin G KO mice.
Conclusions: Our forebrain-specific Ankyrin-G conditional knockout mice may be useful as a model of aspects of the brain changes due to alterations at the *ANK3* locus, associated with bipolar disorder and schizophrenia. The striking alterations of GABA-positive cartridges on the pyramidal axon initial segments suggest that, in this potential model of psychiatric disease, there can be rewiring of cortical microcircuitry even in adulthood. This mouse model may help elucidate psychiatric disease pathophysiology, facilitate development of tools to explore the mechanisms of psychiatric drugs, and make possible the testing of new therapeutic strategies for bipolar and schizophrenia.
Keywords: cerebral cortex, lithium, chandelier cells, Ankyrin-G, expermental therapeutics.
Disclosures: S. Zhu, **Part 1:** Funding from Johnson and Johnson via Johns Hopkins Brain Science Institute, **Part 4:** Funding from Johnson and Johnson via Johns Hopkins Brain Science Institute, ; S. Brown, Nothing to Disclose; V. Bennett, Nothing to Disclose; M. Pletnikov, Nothing to Disclose; C. Ross, **Part 1:** Funding from Johnson and Johnson via Johns Hopkins Brain Science Institute, **Part 4:** Funding from Johnson and Johnson via Johns Hopkins Brain Science Institute.

T22. Activity-based Anorexia in the Rat Induces Reward-related Alterations in Enkephalin Gene Expression and Dopamine Release in the Nucleus Accumbens

Nicole M Avena*, Susan Murray, Nicole Barbarich-Marstellar, Pedro Rada

Columbia University/New York Obesity Research Center, New York, New York

Background: Activity-based anorexia (ABA) is an animal model of core features associated with anorexia nervosa, characterized by limited food access and unlimited access to an exercise wheel, conditions known to elicit excessive running and weight loss. It has been hypothesized that this phenomenon may reflect or result in alterations in reward regions of the brain. To test this, we measured levels of opioid gene expression and dopamine (DA) in the nucleus accumbens (NAc) of ABA and control rats.
Methods: In Exp. 1, rats ($n = 6$/group) were assigned to five groups: ABA, Palatable Food, Recovered ABA, Exercise Control, and Control. RT-PCR was used to measure enkephalin mRNA. In Exp. 2, rats ($n = 7$–8/group) were assigned to an ABA or control group. *In vivo* microdialysis was used to measure extracellular DA levels during baseline, running, running and chow, and post sample.
Results: The results revealed that enkephalin mRNA was elevated in the NAc of ABA rats, rats with access to palatable food, and recovered ABA rats. Higher levels of extracellular DA were also found during running in ABA rats *vs* controls.
Conclusions: These findings suggest alterations in reward-related neurochemicals in ABA rats. This may explain why ABA rats exhibit increased exercise even during the period of food access, as the reinforcing effects of running may be enhanced when coupled with food restriction. These results

may have translational implications for understanding the neurological basis of anorexia nervosa.
Keywords: anorexia nervosa, rat, self-starvation, dopamine, opioids.
Disclosures: N. Avena, Nothing to Disclose; S. Murray, Nothing to Disclose; N. Barbarich-Marstellar, Nothing to Disclose; P. Rada, Nothing to Disclose.

T23. Repeated Ketamine Exposure During Adolescence Produces Long Lasting Stress Resistance in Adulthood

Eric M Parise, Lyonna F Alcantara, Brandon L Warren, Carlos A Bolanos-Guzman*

Florida State University, Tallahassee, Florida

Background: Nearly 50% of adolescents who suffer from Major Depressive Disorder (MDD) are non-responsive to current treatments, thus it is critical that more effective treatments be evaluated. Ketamine, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist has received considerable attention as a rapid-acting and long-lasting treatment for MDD in adults, however little is known about its efficacy in adolescents. Therefore, the effectiveness and functional consequences of ketamine exposure during adolescence were explored.
Methods: Adolescent male rats (postnatal day [PD] 35) received two ketamine (0, 5, 10 or 20 mg/kg) injections, 4 h apart, after exposure to day 1 of the forced swim test (FST). The next day, rats were re-exposed to the FST to assess ketamine-induced antidepressant-like responses. Separate groups were exposed to chronic unpredictable stress (CUS) to confirm findings from the FST. After these initial experiments, adolescent naïve rats were exposed to either 1 or 15 consecutive days (PD35–49) of ketamine (20 mg/kg, twice daily). Ketamine's influence on behavioral reactivity to aversive (ie, elevated plus-maze, FST) circumstances was then assessed 2 months after treatment.
Results: Ketamine treatment reversed the CUS-induced depression-like behaviors in the FST. Repeatedketamine exposure resulted in anxiolytic- and antidepressant-like responses 60 days after drug exposure. None of the ketamine doses used was capable of inducing drug-seeking behaviors as measured by place preference conditioning.
Conclusions: Repeated ketamine exposure induces enduring resilient-like responses regardless of age of exposure. These findings point to ketamine, and its repeated exposure, as a potentially useful antidepressant during adolescence.
Keywords: adolescence, anxiety, depression, ketamine, rats, resilience, stress.
Disclosures: E. Parise, Nothing to Disclose; L. Alcantara, Nothing to Disclose; B. Warren, Nothing to Disclose; C. Bolanos-Guzman, Nothing to Disclose.

T24. Susceptibility to Chronic Social Defeat Stress Increases Morphine Reward

Megan Kechner, Michelle Mazei-Robison*

Michigan State University, East Lansing, Michigan

Background: Stress exposure is known to influence drug craving and relapse in human subjects and these effects can be modeled in preclinical rodent models. Social defeat stress, an ethologically valid model that utilizes the physical and psychological stress imposed by social subordination, has been demonstrated to exhibit excellent face and pharmacological validity for stress-related disorders like depression and post-traumatic stress disorder, inducing long-lasting changes in behavior that are sensitive to chronic, but not acute, antidepressant treatment. The social defeat model has been used to assess cocaine reward, behavioral sensitization, and self-administration, with results generally supporting increased drug reward and self-administration following social defeat stress, consistent with the co-morbidity of drug dependence and mood disorders in human populations. However, the chronic social defeat model, as well as most other rodent models of mood disorders, utilize some form of physical trauma, complicating the study of pain-relieving opiate drugs, a significant hurdle given the escalation in the use and abuse of these drugs. To overcome this, we will also use the recently developed model of emotional stress. In this model, mice witness, but are not physically exposed to, chronic social defeat stress, alleviating the confound of studying pain-relieving opiate drugs. Given the co-morbidity of stress-related disorders and opiate dependence, we sought to examine whether physical or emotional defeat stress increased morphine reward and consumption.
Methods: For physical chronic social defeat stress, we utilized the previously validated paradigm. Briefly, C57Bl6 mice were subjected to a daily 10 min. social defeat episode with a novel CD1 mouse for 10 days. Following each defeat episode, the C57 mouse was separated from the CD1 mouse by a barrier that allows sensory but not physical interaction, and the two mice were co-housed. After the tenth defeat, the mice were singly housed and social interaction (SI) testing was performed 24 h later. Starting on day 12, mice underwent morphine CPP testing using established methods. To assess morphine consumption, we used a two-bottle choice paradigm that takes advantage of the genetic background of C57BL6 mice, which have been shown to exhibit a propensity for morphine drinking. Mice were singly housed with two bottles initially containing just water, allowing acclimation to the bottles. The bottles were then filled with 0.2% sucrose-0.3 mg/ml morphine sulfate and 0.2% sucrose-0.06 mg/ml quinine sulfate, which were weighed daily. Animals were sacrificed at the conclusion of the experiment and tissue (ventral tegmental area, nucleus accumbens, and hippocampus) was dissected and processed for western blot analysis. Emotional stress was completed as described by Warren *et al* (2013). In this modified version of chronic social defeat stress, a second male C57 mouse is placed on the opposite side of the perforated partition during the physical stress, such that the mouse experiences the psychological sensory stress of witnessing an aggressive encounter without physical contact. The 'witness' mouse is then housed across from a novel CD1 mouse for the remaining 24 h.
Results: Following 3 training days using a low conditioning dose of morphine (10 mg/kg) that did not induce significant preference in control mice, we found that susceptible mice exhibit significantly increased morphine CPP compared to control and resilient mice. Importantly, we found that there was a significant negative correlation between SI ratio and

the place preference score, where decreasing SI ratio correlates with an increase in morphine reward. These data support the hypothesis that susceptibility to chronic social defeat stress increases morphine reward. In our pilot study of morphine consumption in naïve mice, we found that mice exhibited a preference of ~75% for the morphine-sucrose solution compared to the quinine solution. Total intake volume did not differ between mice given a choice between quinine and morphine and controls that received 0.2% sucrose alone. Additionally, we observed biochemical changes in the brain consistent with chronic morphine treatment, including a significant increase in delta Fos B in the nucleus accumbens. In a small study, we examined morphine consumption following physical social defeat stress. We found a strong trend for a negative correlation between SI ratio and morphine consumption, where decreasing SI ratio correlates with an increase in morphine consumption.

Conclusions: Together these data suggest that physical social defeat stress increases morphine reward and consumption. Excitingly, we have established a morphine consumption assay that allows us to measure voluntary morphine intake. Intake in this model is sufficient to produce biochemical changes in the mesolimbic reward pathway consistent with chronic morphine administered by ip injection or sc pellet. We are currently testing whether emotional stress produces similar changes in morphine reward and consumption. These studies, along with others to examine the molecular mechanisms underlying resiliency to emotional stress, offer promise of an increased understanding of the neurobiological mechanisms that contribute to opiate dependence, and the potential to uncover novel targets for therapeutic intervention in mood disorders and opiate use.

Keywords: opiate morphine stress ventral tegmental area mood disorder.

Disclosures: M. Kechner, Nothing to Disclose; M. Mazei-Robison, Nothing to Disclose.

T25. The Contribution of Adult Hippocampal Neurogenesis to Fear Memory Generalization

Mazen A Kheirbek*, Liam J Drew, Elizabeth Balough, Christine A Denny, Rene Hen

New York State Psychiatric Institute/RFMH, New York, New York

Background: Maladaptive fearfulness is a hallmark of a number of anxiety disorders. In particular, in disorders such as post-traumatic stress disorder, it is frequently observed that fear becomes expressed in safe situations that are similar to the original trauma. That is to say, fear is overgeneralized to neutral situations. The dentate gyrus (DG) subregion of the hippocampus functions in pattern separation, a process by which representations of similar experiences or events are transformed into non-overlapping representations so as to facilitate their storage as discrete units. We hypothesize that impairments in pattern separation contribute to the overgeneralization of fear seen in certain anxiety disorders. Recently, adult-born granule cells (GCs) in the DG have been implicated in pattern separation. However, it remains unclear how these young neurons facilitate this process, or whether their functional contribution differs from that of mature GCs. Here, we have probed the mechanism by which immature GCs of the DG may act to prevent the generalization of a contextual fear memory. To address the on-line role of immature GCs in contextual encoding, generalization and anxiety, we used optogenetic techniques to selectively and bidirectionally modulate the activity of immature and mature GCs in a region-specific manner.

Methods: To target opsins selectively to immature GCs, a Nestin-CreER^{T2} line was crossed to either a conditional archaerhodopsin-3 (Arch) or a channelrhodopsin-2 (ChR2) line. Tamoxifen injection in adult mice induced recombination in neural stem cells and transit-amplifying progenitors to generate opsin-expressing adult-born GCs. For targeting mature GCs, we crossed the conditional opsin lines with an Arc-CreER^{T2} line, which directs recombination to cells expressing the immediate early gene Arc. Injection of Tamoxifen paired with exploration of a novel environment induced recombination and expression of opsins in a sparse population of mature GCs (but not immature GCs that don't express significant levels of Arc). Six weeks after TMX, mice were tested for behavioral effects of light-induced inhibition or excitation in the dorsal or ventral DG. As this manipulation allows for epoch selective modulation of activity in adult-generated neurons and their mature counterparts, we tested whether these cells play a context-specific role in a pattern separation task that requires mice to discriminate between a shock-paired context and a similar, safe context. Activity in adult-born GCs or a similar number of mature GCs in the dorsal DG was suppressed during exposure to either the conditioning context or the similar, safe context.

Results: Histological analysis showed that Nestin-ChR2 and Nestin-Arch lines expressed opsins in nearly all immature adult-born, GCs, that Arc-ChR2 and Arc-Arch lines expressed opsins almost exclusively in mature GCs and that similar numbers of opsin-expressing neurons were generated in the Nestin-opsin and the Arc-opsin lines. Optical modulation *in vivo*, demonstrated that while none of the manipulations impacted baseline anxiety state, excitation of either cohort of cells impaired context acquisition. Optical inhibition of adult-generated GCs, but not of an equal number of mature GCs in the dorsal DG disrupted the rapid encoding of contextual fear memories, indicating a selective contribution of adult-generated neurons to rapid encoding of contexts. In a contextual fear discrimination experiment, we found that inhibition of young GCs during exposure to the similar, safe context, but not the conditioning context, impaired discrimination. Surprisingly, inhibition of a population of mature GCs in the similar context improved the animals' ability to discriminate between contexts.

Conclusions: This study reveals differential contributions of mature and immature neurons of the DG to contextual fear encoding and discrimination. Specifically, we show that while young GCs are not needed for the maintenance of an already learned contextual memory, they are necessary for the disambiguation of similar information from already learned information. In contrast, inhibiting the activity of mature neurons improves discrimination, indicating oppo-

site functions for these two populations of cells within the DG. Immature neurons in the dorsal DG are required for the rapid encoding of novel contextual information and to disambiguate novel representations from already learned ones, which is consistent with their proposed role in pattern separation. In contrast mature GCs appear to be involved in generalization. We hypothesize therefore that strategies aimed at stimulating neurogenesis or modulating the activity of mature GCs may restrain overgeneralization and may be effective for the treatment of anxiety disorders.
Keywords: anxiety, dentate gyrus, neurogenesis, generalization.
Disclosures: M. Kheirbek, Nothing to Disclose; L. Drew, Nothing to Disclose; E. Balough, Nothing to Disclose; C. Denny, Nothing to Disclose; R. Hen, **Part 1:** Roche and Lundbeck.

T26. Individual Differences in Instrumental Performance in Naïve Rats Predict Distinctive Responses to Chronic Stress

Shigenobu Toda*, Yoshio Iguchi, Yoshio Minabe

Kanazawa University, Kanazawa, Japan

Background: Major depressive disorder (MDD) causes serious social health issues. Yet, the neural circuits and neurochemical systems underlying the MDD pathophysiology have to be fully elucidated. These days many preclinical studies have shed light on the individual differences in the phenotypes that come out after the exposure to various types of repeated stress, since in general these stressors induce MDD-like symptoms in some, but not others, both in humans and rodents. However, at least in rodent studies few studies have explored the relationship of the post-stress individual differences with the pre-stress ones that might genetically or epigenetically determine the fate of each individual after stress. In the present study, we focused on the role of cost/benefit-based decision-making in pre-stress individual diffrences, since it reflects the functional eligibility of prefrontal-limbic interaction that is severely affected and is often difficult to treat in human MDD. We then hypothesized that the stress-induced consequences and the responsiveness to therapeutic approaches of each individual may be destined by the individual differences in the performance of cost/benefit-based decision-making at the pre-stress (= 'naïve') state.
Methods: To verify the hypothesis above, we first tried to classify several distinctive subgroups from total 84 of naive Sprague-Dawley rats based on their performance in a saccharin-rewarded instrumental behavior trained under a time-constrained progressive ratio schedule for 3 daily sessions without food/water deprivation. This experimental paradigm was chosen to monitor the individual difference of motivation-dependent learning and cost/benefit-based flexibility in decision-making. Next, based on the classification described above, chronic unpredictable stress (CUS), that consisted of forced swimming, restraint, and social defeat in a random order for 4 weeks, or chronic handling was loaded onto each subgroup to compare the effects of CUS on several behavioral/biochemical indexes among these subgroups over 4 weeks after CUS.
Results: Based on the performance in the instrumental training as described above, the 'naïve' animals have been subdivided into three subgroups as follows; Low Motivation (LW: characterized by consistent low completed ratio, population = 25%), Switching (SW: high completed ratio in the first session followed by drastic reduction as sessions went on, 48%), and Hyper Motivation (HM: consistent high completed ratio, 27%). HM also displayed higher response rate on inactive lever at the fixed ratio 5, suggesting a shift to habit-like behavior at this stage. These subgroups were also distinctive each other in biological parameters; first, LM marked a significantly higher serum corticosterone than HM at the baseline, and the response of serum corticosterone to acute stress (footshock) was significantly higher in LM than other two subgroups. Second, the body weight of LM was significantly lower compared to those of other two subgroups through the entire experimental period. We next examined if these three subgroups would display different behavioral or biochemical alterations after CUS. Immediately after CUS, all subgroups demonstrated a disturbed reversal learning and enhanced novelty-induced locomotion, however, the latter was continued for more than 3-weeks only in SW. The serum corticosterone at the baseline was significantly elevated when three subgroups were put together, however, when looked closely to each subgroup, only SW displayed the elevated level of serum corticosterone, and this elevation was continued until 3-weeks after CUS despite there was no alteration as a whole stressed group at this point. In addition, with the same instrumental training that was used in pre-stress sessions, we found a significant acceleration in the performance on an FR 1 immediately after CUS in all subgroups. However, only HM, from immediately after CUS, showed a significant reduction in completed ratio, whereas LM showed a significant increase in completed ratio for the first time about 4 weeks after CUS. In contrast, in SW CUS did not affect the completed ratio during the entire after-CUS sessions.
Conclusions: These results imply that the subgroups classified by distinctive pre-stress individual differences were affected differently by CUS, resulting in distinctive post-CUS phenotypes that came out at different time points. Our data also implicate that the more cognitive flexibility the subjects display before stress, the less influence the stress remains. It is also expected that with this approach it is possible to predict the treatment-resistant individuals/symptoms before loading stress to the subjects.
Keywords: pre-stress individual differences, chronic unpredictable stress, cost/benefit-based decision-making.
Disclosures: S. Toda, **Part 4:** Otsuka: about $100 000, Astellas: about $8000, Both of them are indirect grants to the department that the authors belong.; Y. Iguchi, Nothing to Disclose; Y. Minabe, Nothing to Disclose.

T27. Pay Attention: Modelling the Inattentive and Impulsive Subtypes of Adult ADHD in the Rat—Using the 5-Choice Continuous Performance Task (5C-CPT)

Anneka Tomlinson*, Joanna Neill

University of Manchester, Manchester, United Kingdom

Background: The 5-choice continuous performance task (5C-CPT) is an enhanced version of the 5-choice serial

reaction time task (5-CSRTT). The 5C-CPT assesses vigilance in a way that is similar to the human CPT, in comparison to the 5-CSRTT that assesses sustained attention. Disturbances in attention and inhibitory control play a central role in the symptomatology of ADHD. Selection of rats within a normal 'population' that display reduced sustained attention and vigilance may provide a more translational model of ADHD. The aim of the current study was to investigate the effects of psychostimulant and non-stimulant drugs on attention, impulsivity and performance in adult rats separated into high and low attentive and high and low impulsive in the 5C-CPT based on attentive responses.

Methods: The effects of acute methylphenidate (MPH), atomoxetine (ATMX) (0.5, 1.0, 2.0 mg/kg i.p) A-142996 (0.1, 0.3, 1.0 μmol/kg) were assessed in the 5C-CPT in female Lister-hooded adult rats ($n = 40$). Animals were trained for 60 sessions, and then divided into four groups (high and low attentive, and high and low impulsive) based on set criteria. Animals were challenged on test days by increasing the variable inter-trial interval from 5 to 10 s (attentive groups) and from 5 to 20 s (impulsive groups). The impulsive groups only received ATMX and MPH. A within subjects design was utilised.

Results: MPH (2.0 mg/kg) and ATMX (2.0 mg/kg) significantly increased % accuracy ($p < 0.05$). ATMX significantly reduced false alarm rate (1.0, 2.0 mg/kg; $p < 0.05$) in the low attentive group (LA). ATMX (1.0 mg/kg, 2.0 mg/kg) and MPH (0.5, 1.0 mg/kg) significantly increased sensitivity index in LA ($p < 0.05$; $p < 0.01$). MPH significantly decreased correct rejections at all doses in LA animals ($p < 0.01$, $p < 0.05$, $p < 0.05$ respectively). A-142996 (0.6, 1.0 μmol/kg) significantly increased % correct rejections ($p < 0.01$; $p < 0.001$), and sensitivity index ($p < 0.05$; $p < 0.01$) in LA. A-142996 (0.6, 1.0 μmol/kg) significantly reduced false-alarm rate at 0.6, 1.0 μmol/kg ($p < 0.01$; $p < 0.001$) in LA animals. MPH increased premature responding in low impulsive (LI) animals at 1.0 mg/kg compared to vehicle treated LI animals. However in high-impulsive (HI) animals MPH at 1.0 mg/kg ($p < 0.01$) and 2.0 mg/kg ($p < 0.01$) significantly decreased the number of premature responses compared with vehicle treated HI animals. MPH at 1.0 mg/kg significantly reduced the false alarm rate p[FA] in HI animals ($p < 0.05$) compared with vehicle treated animals. Vigilance was also enhanced in HI animals ($p < 0.01$), SI scores significantly decreased at all doses in HI animals compared with vehicle treated HI animals ($p < 0.05$; $p < 0.05$; $p < 0.01$). ATMX at all doses (0.5, 1.0, 2.0 mg/kg) significantly reduced premature responding in HI animals ($p < 0.001$) compared to vehicle treated HI animals. However in LI animals atomoxetine at 1.0 mg/kg significantly ($p < 0.01$) increased the number of premature responses compared with vehicle treated LI animals. AMX at 1.0 mg/kg significantly reduced the p[FA] in HI animals ($p < 0.01$) compared with vehicle treated HI animals. Vigilance as measured by the non-parametric measure SI, was also enhanced in HI animals; SI scores were significantly increased at 1.0 and 2.0 mg/kg ($p < 0.05$) in HI animals compared with vehicle treated HI animals. The total number of processed trials were significantly higher in animals treated with 1.0 mg/kg atomoxetine ($p < 0.01$).

Conclusions: In summary, ATMX and MPH enhanced sustained attention in the 5-CSRTT and vigilance in 5C-CPT in low performers. The compound not currently utilised in the treatment of adult ADHD; A-1422996 (D4-agonist) also enhanced aspects of attention; mainly vigilance in LA only. ATMX reduced impulsivity in HI animals at all doses, and appeared to enhance impulsivity in LI animals. This would suggest that LA animals and HI animals are more sensitive to the effects of both stimulant and non-stimulant drugs. MPH significantly enhanced both sustained attention and vigilance in LA, whilst ATMX significantly reduced impulsivity in HI. Sugguesting that ATMX is more suited for symptoms of impulsivity and MPH is more effective for eleviating symptoms of impaired attention. These data provide validation of a rat model for the inattentive subtype of adult ADHD and a model for the impulsive subtype of adult ADHD. The model utilises the 5C-CPT to select those with deficits in sustained attention and vigilance, and increased impulsivity which can then be enhanced (attention) or reduced (impulsivity) by ADHD medication.

Keywords: impulsivity attention ADHD cognition animal model.

Disclosures: A. Tomlinson, Nothing to Disclose; J. Neill, Nothing to Disclose.

T28. Lesions of the Basolateral Amygdala Induce Elevated Risk-taking in Rats

Caitlin Orsini*, Barry Setlow

University of Florida, Gainesville, Florida

Background: On a daily basis, organisms are continually faced with decisions, most of which include choices that are associated with unfavorable consequences. Most people are able to balance the rewards and risks inherent in these choices and ultimately make adaptive decisions. However, many psychiatric conditions, such as attention deficit hyperactivity disorder (ADHD), schizophrenia, and in particular, addiction, are characterized by maladaptive decision-making, such that choices are biased towards overly risky options. Therefore, it is important to understand the neural basis of risk-based decision making to determine how these capabilities become compromised. The involvement of the basolateral amygdala (BLA) in some forms of decision-making is relatively well-established, but its specific involvement in integrating reward and risk-related information during decision-making is less clear.

Methods: To explore the role of the BLA in risk-based decision-making, we assessed the effects of neurotoxic lesions of the BLA on a rodent model of risky decision-making [the Risky Decision-Making Task (RDT)], in which animals are given choices between a small, 'safe' food reward and a large, 'risky' food reward that is accompanied by variable probabilities of punishment (mild electric footshock). Sixteen male Long-Evans rats were food-restricted and trained on the RDT until stable performance emerged (approximately 30 sessions). After returning to free-feeding, rats received either bilateral injections of N-methyl-D-aspartate (NMDA; 0.3 μl/hemisphere) or sham injections. Upon recovery, rats were food restricted and re-tested on the RDT until stable performance re-emerged (40

sessions). After stable performance emerged, all rats were then given acute intraperitoneal injections of amphetamine (0, 0.3, 1.0 and 1.5 mg/kg) and re-tested on the RDT. Finally, to explore whether the lesion-induced increase in risk-taking was a result of an increase in reward-seeking behavior, we assessed instrumental responding (lever pressing) for food pellets under various fixed ratio (FR) schedules of reinforcement (FR1, 3, 10, 20 and 40, one session/day across five days).
Results: Prior to surgery, there were no differences between sham and lesioned groups in choice of the large risky reward. After surgery, however, BLA-lesioned rats displayed significantly greater choice of the large risky reward (greater risk-taking) relative to sham rats. With respect to acute amphetamine administration, we found that amphetamine dose-dependently decreased risk-taking, irrespective of lesion condition. Finally, BLA-lesioned rats responded significantly less on the FR schedules than the sham rats, particularly on the FR3, FR10 and FR20 schedules, indicating that elevated risk-taking in BLA-lesioned rats is not likely due to an increase in food motivation.
Conclusions: Together, these findings demonstrate an important role for the BLA in risk-based decision-making. In particular, this structure seems to be crucial for guiding choice behavior in response to risk of adverse consequences, so as to select the most advantageous choice. Importantly, BLA lesions spare some processing of the aversive qualities of the punishment, since amphetamine, which typically enhances the impact of conditioned punishment, caused risk aversion even in BLA-lesioned rats. Future work will focus on identifying the specific information encoded by BLA during risk-based decision-making and how this is influenced by its connections with other brain structures, such as the orbitofrontal cortex.
Keywords: basolateral amygdala, risk-taking, decision-making, amphetamine, addiction.
Disclosures: C. Orsini, Nothing to Disclose; B. Setlow, Nothing to Disclose.

T29. Loss Estrogen-related Receptor Alpha Activity Affects Behaviors Related to Eating Disorders in Mice

Huxing Cui, Michael L Lutter*

University of Iowa, Iowa City, Iowa

Background: While genetic factors have been implicated in the psychopathology of eating disorders, such as anorexia nervosa and bulimia nervosa, a clear biological pathway has not been delineated.
Methods: Using next generation sequencing, we have identified two rare missense mutations that segregate with illness in two families with multiple members affected by eating disorders. Biochemical and transcriptional activity assays were used *in vitro* to determine the conseuqences of candidate mutations on protein function. Knockout mice for one of the genes were obtained and behaviors related to the development of eating disorders were measured.
Results: Analysis of 20 members in the first family identified a rare missense mutation in the estrogen-related receptor alpha (*ESRRA*) gene, while analysis of eight members in the second family identified a missense mutation in the histone deacetylase 4 (*HDAC4*) gene. We determined that ESRRA and HDAC4 interact both *in vitro* and *in vivo*, and transcriptional analysis revealed that HDAC4 potently represses the transcriptional activity of ESRRA. Biochemical analysis of the mutations revealed that the identified mutation in ESRRA decreased its transcriptional activity, while the HDAC4 mutation increased transcriptional repression of ESRRA. ESRRA is expressed in several brain regions associated with the development of eating disorders including several components of the corticostriatal-thalamocortical circuit. Mice lacking expression of ESRRA display several behavioral deficits relevant to eating disorders including reduced intake of high fat diet and increased anxiety- and depression-like behaviors.
Conclusions: Our findings suggest mutations that result in decreased ESRRA activity increase the risk of developing eating disorder-related behaviors.
Keywords: anorexia nervosa, eating disorders, estrogen-related receptor alpha, histone deacetylase 4.
Disclosures: H. Cui, Nothing to Disclose; M. Lutter, Nothing to Disclose.

T30. Chronic Phenytoin Administration Prevents Single Prolonged Stress Induced Extinction Retention Deficits and Glucocorticoid Upregulation

Sophie A George*, Dayan K Knox, Mariana Rodriguez, John Riley, Israel Liberzon

University of Michigan, Ann Arbor, Michigan

Background: At present, only two pharmacological agents (both selective serotonin reuptake inhibitors) are FDA approved for the treatment of post-traumatic stress disorder (PTSD). Both have limited efficacy, and the mechanism of action is likely non-specific. Valid animal models help to identify the neurobiological processes underlying psychopathology that can in turn, be targeted by novel therapeutic agents. Recent data obtained using the single prolonged stress (SPS) model of PTSD implicates HPA axis abnormalities in deficits in retention of extinction memories, and also demonstrates altered levels of glutamate in the medial prefrontal cortex (mPFC). We therefore examined the effect of phenytoin (PHE) administration, an antiepileptic agent with anti-glutamatergic properties, on the development of extinction retention deficits (Experiment 1) and GR changes in the mPFC, dorsal and ventral hippocampus (dHPC and vHPC) and amygdala (Experiment 2), using the SPS model.
Methods: Eighty-eight male Sprague Dawley rats were assigned to SPS or control groups. Rats in the SPS group were exposed to serial application of restraint, forced swim and ether exposure. Rats assigned to the control group were left undisturbed. One day later, rats received a subcutaneous injection of high or low dose phenytoin (40 or 20 mg/kg) or vehicle. Injections were repeated once per day for seven days. Experiment 1: Fear conditioning, fear extinction and extinction retention testing were conducted on three consecutive days. Time spent freezing was used as an index of conditioned fear. Experiment 2: A different group of rats underwent the same behavioral procedures as in Experiment 1. One day following extinction retention testing, they were euthanized, and their brains were removed, dissected

and flash frozen for western blot analysis to quantify GR levels.
Results: Experiment 1: Freezing during fear conditioning and extinction was not altered by SPS or PHE treatment, however during extinction recall, ANOVA revealed a significant interaction between SPS and PHE ($F_{(2, 40)} = 3.78$, $P = 0.032$) indicating that phenytoin treatment differentially affected extinction retention in SPS and control rats; PHE administration increased freezing in controls, while decreasing freezing in SPS group. Experiment 2: SPS increased GR expression in the mPFC ($F_{(1, 32)} = 8.29$, $P = 0.007$), and both high and low dose PHE attenuated this enhancement (*post hoc* $P = 0.008$ and .021 respectively). In the dHPC, ANOVA revealed a main effect of stress only ($F_{(1, 32)} = 10.67$, $P = 0.003$). PHE tended to reduce GR expression in SPS animals (*post hoc* $p = 0.088$), but had no effect in controls. There were no significant effects of SPS or PHE in the vHPC or amygdala.
Conclusions: Pharmacologically, the effects of PHE can involve a number of different systems ie sodium channels, glutamatergic neurotransmission and more. If indeed the observed findings are mediated via PHE glutamatergic effects, it suggests that the development of SPS-induced extinction retention deficits may be sensitive to modulation of glutamatergic activity following trauma. Additionally, these data might also suggest an interaction between glutamatergic activity and glucocorticoid receptor changes, as treatment with PHE prevented GR upregulation in the mPFC and dHPC. These data raise the possibility that secondary prevention with compounds that alter excitatory neural transmission may be effective in preventing the development of post-trauma PTSD-like behavioral and physiological abnormalities.
Keywords: post-traumatic stress disorder, animal model, secondary prevention strategies, anti-epileptic, glucocortiocoid receptors.
Disclosures: S. George, Nothing to Disclose; D. Knox, Nothing to Disclose; M. Rodriguez, Nothing to Disclose; J. Riley, Nothing to Disclose; I. Liberzon, Nothing to Disclose.

T31. Independent Effects of Lps and Social Isolation on Forced Swim Behavior in Female Mice

Cristina L Sanchez*, Nicole L Schramm-Sapyta, Cynthia M Kuhn, Florian Daniel Zepf

RWTH Aachen University, Germany

Background: Animal models are valuable experimental tools for investigating mechanisms of and developing therapies for psychiatric illnesses. Although depression is diagnosed more frequently in women, no convincing animal model for depression in female rodents exists. It has been reported that female mice respond to prolonged social isolation and LPS treatment with anhedonia. In addition, it has been suggested that inflammation plays a significant role in the development of depression in both sexes. The purpose of the present study was to analyze the additive effects of LPS and social isolation on the forced swim behavior in female mice.
Methods: Adult female C57BL/6 mice from Jackson Laboratory were treated once with an intra-peritoneal injection of bacterial endotoxin lipopolysaccharide (LPS, 0.83 mg/kg dose) or a vehicle (saline) and immediately housed individually (single housing) or in sets of 5 mice/cage (grouped housing) for 45 days. After this time, anhedonic behavior was assessed with the forced swim test (FST) and three categories of behavioral activity were scored (latency, swimming and immobility). A pre-test of 3 min was conducted the day prior to the experiment; after the session mice were immediately dried and kept in their home cages. The next day, at the same time, mice were recorded and placed for 5 min in the glass cylinder. Time in movement for each mouse was evaluated in time blocks of 30 s for a 5 min session. The effects of treatment and housing were analyzed by repeated measures ANOVA.
Results: Our results indicate that there is a main effect of housing and time point in female mice. A single injection of LPS showed a significant decrease on time in movement in grouped mice compared with saline-treated mice. In contrast, social isolation is able to induce significant changes in some time points during the FST. When both LPS and saline-treated mice were added together, social environment made a significant effect on time in movement and time point. Grouped mice presented a constant movement during the whole session, while changes in the movement pattern of the single-housed mice were shown. An intense drop in movement evoked at the beginning of the test. However, when the time was close to the end of the pre-test (3 min), isolated animals started moving again and immediately after, they shortened the time moving.
Conclusions: The present results show that social environment and inflammatory challenge by LPS seem to produce independent and somewhat different effects to increase immobility and decrease swimming time in female mice in the FST. The FST is a tool that has been used widely for assessing behavior despair and antidepressant effects, suggesting that further investigation of these manipulations as models to study depression in females is worth pursuing. The significant effects during the 1st but not the 2nd three minutes of the test suggest that robust effects of manipulations might be missed without evaluation of the time course of response in this test.
Keywords: social isolation, inflammatory challenge, animal models, mice, depression.
Disclosures: C. Sanchez, Nothing to Disclose; N. Schramm-Sapyta, Nothing to Disclose; C. Kuhn, Nothing to Disclose; F. Zepf, **Part 1:** F.D.Z. was the recipient of an unrestricted award donated by the American Psychiatric Association (APA), the American Psychiatric Institute for Research and Education (APIRE) and AstraZeneca (Young Minds in Psychiatry Award). He also received research support from the German Federal Ministry for Economics and Technology, the German Society for Social Pediatrics and Adolescent Medicine, the Paul and Ursula Klein Foundation, the Dr August Scheidel Foundation, the IZKF fund of the University Hospital of RWTH Aachen University, and a travel stipend donated by the GlaxoSmithKline Foundation. He is the recipient of an unrestricted educational grant, travel support and speaker honoraria from Shire Pharmaceuticals, Germany. He also receives editorial fees from Co Action Publishing, Sweden. In addition, he has received support from the Raine Foundation for Medical Research (Raine Visiting Professorship).

T32. Hippocampal-Prefrontal BDNF Circuits in Fear Extinction

Luis E Rosas-Vidal, Fabricio H Do Monte, Gregory J Quirk*

University Puerto Rico, San Juan, Puerto Rico

Background: Brain-derived neurotrophic factor (BDNF) has been implicated in various learning and memory processes (Cunha *et al*, 2010) including fear extinction (Bredy *et al*, 2007; Chhatwal *et al*, 2006; Heldt *et al*, 2007). We have previously shown that BDNF infused into the infralimbic prefrontal cortex (IL) is sufficient to induce extinction learning even in the absence of training (Peters *et al*, 2010). Additional experiments are needed, however, to probe the relationship between extinction-related BDNF and prefrontal function.
Methods: Rats were implanted with cannulas in either IL or adjacent prelimbic prefrontal cortex (PL). To address whether BDNF is sufficient for fear extinction, rats were infused with BDNF into IL or PL one day after fear conditioning, and returned to their home cage. To address whether BDNF is necessary for fear extinction, conditioned animals were infused with a BDNF-binding antibody into IL or PL prior to extinction training. To assess which regions might constitute a source of neuronal BDNF during extinction, immunohistochemical techniques were used to label BDNF—NeuN co-localization in IL, PL, amygdala, and the ventral hippocampus (vHPC). To characterize the effect of BDNF infused into vHPC on IL activity, rats were implanted with unit recording electrodes in IL together with a cannula in the vHPC.
Results: Replicating our previous findings, infusion of BDNF into IL was sufficient to decrease fear in the absence of extinction training ($p = 0.015$). This occurred for both recent fear memories (1d) and well as older fear memories (14d) ($p = 0.009$). BDNF infused into PL had no effect. Infusions of a BDNF antibody in IL prior to extinction training impaired extinction retention when tested the following day ($p = 0.014$). Infusion of BDNF antibody into PL had no effect on extinction. Extinction training increased neuronal BDNF expression in vHPC ($p = 0.042$), but not in IL, PL, or amygdala. Consistent with facilitated extinction, infusions of BDNF in vHPC caused a subpopulation of IL neurons to increase their responses to conditioned tones.
Conclusions: Our findings show that BDNF in IL, but not PL, is necessary and sufficient for extinction, both for recent and older fear memories. We also showed that extinction training increases BDNF expression in the vHPC, which projects to IL. Because BDNF in vHPC increased IL firing rate, our findings suggest that extinction augments hippocampal modulation of prefrontal extinction areas, via BDNF. Our findings further support the idea that deficient BDNF signaling impairs extinction learning and may contribute to extinction deficits seen in anxiety disorders (Soliman *et al*, 2010).
Keywords: infralimbic, prelimbic, ventral hippocampal, PTSD, exposure.
Disclosures: L. Rosas-Vidal, Nothing to Disclose; F. Do Monte, Nothing to Disclose; G. Quirk, Nothing to Disclose.

T33. Adolescent Cannabinoid Treatment Leads to Persistent Increase in Frontostriatal CB1 Expression Associated with Upregulation of Class I HDACs

Subroto Ghose*, Hersh Trivedi, Kelly Gleason, Marcus Shanks, Shari Birnbaum

UT Southwestern, Dallas, Texas

Background: Cannabis is the most commonly abused illicit drug in the United States. 2.1 million Americans used cannabis for the first time in 2007; of them 62.2% were less than 18 years old. This is of particular health concern given the association between adolescent cannabis use and adult onset of psychosis. Schizophrenia did not develop days or weeks after cannabis use, but years later, suggesting that cannabis use during a critical period of brain maturation may lead to long-term effects.
Methods: We conducted a series of experiments to examine the long-term consequences of adolescent cannabinoid exposure on the endocannabinoid system. Mice were administered with a cannabinoid receptor 1 (CB1) agonist, (WIN 55,212-2) or vehicle for 10 days by I.P. injection at different developmental time points (5 and 9 weeks). At 16–18 weeks of age, behavioral tests were carried out followed by molecular studies 2 week after the last behavioral test.
Results: Mice administered WIN 55,212-2 (WIN) at 5 weeks of age display significant deficits in PPI and fear conditioning learning and memory paradigm. These behavioral deficits were not observed in mice treated with the CB1 agonist at later developmental time points. We previously reported an altered expression profile of genes involved in endocannabinoid signaling in the hippocampus. Here, we extend those studies, examining CB1 protein expression and Class I and II HDACs in the frontal cortex and striatum. Mice treated with WIN 55,212-2 at 5 weeks of age show a significant upregulation of CB1 in both the frontal cortex (control $0.45 + 0.1$; WIN $0.64 + 0.1$ units, $p < 0.01$) and striatum (control $0.53 + 0.1$; WIN $0.69 + 0.1$ units, $p < 0.01$). The increase in CB1 is associated with significant increases in HDAC 2 (control $1.34 + 0.2$; WIN $1.60 + 0.1$ units, $p < 0.01$) and 3 (control $0.035 + 0.3$; WIN $0.4 + 0.05$ units, $p = 0.02$) but not HDAC 4 or 5 in the striatum. Additional measures of histone acetylation and methylation are underway.
Conclusions: These data suggest that adolescent cannabinoid administration leads to persistent changes in the endocannabinoid pathway and raise the possibility that epigenetic mechanisms are at play in mediating this effect. These data may be relevant to understanding the long term sequelae of significant adolescent cannabis use and may be of particular importance in understanding mechanisms by which adolescent cannabinoid exposure leads to molecular changes predisposing to schizophrenia.
Keywords: cannabis, adolescence, epigenetics, endocannabinoids, schizophrenia.
Disclosures: S. Ghose, Nothing to Disclose; H. Trivedi, Nothing to Disclose; K. Gleason, Nothing to Disclose; M. Shanks, Nothing to Disclose; S. Birnbaum, Nothing to Disclose.

T34. Determinants of Conditioned Reinforcing Effectiveness: Implications for Relapse to Cocaine-seeking

Gregory T Collins*, Charles P France

University of Texas Health Science Center, San Antonio, Texas

Background: Drug abuse is a serious public health problem characterized by high rates of relapse, even after repeated attempts to abstain from drug use. Dopamine systems play a central and dynamic role in mediating not only the reinforcing effects of drugs of abuse, but also the conditioned reinforcing effects of drug-associated stimuli. Although the capacity of drug-associated stimuli to promote relapse-related behaviors in laboratory animals has been well-established, relatively little is known about how individual differences in drug history, diet, age, etc. impact the conditioned reinforcing effectiveness of drug-associated stimuli, and in turn vulnerability to relapse. The current studies were aimed at identifying the specific aspect(s) of cocaine-reinforcement history that control relapse-related behaviors in rats. In particular, they investigated how differences in the (1) total amount of responding, (2) number of cocaine-stimulus pairings, (3) total cocaine intake, and (4) reinforcing effectiveness of the self-administered dose of cocaine altered the strength of relapse-related behaviors.
Methods: Two groups of adult male Sprague Dawley rats were trained to lever press for injections of a small (0.1 mg/kg/inj) or large (1.0 mg/kg/inj) dose of cocaine paired with a 5-s light-tone stimulus (conditioned stimulus [CS]). During acquisition, responding was initially reinforced under a continuous reinforcement schedule with response requirement gradually incremented from 1 to a fixed ratio 3, 5, and 10 over the next 22 sessions. Next, a progressive ratio (PR) schedule of reinforcement was used to quantify the reinforcing effectiveness of the 0.1 and 1.0 mg/kg/inj doses of cocaine, as well as the relative contribution of the CS to the maintenance of responding (ie, cocaine delivered without the CS). Subsequently, a series of tests were performed in order to evaluate the involvement of dopamine D2-like (D_2, D_3, and D_4) receptors in mediating the conditioned reinforcing effects of the CS in the absence of cocaine. During this portion of the study, rats were treated with the clinically-used dopamine D_3/D_2 receptor agonist pramipexole (0.1–3.2 mg/kg) prior to sessions in which responding under a PR schedule of reinforcement resulted in one of two outcomes: (1) presentation of the CS alone, or (2) no scheduled consequence.
Results: Despite similar rates of acquisition (75 and 85% for the small and large dose of cocaine, respectively), rats that responded for the small dose of cocaine made significantly more responses, and received significantly more cocaine-stimulus pairings than rats that responded for the large dose of cocaine. Although total cocaine intake was comparable across the two groups of rats, the large dose of cocaine was 8.5 times more reinforcing than the small dose of cocaine as determined by the final ratio completed during the PR phase of the study. During the pretreatment phase, pramipexole dose-dependently increased PR responding for CS presentation alone in both groups of rats; responding occurred at low rates when the CS was omitted. In addition to significant differences in the effectiveness of the CS to maintain PR responding (large dose CS greater than the small dose CS), pramipexole was more potent at enhancing CS-maintained responding in rats that responded for the large dose of cocaine than in rats that responded for the small dose of cocaine.
Conclusions: The current study quantified the amount and strength of behavior maintained by a small dose or a large dose of cocaine in order to identify the determinants of the conditioned reinforcement which is known to be important in vulnerability to relapse. The results of this study provide clear evidence that the magnitude of the conditioned reinforcing effectiveness of the CS is directly related to the reinforcing effectiveness of the self-administered dose of cocaine, and not the total amount of responding, or the number of cocaine-stimulus pairings. Dopamine systems are dynamically regulated, and future studies will examine how D_3 and D_2 receptor-selective agonists and antagonists affect the strength of relapse-related behaviors under a variety of conditions. By identifying how an individual's history alters the relative contribution of D_2 and D_3 receptors in vulnerability to relapse, this work has the potential to guide efforts to develop individualized treatment strategies to help people remain abstinent.
Keywords: cocaine self-administration; conditioned reinforcement; relapse-related behavior; dopamine D2-like receptors.
Disclosures: G. Collins, Nothing to Disclose; C. France, Nothing to Disclose.

T35. Ketamine Alters Socially-evoked Activity in the Amygdala

Takuma Mihara*, Rosanna Sobota, Robert Lin, Robert Featherstone, Steven J Siegel

Astellas Pharma Inc., Tsukuba-shi, Japan

Background: Social impairments are common in schizophrenia and are not remediated by current treatments. Recently, many studies have suggested that the amygdala is a key brain region for regulation of social function. Therefore, dysfunction of the amygdala has been proposed as a potential mechanism for negative symptoms in schizophrenia. This in turn may be due to NMDA receptor-mediated hypofunction, which is also thought to be related to the pathogenesis of schizophrenia. Specifically, NMDA receptor antagonists have been widely used to produce or disrupt rodent behaviors thought to be correlated to the positive, negative, and cognitive symptoms of schizophrenia. In this study, electroencephalographic amygdala activity was assessed in mice during the choice and interaction phases of a three-chamber social test. This activity was also evaluated following exposure to the NMDA receptor antagonist ketamine, in order to determine the degree to which NMDA receptor activity in the amygdala is related to social function.
Methods: Bipolar electrodes were implanted into the right peri-amydala region of each mouse. Mice were then tested in three-chamber social interaction apparatus with a target mouse and a dummy mouse in the two outer chambers.

Each entry into either the social or non-social chamber was marked on the EEG recording using Spike2 software and video recordings. Social interaction scores were later analyzed by a blind experimenter, who recorded the time spent and number of entries into each chamber. The ketamine group was exposed to 20 mg/kg IP before the social choice test, while the vehicle group was injected with a commensurate volume of saline. FFT EEG analysis was performed with Spike2 software and time-frequency analyses were performed using Matlab software. After completion of all experiments, electrode placements were verified and data was included from all animals with peri-amygdala placement.

Results: Vehicle-treated mice spent significantly more time in the social chamber than in the non-social one. This selective social preference was eliminated by ketamine. The power spectrum of the socially-evoked potentials was analyzed using time-frequency analyses during the choice phase (450–200 msec before entering the social and non-social sides). Power in the theta (4–8 Hz), alpha (8–12 Hz), and high gamma (80–120 Hz) ranges was significantly higher prior to entering the social side than the non-social side. Ketamine significantly reduced the difference power between social and non-social choices in theta, gamma (30–80 Hz), and high gamma ranges. Alternatively, ketamine significantly increased power in both the gamma and high gamma ranges during time spent in the social chamber. This finding was not present during the time that the subject remained in the non-social chamber.

Conclusions: These results suggest that impaired function of NMDA receptor-mediated glutamate transmission can induce social impairments and dysfunction of the amygdala, similar to the pattern seen in schizophrenia. Future studies will utilize this method to evaluate the potential mechanism of action for social dysfunction and for development of novel agents for the treatment of social impairments in schizophrenia.

Keywords: social impairment, amygdala, ketamine, EEG.

Disclosures: T. Mihara, **Part 5:** Astellas Pharma Inc.; R. Sobota, Nothing to Disclose; R. Lin, Nothing to Disclose; R. Featherstone, Nothing to Disclose; S. Siegel, Nothing to Disclose.

T36. Modulation of Fear-related Behaviours by Prefrontal Cortical Gabaergic Transmission and Its Relevance to Schizophrenia

Stan B Floresco, Patrick B Piantadosi*

University of British Columbia, Vancouver, British Columbia, Canada

Background: Individuals with schizophrenia are hypothesized to have a 'noisy' prefrontal cortex (PFC), resulting from deficient gamma-aminobutyric acid (GABA) inhibitory neurotransmission. This aspect of schizophrenic pathophysiology is thought to contribute to the cognitive deficits in the disorder. However, given that the PFC regulates emotional functioning, it is possible that neuropathological alterations in PFC GABA function may also contribute to deficits in affective regulation in schizophrenia. In particular, schizophrenic patients have been suggested to apply aberrant emotional/motivational salience to cues, often being hypo-responsive to cues that predict aversive consequences and overly sensitive to cues that do not predict reinforcement. In addition, individuals with schizophrenia inappropriately apply attentional resources to motivationally-irrelevant stimuli following repeated non-reinforced preexposure to a stimulus, as evidenced by deficits in latent inhibition. These processes can be assessed in non-human animals using analogous discriminative Pavlovian fear conditioning and latent inhibition assays. To further explore how perturbations in PFC GABA transmission may relate abnormal emotional processes in schizophrenia, the present study probed the how pharmacological reduction in PFC $GABA_A$-transmission alters the allocation of emotional/motivational salience to conditioned stimuli in rats.

Methods: Separate groups of rats were trained initially to lever press for sucrose reward on a variable-interval 60 s (VI60) schedule, after which they were subjected to one of two aversive conditioning procedures. (1) Discriminative fear-conditioning: in this task, rats were exposed to one conditioned stimulus (30 s, 9 kHz tone and cue light illumination) co-terminating with an aversive foot-shock (0.5 mA/0.5 s; CS+) and another neutral conditioned stimulus (30 s, 1 kHz tone and flashing house light) that was never paired with a foot-shock (CS−). Forty-eight hrs after conditioning, animals underwent a test session during which they were re-exposed to the CS− and CS+ while lever pressing for food on VI60 schedule. (2) Latent inhibition of conditioned fear: during the conditioning portion of this experiment, separate groups were either pre-exposed (PE) to a CS (tone and cue light) or non-preexposed (NPE), residing in the chamber for an equivalent period. At the end of the (non)preexposure period, all rats experienced three presentations of the CS paired with foot-shock. During the latent inhibition test session 48 h later, rats received presentations of the CS while lever pressing for food. For both tasks, conditioned suppression of lever pressing served as an index of learned fear. For each experiment, separate groups of rats received intra-PFC infusion of either saline vehicle or a low dose of the $GABA_A$ receptor antagonist bicuculline (50 ng) prior to either the conditioning or test phases of the task.

Results: During discriminative fear tests, control subjects applied normal emotional salience, characterized by an appropriate fear response to the aversive CS+, and little fear to the neutral CS−. In contrast, reducing PFC $GABA_A$-transmission prior to the conditioning or test phase of discriminative fear eliminated the ability to discriminative between the CS+ and CS−, in a manner striking similar to that observed in schizophrenic patients. Both treatments reduced fear in response to the CS+, whereas only pre-conditioning infusions also elevated fear to the CS−, suggesting that abnormal hyperresponsivity to a neutral cue is related to deficient GABA function during acquisition, but not recall. With respect to latent inhibition, controls learned the irrelevance of a preexposed stimulus, showing reduced fear to CS presentation during latent inhibition tests. Blockade of $GABA_A$-receptors prior to conditioning had no effect on latent inhibition, and actually enhanced fear in animals that were non-preexposed to the CS. In contrast, latent inhibition was abolished following GABA-blockade prior to the latent inhibition test.

Conclusions: Taken together, these deficits suggest that PFC GABA transmission is critical for adaptive behavior instilled by, and resultant from, aversive conditioning. Of particular note, these findings show that reducing PFC GABA transmission alters aversive condition in a manner that bears a striking resemblance to abnormalities observed in schizophrenia. As such, these data suggest that such emotional abnormalities observed in the disorder may be causally related to PFC GABA deficiency, predisposing these individuals to aberrant attributions of emotional/motivational salience to environmental stimuli.
Keywords: prefrontal cortex; GABA; fear conditioning; schizophrenia; emotional regulation.
Disclosures: S. Floresco, **Part 1:** Contract work for Pfizer Inc., **Part 4:** Contract work for Pfizer Inc., not related in any way to the data presented in this poster.; P. Piantadosi, Nothing to Disclose.

T37. Central CRTH2/GPR44, a Second Prostaglandin D2 Receptor, Mediates Emotional Impairment in the Lipopolysaccharide and Tumor-induced Sickness Behavior Model

Hitoshi Hashimoto*, Ryota Haba, Norihito Shintani, Yusuke Onaka, Hiroyuki Hirai, Kin-ya Nagata, Masataka Nakamura, Akemichi Baba

Laboratory of Molecular Neuropharmacology; iPS Cell-based Research Project on Brain Neuropharmacology and Toxicology, Osaka, Japan

Background: The physiological and pathophysiological roles of prostaglandins in the central nervous system have been extensively studied, and include regulation of sleep and wakefulness, body temperature, pain and inflammation. Nevertheless, their significance in higher brain functions remains a hotly debated topic. In the periphery, the chemoattractant receptor-homologous molecule expressed on T helper type 2 cells (CRTH2, also known as GPR44) mediates the allergic response and has been identified as a second prostaglandin D_2 receptor, yet its central function is not known. In this study, we sought to determine the role of CRTH2 in the brain.
Methods: Conspecific male wild-type ($CRTH2^{+/+}$) and CRTH2-deficient ($CRTH2^{-/-}$) mice, obtained from intercrossing CRTH2 heterozygous mice were intraperitoneally injected with lipopolysaccharide (LPS) or subcutaneously injected with murine colon 26 adenocarcinoma, and their social interaction and object exploratory behavior were recorded. Immunohistochemical staining was performed on coronal brain sections with antibodies against-c-Fos, COX-2, CD31 and NeuN.
Results: Lipopolysaccharide (LPS)-induced decreases in social interaction and novel exploratory behavior were observed in $CRTH2^{+/+}$ mice but not $CRTH2^{-/-}$ mice, although both genotypes similarly showed hypolocomotion and anorexia following LPS injection. Colon 26 inoculation, a more pathologically relevant model, induced decreases in social interaction and novel exploratory behavior in $CRTH2^{+/+}$, but not $CRTH2^{-/-}$ mice. In addition, the CRTH2 antagonist CAY10471, reversed impaired social interaction and novel exploratory behavior after either LPS or tumor inoculation in $CRTH2^{+/+}$ mice. Finally, LPS-induced c-Fos expression in neurons in the central amygdala was completely abolished in $CRTH2^{-/-}$ mice.
Conclusions: These results suggest that under the conditions modeled by LPS injection or tumor inoculation, prostaglandins produced act on CRTH2 in brain regions, including the central amygdala, which may be involved in the regulatory pathway of emotional aspects of sickness behavior, such as social interaction and exploration behaviors. Thus, our study provides the first evidence that central CRTH2 is a key molecule regulating specific emotional behaviors, and offers CRTH2 antagonism as a promising potential mechanism with therapeutic relevance for relief of behavioral symptoms associated with tumors and infectious diseases.
Keywords: CRTH2, GPR44, prostaglandin D2, emotional impairment, social interaction.
Disclosures: H. Hashimoto, Nothing to Disclose; R. Haba, Nothing to Disclose; N. Shintani, Nothing to Disclose; Y. Onaka, Nothing to Disclose; H. Hirai, **Part 5:** Bio Medical Laboratories, Inc.; K. Nagata, **Part 5:** Bio Medical Laboratories, Inc.; M. Nakamura, Nothing to Disclose; A. Baba, Nothing to Disclose.

T38. Timing May Matter: Vulnerability and Resilience to Acute Trauma Vary According to the Circadian Phase at Which Exposure Occurs

Hagit Cohen*, Shlomi Cohen, Aleksander Mathé, Joseph Zohar

Ben-Gurion University, Beer-Sheva, Israel

Background: The HPA-axis displays a characteristic circadian pattern of corticosterone release with lower levels during the onset of the inactive phase and higher levels during the active phase. Since corticosterone levels may influence the response to stress and thus could influence the susceptibility to and/or the severity of stress-related sequelae, this study examined the effects of acute psychological trauma applied at different circadian phases on patterns of behavioral stress responses and on the prevalence rates of PTSD-like responses.
Methods: Rats were exposed to predator scent stress (PSS) at the beginning of the inactive phase or at the beginning of the active phase. Behaviors in the elevated plus-maze and acoustic startle response tests were assessed 7 days post-exposure, and served for retrospective classification into behavioral response groups. Circulating corticosterone concentration was assessed and the PVN expression of the neuropeptide Y (NPY) and NPY-Y1 receptor was assessed by immunohistochemical technique. The interplay between behavioral responses, corticosterone and NPY/NPY-Y1R was assessed. The dexamethasone suppression test was used to assess feedback inhibition of the HPA axis.
Results: The time of day of PSS-exposure markedly affected the pattern of behavioral stress responses and the prevalence rates of extreme behavioral responders: exposure at the onset of the active phase significantly reduced the percentage of extreme-responders as compared to exposure at the onset of the inactive phase. Corticosterone decay rate after PSS was significantly lower during active phase.

Endogenous NPY/NPY-Y1R expression in the PVN area were found to vary significantly in relation to the circadian cycle, showing a sharp rise at the onset of the active phase and a sharp decline before the inactive onset. The observed decline in NPY expression in response to stress was of less consequence during the active-phase than in the inactive phase.
Conclusions: The circadian phase at which traumatic stress impacts significantly affects the character of behavioral stress response. At the beginning of the active phase animals appear more resilient to stress and at the onset of the inactive phase more vulnerable. This effect is associated with endogenous expression of NPY/NPY-Y1R.
Keywords: posttraumatic stress disorder, animal model, corcadian cycle, neuropeptide Y, corticosterone.
Disclosures: H. Cohen, Nothing to Disclose; S. Cohen, Nothing to Disclose; A. Mathé, Nothing to Disclose; J. Zohar, Nothing to Disclose.

T39. Variations in a Stress Paradigm on Top of Early Interference with the Expression of Multiple Genes Leads to Disparate Behavioral Responses in Rats—A Possible Role for Essential Amino Acids

Eyal Asor, Avi Avital, Ehud Klein, Dorit Ben-Shachar*

Rambam Health Care Campus and Technion, IIT, Haifa, Israel

Background: It is currently accepted that complex behavior and mental disorders results from a combination of biological susceptibility and exposure to environmental stimuli. However, most of the gene-environment interaction models focus on the interaction between environmental stimuli and several candidate genes. We suggest an alternative approach of interference with the expression of multiple genes followed by exposure to environmental insults, which better reflects real life.
Methods: Early interference with gene transcription was performed by treatment of 7 days old Wistar male rats for 4 days with the Sp1/DNA binding inhibitor, mithramycin. Environmental insult was mimicked by exposing these rats during adulthood (34 days) to sub-chronic (12 days) or chronic stress (28 days). The effects of mithramycin and stress treatment on the behavioral response and serum corticosterone levels were assessed. Monoamines and amino acids levels were analyzed by HPLC in brain and serum. RNA was extracted from the right prefrontal cortex for whole genome cDNA array analysis. Various in silico analyses were performed by Genome studio software, Ingenuity Pathways Analysis (IPA) and metabolic pathway analysis. Candidate genes were validated by qRT-PCR and Western blotting.
Results: Exposure of mithramycin treated rats to sub-chronic stress led to anxious behavior in the open field test, high startle response, low sucrose preference, indifference to novel objects and high serum corticosterone levels. Whereas their exposure to chronic stress normalized sucrose preference, startle response and serum corticosterone, and induced novelty seeking behavior and reduced anxiety. Thus, depending on stress duration, two opposite 'apathetic' anhedonic *vs* 'daring' and novelty seeking phenotypes were obtained. In saline treated rats extension of stress duration led to behavioral and hormonal adaptation to stress. Data from the in-silico analyses pointed at brain amino acids. Indeed, exposure of mithramycin treated rats to chronic stress (combined treatment), but not sub-chronic stress, led to a significant decrease in brain tryptophan levels and in mRNA and protein levels of its transporter LAT1 (large neutral amino acid transporter), which was associated with reduced serum tryptophan/branched chain amino acid ratio. No change was observed in serotonin or its metabolite 5-HIAA.
Conclusions: This study suggests that postnatal temporal interference with multiple gene expression can induce hyper-responsiveness to later in life environmental insults, the features of which impacts the phenotypic outcomes. Essential amino acids may serve as a key link between the genetic background and the environment. They can be responsible for the behavioral changes, as they are the building blocks of proteins, precursors of neurotransmitters and are also important regulators of the mTOR complex, which integrates inputs from many environmental stimuli. The data of the present study suggest that our experimental paradigm may be used to model gene—environmental interaction in the etiology of mental disorders and may pave the way to new treatment strategies.
Keywords: Gene-environmental interaction; Sp1; Stress; Essential amino acids; Rats; Mental disorder.
Disclosures: E. Asor, Nothing to Disclose; A. Avital, Nothing to Disclose; E. Klein, Nothing to Disclose; D. Ben-Shachar, Nothing to Disclose.

T40. Neural Disconnectivity and Loss of Connections Symmetry in Brains of Mice Knockout for the Neurodevelopmental Gene Ahi1

Amit Lotan*, Tzuri Lifschytz, Omer Lory, Gadi Goelman, Bernard Lerer

Hadassah Hebrew University Medical Center, Jersualem, Israel

Background: The Abelson helper integration site 1 (*AHI1*) gene plays a pivotal role in brain development. Studies by our group and others have demonstrated association of *AHI1* with schizophrenia and autism. Recently, we showed that *Ahi1* heterozygous knockout ($Ahi1^{+/-}$) mice displayed an anxiolytic-like phenotype across different converging modalities. Using behavioral paradigms that involve exposure to environmental and social stress, decreased anxiety was evident in open field, elevated plus maze and dark light box, as well as during social interaction in pairs. Assessment of core temperature and corticosterone secretion revealed a significantly blunted response of the autonomic nervous system and the hypothalamic-pituitary-adrenal axis in Ahi1 + /− mice exposed to environmental and visceral stress. On resting-state functional MRI based on seed-voxel correlation maps, connectivity of the amygdala with other brain regions involved in processing of anxiogenic stimuli and inhibitory avoidance learning, such as the lateral entorhinal cortex, ventral hippocampus and ventral tegmental area (VTA), was significantly reduced in the mutant mice. Upon these results, we hypothesized that

alterations in the strength of functional connections within a specific integrated circuit underlie the anxiolytic-like phenotype displayed by the knockout mice. The present work describes a complementary and more global approach to examining between-group differences in functional connectivity based upon connections between predefined regions of interest (ROIs).

Methods: *Acquisition*. Mice ($n = \sim 20$/genotype) were anesthetized with isoflurane (1.5–2.0%) and a 30 : 70 O_2:N_2O mixture. Mice were placed inside a water heated surgical bed while respiration rate (65 ± 5)/min was continuously monitored. Data was collected with a 4.7T Bruker BioSpec scanner (Bruker Biospin Ettlington, Germany) using a passively RF decoupled surface coil, 1.2-cm in diameter (Doty Scientific, Columbia, SC, USA), and a birdcage transmission coil (Bruker Corp.). Functional BOLD contrast MRI was collected with EPI-FID (TR = 3.5, TE = 20 ms, 200 repetitions, matrix = $128 \times 128 \times 12$, FOV = $1.8 \times 1.8\,cm^2$, 1 mm slice width). *Pre-processing*. Analysis was performed using custom-made IDL software and SPM8 software[1]. It included: (i) Motion and slice time correction. (ii) regressing out the 6 motion functions created in step (i). (iii) Spatial smoothing by a 3-point-Gaussian kernel and band pass filtering (0.01–0.1 Hz) and (iv) normalization to the Paxinos mouse brain atlas[2]. *Network analysis*. 71 ROIs (16 cortical and 55 non-cortical) were preselected in the mouse atlas covering most of the limbic related structures. Correlations between the predefined ROIs were obtained by calculating the Pearson correlation coefficients between all possible pairs and applying the Fischer's z transformation. Using this data, a second order random effect analysis between subjects was undertaken. Only connections with correlation values significantly higher or lower than the global mean (passing a False Discovery Rate based cutoff) were included. Significant connections whose mean values were above the global mean were termed positive-correlation connections, and those with mean values below the global mean value were termed negative-correlation connections.

Results: Connectivity maps generated between predefined ROIs indicated that significant connections with positive correlation coefficients, which typically represented bilateral connections, tended to be similar across genotypes. However, connections with negative correlation coefficients, which typically represented connections between cortical and subcortical structures, were significantly fewer and less symmetrical among the $Ahi1^{+/-}$ group compared to wildtype controls ($p < 0.001$).

Conclusions: Complementing previous results obtained through seed based analysis, the current approach highlights global network differences in functional connectivity. The data obtained through ROIs analysis suggests that relative to wildtype mice, the mutant mice display a global disconnectivity pattern most prominent with respect to cortico-subcortical connections. To the best of our knowledge there are no other reports describing the use of this novel method in genetically modified mouse models relevant to neuropsychiatric disorders. The high sensitivity of the method was demonstrated by its ability to detect global network alterations even for modest behavioral changes, especially when structural brain anomalies were not detected. *Ahi1* is currently assumed to play a major role in neurodevelopment. Moreover, recent data from our group suggests that altered expression of *Ahi1 in uteru* had a detrimental effect on neuronal migration. Based upon these data, we assume that during the critical neurodevelopmental period, aberrant neuronal migration in Ahi1 + /− mice leads to long-lasting changes in cortico-subcortical connectivity, ultimately leading to an endophenotype related to cognitive-emotional interactions. The current findings highlight the contribution of translational approaches to understand the genetic basis of emotional regulation and its associated neurocircuitry, with possible relevance to personality and anxiety disorders in humans.

Keywords: AHI1, anxiety disorders, resting state functional MRI, connectivity.

Disclosures: A. Lotan, Nothing to Disclose; T. Lifschytz, Nothing to Disclose; O. Lory, Nothing to Disclose; G. Goelman, Nothing to Disclose; B. Lerer, Nothing to Disclose.

T41. Epigenetic Regulation of Dopamine D2 Receptor in the Core of the Nucleus Accumbens Contributes to Addiction Liability

Shelly B Flagel*, Sraboni Chaudhury, Maria Waselus, Stanley J Watson, Huda Akil

University of Michigan, Ann Arbor, Michigan

Background: We have spent the last several years studying rats that are selectively bred for differences in locomotor response to novelty and found that these animals differ on a number of traits related to addiction. Relative to selectively-bred low-responder rats (bLRs), bred high-responder rats (bHRs) exhibit increased locomotor response to novelty, increased impulsivity, higher susceptibility to control by food- and drug-related cues, and hypersensitivity of their dopamine system. Following prolonged cocaine self-administration, bHRs are more likely to seek drug when it is no longer available and show a greater propensity to relapse. We took advantage of this unique genetic animal model to examine the epigenetic regulation of neurobiological antecedents and consequences of addiction. Here we focus on the dopamine D2 receptor, a molecule that has previously been implicated in addiction via animal models, human neuroimaging, and genetic studies.

Methods: bHR and bLR rats from the 26th generation of an in-house selective breeding colony were used. A subset of rats ($n = 6$/phenotypes) were sacrificed under basal conditions for analysis of neuromolecular antecedents of addiction. Another group of animals ($n = 6$/phenotype) were exposed to ≈ 60 days of drug self-administration wherein all rats received the same amount of cocaine. Brains were obtained after ≈ 1 month of abstinence for analysis of neuromolecular consequences of addiction. Tissue was collected in serial sections (10 μm) using a cryostat and the core of the nucleus accumbens was isolated using laser capture microdissection. A chromatin immunoprecipitation assay was used to examine levels of association of the repressive modified histone H3K9me3 at the dopamine D2 receptor gene promoter. *In situ* hybridization histochemistry was used to examine levels of D2 mRNA in the core of the nucleus accumbens. Significant differences between phenotypes under either basal conditions or following prolonged cocaine self-administration were assessed with independent *t*-tests. Correlational analyses were conducted to examine relationships between variables.

Results: Although all rats received the same amount of cocaine during the prolonged self-administration procedures, only bHRs showed addiction-like behavior. Relative to bLRs, bHRs exhibited increased drug-seeking behavior when the drug was no longer available and greater responding during drug- and cue-induced reinstatement tests. Thus, bHR rats represent an addiction-prone phenotype. Here we replicate previous findings demonstrating lower levels of D2 mRNA in the core of the nucleus accumbens in bHR rats relative to bLR rats under basal conditions. We extend these findings by demonstrating that bHRs also show enhanced association of the repressive histone, H3K9me3, at the dopamine D2 receptor gene promoter under basal conditions. Further, there was a significant negative correlation between levels of D2 mRNA and the association of H3K9me3 at D2 under basal conditions across phenotypes ($n=12$, $r=-0.9$, $P<0.0001$). Interestingly, there were no significant phenotypic differences in D2 mRNA or the epigenetic marker following prolonged cocaine exposure. However, there was a significant positive correlation between levels of association of H3K9me3 at D2 and responding during cue-induced reinstatement in brains taken following the prolonged self-administration experience ($n=12$, $r=0.8$, $P<0.001$).
Conclusions: These findings support and extend previous findings implicating dopamine D2 receptor in addiction liability. Unique to this study, however, we were able to assess pre-existing differences in D2 mRNA and epigenetic regulation of this molecule prior to drug exposure. We report that an addiction-prone phenotype exhibits lower levels of D2 mRNA in the core of the nucleus accumbens and greater levels of association of H3K9me3 at D2. A significant correlation between these variables suggests that the repressive histone is important in the regulation of D2 expression in this brain region, at least under basal conditions. These neuromolecular differences between phenotypes disappear following prolonged cocaine self-administration, despite the fact that behavioral differences persist throughout the paradigm. Perhaps of greatest interest is the significant relationship revealed between H3K9me3 at D2 and behavior during the cue-induced reinstatement test. These findings suggest that the epigenetic regulation of D2 may be a critical substrate underlying addiction liability and propensity to relapse.
Keywords: dopamine, epigenetics, nucleus accumbens, animal model, addiction.
Disclosures: S. Flagel, Nothing to Disclose; S. Chaudhury, Nothing to Disclose; M. Waselus, Nothing to Disclose; S. Watson, Nothing to Disclose; H. Akil, Nothing to Disclose.

T42. Positive Allosteric Modulation of mGluR5 Reverses the Akt Signaling Deficits in Serine Racemase Knockout Mice, a Genetic Model of Schizophrenia Due to NMDA Receptor Hypofunction

Darrick T Balu*, Shunsuke Takagi, Thomas Steckler, Jose Manuel Bartolome, Carrie K Jones, Jeffrey Conn, Joseph T Coyle

Harvard University, Belmont, Massachusetts

Background: There is substantial evidence that hypofunction of the *N*-methyl-D-aspartate receptor (NMDAR) is a core pathophysiological mechanism underlying schizophrenia. We have previously demonstrated that serine racemase knockout (SR−/−) mice exhibit neuroanatomical and behavioral similarities to schizophrenia, as well as reductions in hippocampal Akt/glycogen synthase kinase 3 (GS3K)/mammalian target of rapamycin (mTOR) signaling that can be reversed with three weeks of D-serine treatment. Traditional metabotropic glutamate receptor 5 (mGluR5) positive allosteric modulators (PAMs) enhance NMDAR activity and are currently being developed to ameliorate the NMDAR hypofunction associated with schizophrenia. However, a novel mGluR5 PAM, VU0409551 (VU551), has been developed that can selectively modulate coupling to some, but not all signaling pathways.
Methods: Wild-type (WT) mice were given once daily (q.d.) intraperitoneal injections (10 ml/kg) of vehicle (20% β-cyclodextrin in sterile water) or the mGluR5 PAM VU551 (10, 30 mg/kg) for 5 days. A separate cohort of WT and SR−/− mice were given q.d. injections of either vehicle, D-serine (150 mg/kg), or VU551 (30 mg/kg) for 5 days. All mice were sacrificed 2 h after the last injection for subsequent analyses. Plasma and cortical levels of VU551 were determined by mass spectrometry, while D-serine brain tissue content was measured using high-performance liquid chromatography. Western blot was used to measure changes in brain proteins.
Results: In WT mice, we found a dose-dependent increase in the plasma and brain levels of VU551 that was accompanied by a dose-dependent increase in the amount of phosphorylated Akt (p-Akt; active state), without affecting the total amount of Akt protein. In addition, the amount of p-GS3Kα/β and p-mTOR were increased, both of which are downstream targets of Akt. Furthermore, administration of D-serine or VU551 for 5 days to SR−/− mice reversed their deficits in Akt, GS3K, and mTOR phosphorylation, a measure of their activation.
Conclusions: These data demonstrate that impairments in Akt/mTOR signaling caused by a lack of D-serine and consequent NMDAR hypofunction, can be corrected by subchronic administration of D-serine or VU551, an mGluR5 PAM. These results support augmenting NMDAR and/or mGluR5 function as viable mechanisms by which to reverse the deficits in signaling cascades that are known to be perturbed in schizophrenia. Finally, our findings highlight the utility of a new class of mGluR5 PAMs as potential novel therapeutics for treating the cognitive and negative symptoms of schizophrenia.
Keywords: NMDA receptor, D-serine, mGluR5, schizophrenia, Akt.
Disclosures: D. Balu, Nothing to Disclose; S. Takagi, Nothing to Disclose; T. Steckler, **Part 5:** Janssen Research and Development; J. Bartolome, **Part 5:** Janssen Research and Development; C. Jones, **Part 4:** Receives research support from Bristol Myers Squibb and Astrazeneca and are inventors on composition patents protecting mGlu5 and other mGlu receptor PAMs. ; J. Conn, **Part 4:** Receive research support from Bristol Myers Squibb and Astrazeneca and is an inventor on composition patents protecting mGlu5 and other mGlu receptor PAMs. Consultant for Karuna Pharmaceutical Company.; J. Coyle, **Part 3:** Consultant for Abbvie and Envivo.

T43. Age-related Sperm DNA Methylation Changes are Transmitted to Offspring and Associated with Abnormal Behavior and Dysregulated Gene Expression

Maria H Milekic*, Yurong Xin, Anne O'Donnell, Victoria Fatemeh Haghighi, Jay A Gingrich, John Edwards, Timothy Bestor

Columbia University, New York, New York

Background: Accumulating evidence support that advanced paternal age (APA) poses an increased risk in children for psychiatric disorders such as schizophrenia (SZ), bipolar and autism spectrum disorders (ASD). There is clear evidence that *de novo* single nucleotide and copy number variations contribute to SZ and ASD, and that APA is associated with an increased rate of these types of mutations. Likewise, aging is associated with altered DNA methylation in both mammalian somatic and germ cells, and epigenetic abnormalities have been observed in the psychiatric disorders associated with APA. Accordingly, we hypothesized that DNA methylation abnormalities arising in the sperm of older fathers are inherited by the offspring and result in altered gene expression and behavior.
Methods: Old (12mo) and young (3mo) male 129SvEv mice were bred with two female (3mo) 129SvEv mice to generate old (OFO) and young (YFO) father offspring. The males were removed after 2 weeks to prevent direct contact with the offspring and the females were separated to control for maternal and litter effects. At 3mo the offspring were tested on a behavioral battery, including tasks such as open field, startle activity and prepulse inhibition. We determined DNA methylation using a whole genome sequencing approach called Methylation Mapping Analysis by Paired-end Sequencing (Methyl-MAPS) (Edwards *et al Genomic Res.* 2010). Epididymal sperm from old and young male mice ($n = 4$/group) was collected after the breeding. The brains of OFO and YFO were harvested at the end of behavioral testing. Methyl-MAPS libraries were prepared for both the fathers' sperm ($n = 4$/group), as well as from one hemisphere from OFO and YFO ($n = 4$/group). Mate-pair libraries were prepared for sequencing on the ABI SOLiD platform according to methods described by Edwards *et al* (*Genomic Res.* 2010). Data was processed and analyzed as described by Xin *et al* (*Epigenetics*, 2011). Transcriptome RNA-seq was performed on the remaining hemisphere from the same OFO and YFO samples used for Methyl-MAPS. Library construction and sequencing were performed by the Columbia Genome Center on an Illumina HiSeq2000. Off-Line Basecaller (OLB-1.9.4) was used for base calling and the pass filter reads were mapped to the mouse genome (NCBI37/mm9) using Tophat. We estimated the relative abundance (aka expression level) of genes and splice isoforms using cufflinks with default settings.
Results: To determine whether aging alters sperm DNA methylation patterns, we performed genome-wide methylation profiling of epididymal sperm DNA pooled from young or old male mice using Methyl-MAPS. Mapping the methylation difference between the two groups across the regions up- and downstream of the transcription start site (TSS) and the first, internal and last exons of 20 496 RefSeq genes, we found that old mice had a significant loss of methylation at the regions flanking the TSS compared to young males. Comparing CpG island (CGI) and non-CGI promoters, the methylation difference was more profound at the regions up- and downstream of CGI promoter, so called CGI shores. Behavioral testing of the offspring of these old and young males revealed that OFO have significantly reduced exploratory, startle amplitude and prepulse inhibition compared to YFO. Performing Methyl-MAPS on brain DNA from OFO and YFO we found that, similar to the old fathers, OFO have significantly reduced DNA methylation at the regions flanking the TSS. This difference was specific to promoter CGI shores. Because DNA methylation patterns were altered in regions known to regulate transcription, we performed transcriptome RNA-seq on the remaining hemisphere of OFO and YFO. Differential gene expression analysis revealed a significant change in the expression of genes previously implicated in ASD and mental retardation (*En2* and *CA8*), as well as genes regulating neural development (*NeuroD1*), synaptogenesis (*Cbln1* and *Cbln3*) and cell signaling (*Gabra6*).
Conclusions: Similar to the epidemiological findings in humans, increased paternal age in mice is associated with behavioral alterations in the offspring. This seems to be mediated, in part, by germ line transmission of DNA methylation abnormalities arising in the sperm of older fathers. Our whole genome methylation experiments on sperm DNA from old and young mice, revealed that there is a loss of methylation at promoter CGI shores with aging and that this specific signature is also present in the OFO. These CGI shores have been shown to contain cell-, tissue- and species specific DNA methylation differences (Irizarry, Nat. Genet, 2009), which are associated with gene expression. RNA-seq on brains from OFO and YFO revealed significant changes in genes implicated in ASD, and known to regulated neural development and synaptic functions. These findings indicate novel pathways and mechanisms that may contribute to ASD and SZ and which may eventually lead to the development of new and more effective therapeutic interventions.
Keywords: paternal age, DNA methylation, gene expression, schizophrenia, autism, mouse model.
Disclosures: M. Milekic, Nothing to Disclose; Y. Xin, Nothing to Disclose; A. O'Donnell, Nothing to Disclose; V. Haghighi, Nothing to Disclose; J. Gingrich, Nothing to Disclose; J. Edwards, Nothing to Disclose; T. Bestor, Nothing to Disclose.

T44. Single Prolonged Stress Decreases Sign-tracking Conditioned Responses and Attenuates Cue-induced Reinstatement of Cocaine-seeking Behavior

Christopher Fitzpatrick, Terry E Robinson, Jonathan D Morrow*

University of Michigan, Ann Arbor, Michigan

Background: Prolonged, uncontrollable stress induces anhedonia-like decreases in reward-seeking behaviors. An internal desire to obtain reward can motivate appetitive behavior; however, many reward-seeking behaviors are primarily instigated and maintained by reward-associated, conditioned cues. A Pavlovian conditioned approach (PCA) procedure can be used to separate these two aspects of

reward-seeking behavior by physically separating the conditioned stimulus (CS) from the reward location. In this paradigm, cue-directed behavior (ie, sign-tracking conditioned responses [CRs]) can be measured separately from reward-directed behavior (ie, goal-tracking CRs).Sign-tracking and goal-tracking appear to be mediated by somewhat different neural mechanisms, eg sign-tracking is dopamine-dependent while goal-tracking is not. In this experiment, we sought to test whether the effects of prolonged stress on reward-seeking behavior may be mediated primarily through a reduction in cue-driven behavior, as opposed to a reduction in behavior driven by primary rewards.

Methods: Sprague-Dawley rats underwent a single prolonged stress (SPS) procedure, which consists of 2-h restraint, 20-min forced swim, and brief anesthesia with ether. Control rats were left undisturbed in a novel room for an equivalent time period. The rats then underwent 5 training sessions using a PCA procedure in which a retractable lever served as a cue or 'sign' predicting delivery of a food reward. Each test session consisted of 25 trials, and each trial during a test session consisted of presentation of the lever (CS) into the chamber for 8 s. Retraction of the lever was immediately followed by the response-independent delivery of one food pellet (US) in the food receptacle. Following PCA training, the rats were implanted with indwelling jugular venous catheters for IV cocaine delivery. Rats were then trained to self-administer cocaine by nose-poking for IV delivery of cocaine paired with a cue light. Following self-administration, rats underwent extinction, during which nose-pokes no longer had any programmed consequences. Finally, rats were tested for cue-induced reinstatement by reinforcing nose-pokes with presentation of the cue light, but no cocaine delivery. In order to assess the effects of SPS on dopaminergic activity, an additional group of rats underwent SPS or the undisturbed control procedure, and after a 2-week incubation period, basal and evoked dopamine levels were measured by microdialysis in the nucleus accumbens.

Results: In the PCA paradigm, SPS caused selective decreases in cue-directed (sign-tracking) behavior without affecting behavior directed toward the reward itself (goal-tracking). In addition, SPS did not affect the acquisition or extinction of cocaine self-administration, but significantly attenuated cue-induced reinstatement of cocaine-seeking behavior after extinction. Preliminary results from microdialysis suggest that SPS lowered evoked dopamine responses without affecting basal levels of dopamine in the nucleus accumbens.

Conclusions: These results suggest that SPS decreases reward-seeking behavior primarily by diminishing the motivational properties of predictive cues, rather than by reducing motivation for rewards themselves. This may be because SPS causes long-term reductions in evoked dopamine responses, which would affect cue-directed sign-tracking behavior more than the relatively dopamine-independent goal-tracking responses directed toward primary rewards.

Keywords: addiction; reward; autoshaping; anhedonia; stress.

Disclosures: C. Fitzpatrick, Nothing to Disclose; T. Robinson, Nothing to Disclose; J. Morrow, Nothing to Disclose.

T45. Empathic Fear Responses in Mice are Triggered by Recognition of a Shared Experience

Jeff Sanders*, Mark Mayford, Dilip V Jeste

Emory Univeristy, Atlanta, Georgia

Background: Empathy is an important capacity that involves the ability to recognize and share emotions with others. Empathy for others is facilitated by having had a similar prior experience. It increases with the intensity of distress that observers believe is occurring to others, and is associated with acute emotional responses to witnessing others' distress. We sought to develop a mouse model of human empathy that modeled these characteristics.

Methods: We recorded the freezing of an 'observer' mouse while it witnessed the experience of a 'subject' mouse. We studied four experimental groups: (1) SH_{obs} = observers that received footshocks in context A on day 1, and then observed footshocks given to a subject in context B on day 2. (2) SHN_{obs} = observers that received footshocks in context A on day 1, and then observed a subject in context B, where footshocks were not delivered on day 2. (3) SW_{obs} = observers that underwent forced swim stress on day 1, and then observed footshocks given to a subject in context B on day 2. (4) Naïve = observers that remained in their homecage on day 1, and that observed footshocks given to a subject in context B on day 2. Footshocks were delivered within a contextual fear conditioning session consisting of 120 s of free exploration followed by six non-signaled foot shocks (duration 1 s, intensity 0.7 mA) with an interstimulus interval of 15 s for a total duration of 216 s.

Results: All groups showed low levels of freezing during a 120 s baseline period in context B ($\sim$0–6%). SH_{obs} behaved significantly different from all other groups during the subsequent period when they observed cagemates receiving footshocks (main effect for group ($F(3,52) = 12.1$, $p < 0.001$). During footshock observation SH_{obs} froze significantly more than baseline levels and SH_{obs} freezing increased with consecutive shocks delivered to subjects ($F(6,312) = 2.97$, $p < 0.05$), reaching up to 40%. SH_{obs} froze more in the 5 s interval immediately after witnessing subject footshock, with lower freezing during the remaining 10 s ($p < 0.05$). In contrast, none of the other groups showed freezing levels different from baseline. Minimal freezing in SHN_{obs} excluded the recognition of contextual cues as a source of observer freezing. Observer freezing was also minimal in SW_{obs}, ruling out a non-specific effect of heightened anxiety from a prior stressful experience. As an added control observers witnessed a subject in context B where shocks were delivered, but where the subject was protected from shock by a thin barrier placed upon the shock grid (SH_{obs}-Block). SH_{obs}-Block did not show freezing different from baseline. Therefore, we also ruled out observer freezing as an artifactual response to cues coming from the footshock equipment.

Conclusions: Our experiments indicate that SH_{obs} freezing was specifically triggered by their recognition of a shared footshock experience with subject mice. Our assay models several aspects of human empathy in mice. These include emotional responses in observer mice that were modulated by a shared life experience with subjects and by the intensity of aversive experience occurring to subjects. These

also include acute emotional responses to observing the aversive experience of others. This model may allow for an improved understanding of neurobiological systems for the ability to recognize and share emotion with others, a core feature of empathy that is oftentimes impaired in clinical disorders.
Keywords: model, empathy, fear, emotion, mouse.
Disclosures: J. Sanders, Nothing to Disclose; M. Mayford, Nothing to Disclose; D. Jeste, Nothing to Disclose.

T46. Selective Removal of Parvalbumin Interneurons from Striatal Networks to Model the Pathophysiology of Tourette Syndrome

Meiyu Xu, Vladimir Pogorelov, Lina Li, Christopher Pittenger*

Yale University, New Haven, Connecticut

Background: Although they constitute only about 1% of the neurons in the striatum, the parvalbumin interneurons (PV-positive interneurons) critically regulate striatal function. A recent postmortem study revealed a reduction in PV-positive interneuron number in patients with severe Tourette syndrome (TS), suggesting a link between interneuron pathology and the symptoms of TS. Despite the importance of PV interneurons in striatal information processing, experimental tools to selectively manipulate this cell population, without disrupting other striatal cell types, are limited. We have achieved the targeted cell type-specific and temporally controlled ablation of striatal PV -positive neurons in adult mice, thereby generating a potential model of circuit dysfunction in TS.
Methods: We engineered a recombinant adeno-associated virus (AAV) using the FLEX system to express the diphtheria toxin receptor (DTR) in specific interneuronal subtypes in transgenic mice. In this extremely flexible system, interneuronal specificity is defined by the transgenic cre-expressing mouse line used, while regional specificity is defined by the brain region into which the virus is infused. We ablated PV interneurons in the dorsal striatum and characterized the cellular, neurochemical, and behavioral consequences.
Results: Infusion of this virus into the dorsal striatum of PV-cre transgenic mice renders the PV-expressing interneurons susceptible to ablation by systemic diphtheria toxin (DT) administration, while neighboring neurons and glia do not express DTR and are resistant to ablation. Since ablation is triggered by a systemic injection after recovery from surgery, behavioral analysis can be performed before and after ablation without the confounding effects of intervening surgery or anesthesia. Pilot experiments suggest that this ablation produces an increased stereotypical grooming after stress as compared to control group of mice, mimicking worse tics observed in patients with TS after stress of life event.
Conclusions: These experiments seek to confirm the causal conncetion between a deficit in parvalubmin-expressing interneurons, which has been observed in human postmortem tissue from individuals with TS, and the phenomenology of the disorder. By so doing they establish a novel animal model with clear construct validity, grounded in the pathophysiology of the disorder. This model is likely to be fruitful in subsequent examinations of downstream pathphysiological events and may guide the development of novel therapeutics.
Keywords: tourette syndrome; basal ganglia; striatum; interneuron; parvalbumin; tic; animal model.
Disclosures: M. Xu, Nothing to Disclose; V. Pogorelov, Nothing to Disclose; L. Li, Nothing to Disclose; C. Pittenger, Nothing to Disclose.

T47. Effects of Prenatal and Postnatal Hypoxia on Brain Derived Neurotrophic Factor Signaling in Mice

Anilkumar Pillai*, Kristy R Howell, Sarah Mehta

Georgia Regents University, Augusta, Georgia

Background: Obstetric complications such as hypoxia have been implicated in the pathophysiology of neuropsychiatric disorders including schizophrenia. Studies in rodents have shown that chronic hypoxia leads to anatomical changes such as volume loss, decreased myelination, and enlarged ventricles often observed in preterm babies. Although a large number of genes have been shown to be regulated by hypoxia challenge, the Brain derived neurotrophic factor (BDNF) is a potent hypoxia-regulated gene. BDNF is a neuroprotective molecule, and alterations in both BDNF and its receptor TrkB have been found in subjects with schizophrenia. In the present study, we examined the effects of prenatal as well as postnatal hypoxia on BDNF signaling in mice.
Methods: Pregnant dams were exposed at embryonic day 17 (E17) for the prenatal hypoxia experimental paradigm. Postnatal hypoxia was performed on pups of age P4–P11. The hypoxia protocol used was 9% Oxygen for 2 h in each design. BDNF and TrkB protein levels were determined at 3 months of age via western blotting. Statistical analysis between groups was performed by unpaired two-tailed Student's *t* test. Data are presented as means ± S.E.
Results: We found that prenatal hypoxia induced significant increase in TrkB levels in hippocampus where as no change was found in frontal cortex. In contrast, postnatal hypoxia induced increases in TrkB levels in both frontal cortex and hippocampus. We found significant reductions in truncated TrkB levels in frontal cortex and hippocampus following postnatal hypoxia. Interestingly, BDNF protein levels were higher in frontal cortex and hippocampus of prenatal hypoxia-induced animals. These changes were accompanied by alterations in cortisol as well as glucocorticoid receptor levels in the above mice.
Conclusions: Prenatal and postnatal hypoxia have differential effects on BDNF/TrkB signaling in mice. Thus, alteration in BDNF signaling might be a potential mechanism for hypoxia-induced neurochemical as well as behavioral changes associated with schizophrenia.
Keywords: hypoxia, BDNF, schizophrenia, animals.
Disclosures: A. PILLAI, Nothing to Disclose; K. Howell, Nothing to Disclose; S. Mehta, Nothing to Disclose.

T48. Cocaine-induced Adaptations in Alpha2delta-1 Calcium Channel Subunit in Nucleus Accumbens Contribute to Cocaine-induced Drug Seeking

Sade Spencer*, Robyn M Brown, Gabriel Quintero, Yonatan M Kupchik, Kathryn Reissner, Peter W Kalivas

Medical University of South Carolina, Charleston, South Carolina

Background: Chronic use of drugs of abuse produces enduring neuroadaptations in brain reward circuitry believed to contribute to compulsive relapse. Specifically, chronic cocaine induces enduring pathological changes in cortico-accumbens glutamatergic synaptic plasticity that underlie relapse in animal models. The cellular mechanisms mediating cocaine-induced synaptic adaptations are partly understood, with studies revealing enhancement of prefrontal cortex (PFC) release probability, changes in glutamate receptor expression and currents, and alterations in dendritic spine morphology in nucleus accumbens (NAc). Many of the enduring neuroadaptations after chronic drug exposure may be regulated by voltage gated calcium channel (VGCC) activity including changes in release probability and excitability. Recently, a role for the VGCC auxiliary subunit $\alpha_2\delta$-1 in drug reward has been suggested as $\alpha_2\delta$-1 is induced by noncontingent administration of multiple drugs indicating it might represent a common mechanism between different classes of abused substances. The $\alpha_2\delta$-1 subunit modulates VGCC kinetic properties and regulates channel trafficking and stability. Moreover, $\alpha_2\delta$-1 serves Ca^{2+} channel-independent functions including promoting excitatory synaptogenesis via astrocyte-secreted thrombospondin (TSP) proteins with possible implications for drug-induced morphological plasticity. Gabapentin (GBP) binds with high affinity to $\alpha_2\delta$-1 and antagonizes binding of endogenous ligands. Importantly, intracerebroventricular GBP blocks methamphetamine-induced sensitization and conditioned place preference (CPP), via binding to $\alpha_2\delta$-1. In the present study, we hypothesized that $\alpha_2\delta$-1 signaling would be upregulated by cocaine self-administration and postulated that disrupting this signaling with gabapentin treatment would inhibit reinstatement.

Methods: Adult male Sprague-Dawley rats ($\sim$300 g) were trained to self-administer cocaine on an FR1 schedule (2 h per day) where responses on an active lever resulted in a drug infusion (0.2 mg) paired with a discrete light and tone cues followed by extinction training. A yoked saline group received saline infusions noncontingent on their responses. For comparison of protein levels, rats were rapidly decapitated and $\alpha_2\delta$-1 protein levels were determined by Western Blot. To clarify the role of $\alpha_2\delta$-1 in reinstated cocaine-seeking behavior, microinjections of GBP or aCSF were made through bilateral guide cannulas aimed at the nucleus accumbens core (NAcore) prior to reinstatement trials.Cocaine priming injections (10 mg/kg, i.p.) and/or cocaine conditioned cues were used to reinstate active lever responding after extinction. Novelty- and cocaine- induced locomotor activity was assayed to control for gabapentin's effects on locomotion. Whole cell patch clamp electrophysiology was performed in NAcore on acute slices from cocaine-trained or yoked saline animals with GBP washed onto the slice. All experimental protocols in animal studies were approved by the Institutional Animal Care and Use Committee at MUSC and were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Results: Gabapentin protein was increased in the NAcore after cocaine self-administration and extinction compared to yoked saline controls ($T_{(33)} = 2.087$, $^*p = 0.045$). Novelty induced locomotor activity was not affected by GBP (10 μg or 33 μg) microinjected to NAcore, but GBP reduced cocaine-induced locomotor activity at the higher concentration (two-way ANOVA with repeated measures, interaction $F_{(23, 460)} = 3.67$, $p < 0.001$; $n = 12$ for aCSF, $n = 10$ for gabapentin). Accordingly, the lower 10 μg GBP dose was used to assay effects on reinstatement. GBP to the NAcore reduced cocaine-primed reinstatement (10 mg/kg i.p.) but did not alter cue-induced reinstatement (one-way ANOVA with repeated measures, $F_{(2, 23)} = 22.55$, $p < 0.001$; $n = 13$). Slice electrophysiology revealed that GBP inhibited glutamate release in cocaine-extinguished rats but not in yoked saline controls (two-way ANOVA with repeated measures, interaction $F_{(1, 14)} = 11.24$, $p = 0.0047$; $n = 5$ cells saline, $n = 4$ cells cocaine), and the paired pulse ratio (PPR) was selectively increased in cocaine-extinguished rats (two-way ANOVA with repeated measures, interaction $F_{(1, 14)} = 4.88$, $p = 0.0442$; $n = 5$ cells saline, $n = 4$ cells cocaine).

Conclusions: Here we show that the VGCC $\alpha_2\delta$-1 subunit is elevated in NAc after cocaine self-administration and extinction. When we acutely antagonize TSP binding to $\alpha_2\delta$-1 with gabapentin we observe a decrease in reinstated cocaine-seeking behavior initiated by a cocaine priming injection but not cocaine-conditioned cues. Importantly, gabapentin's effects on drug-seeking at this dose were not due to a general depression of spontaneous or cocaine-induced locomotor activity. At the electrophysiological level, GBP's reduction of evoked EPCS in cocaine-trained rats compared to yoked saline controls is consistent with an increased sensitivity to GBP. Moreover, the increased PPR indicates that a presynaptic mechanism is contributing to these effects. These data indicate that $\alpha_2\delta$-1 may contribute to cocaine-reinstated drug-seeking, and identify this protein as a potential target for the development of cocaine relapse medications.

Keywords: cocaine, reinstatement, gabapentin, Alpha2Delta-1, calcium channel.

Disclosures: S. Spencer, Nothing to Disclose; R. Brown, Nothing to Disclose; G. Quintero, Nothing to Disclose; Y. Kupchik, Nothing to Disclose; K. Reissner, Nothing to Disclose; P. Kalivas, Nothing to Disclose.

T49. Chronic Prenatal Kynurenine Elevation in Rats: A Naturalistic Model of Schizophrenia with Biochemical Abnormalities and Deficits in Hippocampal-mediated Learning and Memory

Robert Schwarcz, Ana Pocivavsek*, Greg I Elmer, John Bruno

University of Maryland School of Medicine, Baltimore, Maryland

Background: Schizophrenia (SZ), a catastrophic psychiatric disorder, results from a combination of genetic

and environmental factors. Kynurenic acid (KYNA), an endogenous antagonist of α7 nicotinic acetylcholine (α7nACh) and NMDA receptors, has been implicated in the pathology of SZ. Thus, brain and CSF KYNA levels are increased in SZ; genetic links exist between KYNA metabolism and SZ; and acute KYNA elevations cause cognitive dysfunction in rodents. As SZ has developmental origins, and as both α7nACh and NMDA receptors play important roles in neurodevelopment and cognition, we recently began to investigate the long-term consequences of perinatal elevations in brain KYNA levels in rats. In light of encouraging results (Eur. J. Neurosci., 35: 1605–1612; 2012; Neuroscience, 238: 19–28, 2013), we now explored the critical time period for KYNA elevation in greater detail. In the present study, KYNA levels were experimentally increased during (1) the prenatal period, ie from gestational day (GD) 15 to GD 22 or (2) adolescence/young adulthood, ie from postnatal day (PD) 42 to PD 49.
Methods: For the prenatal paradigm, the KYNA precursor L-kynurenine (kyn) was added daily to chow fed pregnant rats (100 mg/day) (control: ECon; kyn-treated: EKyn). On the last day of treatment, KYNA levels in the forebrain of embryos were significantly elevated (ECon: 1344 ± 233 fmoles/mg protein, EKyn: 7654 ± 656 fmoles/mg protein, $n = 7$ litters per group). For the manipulation in adolescence/young adulthood, 300 mg kyn/kg/day were added daily to chow (control: AdCon; kyn-treated: AdKyn). On the last day of treatment, ie PD 49, hippocampal KYNA levels were elevated (AdCon: 76 ± 21 fmoles/mg protein, AdKyn: 443 ± 44 fmoles/mg protein, $n = 7$ per group). Upon termination of the treatment, all animals were fed normal rodent chow until testing at PD 56–85 (Econ and EKyn) and PD 70–85 (AdCon and AdKyn).
Results: KYNA levels were significantly elevated in the hippocampus of EKyn offspring (ECon: 47 ± 4 fmoles/mg protein, EKyn: 104 ± 24 fmoles/mg protein, $n = 4$–6 litters/group), but no such differences were observed between AdCon and AdKyn animals (AdCon: 60 ± 9 fmoles/mg protein, AdKyn: 52 ± 5 fmoles/mg protein, $n = 6$ per group). Behavioral testing in adult rats was performed using the passive avoidance paradigm (PAP) and the Morris water maze (MWM). Prenatal kyn treatment caused significant PAP deficits, evidenced as decreased avoidance latency during the retention trial (ECon: 157 ± 32 s; EKyn: 43 ± 10 s; $n = 7$ litters per group), and increased escape latency to find the hidden platform across days in the MWM ($n = 7$ litters per group). In contrast, adult kyn manipulation did not cause PAP deficits (AdCon: 151 ± 29 s, AdKyn: 153 ± 30 s; $n = 15$ per group) or impairment in the MWM ($n = 11$–12 per group).
Conclusions: Collectively, these studies provide evidence that chronic KYNA elevation during the prenatal period, but not in adolescence/young adulthood, induces abnormal brain KYNA production and hippocampus-related cognitive dysfunctions later in life. Increased KYNA formation during a vulnerable stage in brain development may therefore play a significant role in the pathophysiology of SZ.
Keywords: cognitive deficits, development, hippocampus, kynurenic acid.
Disclosures: R. Schwarcz, **Part 1:** Research grants/contracts from: Mitsubishi-Tanabe, Bristol-Myers-Squibb and Lundbeck, No personal income, **Part 4:** Research grants/contracts from: Mitsubishi-Tanabe, Bristol-Myers-Squibb and Lundbeck,; A. Pocivavsek, Nothing to Disclose; G. Elmer, Nothing to Disclose; J. Bruno, Nothing to Disclose.

T50. Evidence for a Novel Role of Acid Sensing Ion Channel, Asic1a in the Molecular Biology of Mood and Anxiety

James R Shoblock*, Natalie Welty, Yi Liu, Changlu Liu, Timothy Lovenberg, Guang Chen

Janssen Pharmaceutical R&D, LLC, San Diego

Background: The acid sensing ion channel ASIC1a is highly expressed in the CNS, especially in limbic regions. Although brain pH is well buffered, it could be transiently lowered locally at the synapse during synaptic activity since neurotransmitter vesicles are maintained at a pH of about 5.5. In addition, several endogenous potentiators of ASIC1a exist, such as nitric oxide, corticosterone, cytokines, dynorphin, and arachidonic acid. Interestingly, many of these molecules have been implicated in mood disorders. Furthermore, human MRS studies suggest that lower brain pH occurs in patients with mood disorders. ASIC1a blockade causes an elevation of activated, phospho-ERK in cortical neurons; similar effects are known to be induced by BDNF, acute ketamine treatment, and chronic lithium or valproate treatment. Altogether, this suggests that an overactive ASIC1a channel could contribute to mood disorders. Indeed, ASIC1a was upregulated in post-mortem depressed brain and preclinical data suggest that the knockout (KO) have an antidepressant- and anxiolytic- like phenotype. The aim of the present study was to confirm and expand on these findings.
Methods: The acid sensitivity of and effect of psalmotoxin-1 (PcTx1) on mouse ASIC1a (mASIC1a) were studied using QPatch electrophysiology. Wild type (WT) and fully backcrossed ASIC1 KO mice, and effects of PcTx1 treatment were studied in a battery of depression, mania, or anxiety related tests including the tail suspension, social interaction in novel chamber under bright light, amphetamine-induced hyperactivity and sensitization, and lipopolysaccharide (LPS, a bacteria endotoxin)-induced behavioral despair and pleasure seeking deficit paradigms.
Results: mASIC1a currents were activated with a pH50 of 6.6. PcTx1 blocked acid-induced currents with an IC50 of ~1 nM. PcTx1 significantly reduced the immobility times in the tail suspension test (TST). KO mice displayed significant lower immobility compared to those of WT, an effect additive with the anti-immobility effects of imipramine. LPS induced significant increases in immobility times and a reduction of sexual pleasure seeking activities in the female urine sniffing test. KO mice treated with LPS were similar to non-LPS treated control WT mice. KO mice displayed significant more social activity in the social interaction test. The KO had a slightly, but significantly, decreased sensitized amphetamine response on day 5.
Conclusions: Our data replicate that PcTx1 is a potent ASIC1a antagonist and can produce anti-depressant-like effects in the TST, a behavioral effect that can also be induced by ketamine, scopolamine, and lithium. KO mice also displayed anti-depressant-like effects in the TST. These data further indicate that the anti-depressant-like effects of

PcTx1 and ASIC1a gene deletion are unlikely due to off target effect of PcTx1 or developmental effects of ASIC1a gene deletion. Recent data showed that ketamine can reverse LPS-induced increases in behavioral despair and reduction in pleasure seeking activity. Given the concern on the theoretical and predictive validities surrounding the TST, we further examined anti-depression-like activity of ASIC1a deletion in the LPS model and found that the deletion effectively reduced the level of behavioral despair and reduction of pleasure seeking activity. Considering that LPS causes cytokine elevation and that cytokines stimulate ASIC1a activity, our KO data suggest a notion that ASIC1a is one of the sufficient mediators in LPS-induced depression-like deficits. KO mice displayed more social interaction in an anxiety-provocative condition (bright lighting), suggesting that ASIC1a might also be involved in fear and anxiety. Amphetamine-induced locomotor sensitization is considered to simulate the pathogenesis process of bipolar/mania. We found that ASIC1a deletion produced modest calming effects in this model. Thus, the clinical observations, confirmed previous preclinical findings, and new evidences from our current studies are coherent support for the role of ASIC1a in the mood regulation.

Keywords: ASIC1a, anxiety, depression, mania, phenotype.

Disclosures: J. Shoblock, **Part 1:** Employee of Janssen Research & Development, L.L.C., **Part 2:** Employee of Janssen Research & Development, L.L.C., **Part 3:** Employee of Janssen Research & Development, L.L.C., **Part 4:** Employee of Janssen Research & Development, L.L.C., **Part 5:** Employee of Janssen Research & Development, L.L.C.; N. Welty, **Part 1:** Employee of Janssen Research & Development, L.L.C., **Part 2:** Employee of Janssen Research & Development, L.L.C., **Part 3:** Employee of Janssen Research & Development, L.L.C., **Part 4:** Employee of Janssen Research & Development, L.L.C., **Part 5:** Employee of Janssen Research & Development, L.L.C.; Y. Liu, **Part 1:** Employee of Janssen Research & Development, L.L.C., **Part 2:** Employee of Janssen Research & Development, L.L.C., **Part 3:** Employee of Janssen Research & Development, L.L.C., **Part 4:** Employee of Janssen Research & Development, L.L.C., **Part 5:** Employee of Janssen Research & Development, L.L.C.; C. Liu, **Part 5:** Janssen Research & Development, L.L.C.; T. Lovenberg, **Part 1:** I am a full time employee of Janssen Pharmaceutical R&D, **Part 2:** I am a full time employee of Janssen Pharmaceutical R&D, **Part 3:** I am a full time employee of Janssen Pharmaceutical R&D, **Part 4:** I am a full time employee of Janssen Pharmaceutical R&D, **Part 5:** I am a full time employee of Janssen Pharmaceutical R&D; G. Chen, **Part 1:** Janssen employee, **Part 2:** Janssen employee, **Part 3:** Janssen employee, **Part 4:** Janssen employee, **Part 5:** Janssen employee.

T51. Repeated Administration of an Acetylcholinesterase Inhibitor Attenuates Nicotine Taking in Rats

Adrian Arreola, Blake Kimmey, Laura Rupprecht, Alycia Lee, Matthew Hayes, Heath D Schmidt*

University of Pennsylvania, Philadelphia, Pennsylvania

Background: Nicotine craving and cognitive impairments represent core symptoms of nicotine withdrawal that predict relapse in abstinent smokers. These findings suggest that cognitive-enhancing medications may prevent drug craving and relapse, in part, by reversing or normalizing nicotine withdrawal-induced cognitive impairments. Acetylcholinesterase inhibitors (AChEIs) are FDA-approved to treat cognitive deficits in patients with Alzheimer's disease. Previously, we demonstrated that acute administration of the AChEIs galantamine and donepezil attenuates nicotine taking and seeking in rats. However, chronic studies of AChEI administration on nicotine taking are needed in order to model the dosing regimen that is likely to be prescribed to smokers attempting to quit. Therefore, the goal of these experiments was to determine the effects of repeated AChEI administration on nicotine self-administration in rats, an animal model of voluntary nicotine taking in human smokers.

Methods: Rats were trained to self-administer intravenous infusions of nicotine (0.03 mg/kg/0.59 ml) on a fixed-ratio 5 (FR5) schedule of reinforcement. Once rats maintained stable nicotine taking, galantamine (0, 0.5 and 5.0 mg/kg, i.p.) was administered 20 min prior to 10 consecutive daily nicotine self-administration sessions.

Results: Repeated administration of 5.0 mg/kg galantamine attenuated nicotine taking in rats. In order to determine if the effects of repeated galantamine generalize to other reinforced behaviors, a separate group of rats was trained to self-administer sucrose pellets. No differences in sucrose self-administration were noted between rats pretreated with galantamine when compared to saline treated controls. Malaise symptoms, including nausea and vomiting, are commonly reported adverse effects of AChEIs administration in humans. Therefore, the effects of repeated galantamine administration on *ad libitum* food intake and pica, an animal model that is used to assess rodent consumption of non-nutritive substances (ie kaolin clay) in response to emetic agents, were tested in a separate cohort of rats. No effects of repeated galantamine administration on pica or normal feeding behavior were observed.

Conclusions: Taken together, these results indicate that repeated AChEI administration attenuates nicotine taking and that these effects are not due to adverse malaise-like symptoms.

Keywords: smoking, nicotine, self-administration, cognition, acetylcholine.

Disclosures: A. Arreola, Nothing to Disclose; B. Kimmey, Nothing to Disclose; L. Rupprecht, Nothing to Disclose; A. Lee, Nothing to Disclose; M. Hayes, Nothing to Disclose; H. Schmidt, Nothing to Disclose.

T53. Effects of Pharmacogenetic Manipulation of the Nucleus Accumbens on Neuronal Activity and Alcohol-related Behaviors

Angela Ozburn*, Ryan Logan, Puja Parekh, Jake Bosin, Colleen A McClung

University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania

Background: Chronic alcohol intake leads to long lasting changes in reward- and stress-related neuronal circuitry. The nucleus accumbens (NAc) is an integral component of

this circuitry. Promising clinical trials have shown that deep brain stimulation of the NAc decreases alcohol craving and relapse in alcohol dependent subjects. Here, we used a cutting edge pharmacogenetic approach to induce activity in the NAc to reduce alcohol-related behaviors in mice. We used the mutagenized muscarinic G protein-coupled receptors hM3Dq and hM4Di that are selectively activated by the pharmacologically inert and orally bioavailable drug, clozapine-N-oxide (CNO). We tested the ability of these channels to change NAc activity and assessed the effects of altered NAc activity to alter binge-like alcohol drinking, tastant intake, and reward.

Methods: Mice were stereotaxically injected with AAV2 hSyn-HA hM3Dq, -hM4Di, or -eGFP bilaterally into NAc. Experiments were carried out to verify CNO induced changes in NAc activity (via *ex-vivo* whole cell electrophysiological recordings). We tested the effect of altering NAc activity on binge ethanol intake (or intake of sucrose, quinine, and water) using the drinking in the dark paradigm ($n = 9$–10/group). We also evaluated the effects of altering NAc activity on the rewarding properties of ethanol using conditioned place preference ($n = 7$–10/group).

Results: CNO increased NAc firing in hM3Dq positive cells and decreased firing in hM4Di cells, confirming the ability of these channels to spatially and temporally alter neuronal activity. Increasing NAc activity significantly decreased binge drinking ($p < 0.05$) without altering intake for other tastants. Increasing NAc activity is not rewarding and altering NAc activity does not change the rewarding properties of ethanol.

Conclusions: These experiments demonstrate that neuronal activity can be controlled in a spatial and temporal manner using pharmacogenetics. We find that increasing NAc activity decreases binge drinking without altering the rewarding properties of ethanol. Ongoing experiments aim to identify the the effects of altering NAc activity on the aversive properties of ethanol, and identifying transcriptional changes induced by this pharmacogenetic manipulation. These findings could have promising implications for treatment.

Keywords: DREADD, CNO, alcohol, nucleus accumbens.

Disclosures: A. Ozburn, Nothing to Disclose; R. Logan, Nothing to Disclose; P. Parekh, Nothing to Disclose; J. Bosin, Nothing to Disclose; C. McClung, Nothing to Disclose.

T54. Re-exposure to Nicotine After Chronic Nicotine Exposure and Withdrawal Potentiates Reward Responsiveness in Rats: Implications for Relapse

Andre Der-Avakian*, Manoranjan S D'Souza, Diego A Pizzagalli, Athina Markou

University of California San Diego, La Jolla, California

Background: Tobacco smoking is one of the leading causes of disability and mortality worldwide, yet most smokers are unable to abstain from and quit smoking. Changes in reward processing are hypothesized to play an important role in relapse amongst abstinent smokers. We recently demonstrated that nicotine withdrawal diminished reward responsiveness in rats and humans. Reward responsiveness (ie, the propensity to modulate behavior as a function of prior reinforcement experience) was assessed using a translational behavioral task originally developed for humans and recently adapted for use in rats (Der-Avakian *et al*, *Translational Psychiatry*, in press). The aim of the present study was to determine whether acute nicotine re-exposure after withdrawal from chronic nicotine administration would alter reward responsiveness in rats.

Methods: Male Wistar rats ($n = 34$) were trained in a signal detection task to discriminate two tones varying in duration. Correct discriminations were reinforced with food pellets. Rats were then exposed to either nicotine (6.32 mg/kg/day, base) or saline delivered for 28 days via subcutaneous osmotic minipumps. Two to eight weeks after termination of chronic nicotine or saline administration, the effects of acute nicotine administration (0, 0.125, 0.25, 0.5 mg/kg, base, sc, administered in a Latin-square design) were assessed during test sessions. A probabilistic reinforcement schedule was introduced during test sessions such that one tone (rich) was reinforced three times more frequently than the other tone (lean). Increased reward responsiveness was defined as an increased response bias for the rich stimulus irrespective of which stimulus was presented.

Results: Acute nicotine administration dose-dependently decreased response bias for the rich stimulus (ie, decreased reward responsiveness) in rats previously exposed to chronic saline administration. Conversely, acute nicotine administration dose-dependently increased response bias for the rich stimulus in rats previously exposed to chronic nicotine administration, reflecting enhanced reward responsiveness induced by acute nicotine in rats with previous nicotine experience.

Conclusions: The potentiation of reward responsiveness by acute nicotine in rats two to eight weeks after chronic nicotine exposure and withdrawal suggests that chronic nicotine exposure induces a long-lasting dysregulation of the processing of natural rewards. This condition may contribute to relapse to tobacco smoking during withdrawal of nicotine in order to re-instate responsiveness to natural rewards, and to experience potentiated nicotine-induced reward responsiveness. Thus, treatment of deficits in reward responsiveness in abstinent smokers may facilitate smoking cessation.

Keywords: nicotine, reward, relapse, anhedonia, animal model, rat.

Disclosures: A. Der-Avakian, Nothing to Disclose; M. D'Souza, Nothing to Disclose; D. Pizzagalli, **Part 1:** Dr Pizzagalli has received contract research support from Advanced Neurotechnology North America and honoraria/consulting fees from AstraZeneca, Ono Pharma USA, Servier, and Shire for studies unrelated to this project over the past 2 yrs., **Part 4:** Dr Pizzagalli has received contract research support from Advanced Neurotechnology North America for studies unrelated to this project over the past 2 yrs.; A. Markou, **Part 1:** Dr Markou has received contract research support from Bristol-Myers Squibb Co., Forest Laboratories and Astra-Zeneca and honoraria/consulting fees from AbbVie for studies unrelated to this project over the past 2 yrs., **Part 4:** Dr Markou has received contract research support from Bristol-Myers Squibb Co., Forest Laboratories and Astra-Zeneca for studies unrelated to this project over the past 2 yrs.

T55. Effects of Maternal Separation on Depressive- and Anxiety- Like Behaviors and Cardiovascular Function in Stress-susceptible Rats

Ilan A Kerman*, Samir Rana, Nateka Jackson, Phyllis C Pugh

University of Alabama Birmingham, Birmingham, Alabama

Background: Adverse life experiences during the early developmental period have been shown to have long-term effects on brain, behavior, physiological, and autonomic system in various animal species. Wistar Kyoto (WKY) rats are a proposed model of neuropsychiatric disorders that exhibit depressive and anxiety like behavior with enhanced stress reactivity. This study utilized maternal separation paradigm, a model of adverse early life experience on stress prone WKY rats to investigate the long-term effects on behavior and cardiovascular parameters. The purpose of the study was to investigate the long term effects of maternal separation (180 min separation) *vs* handling (15 min separation) on the behavior and cardiovascular system.
Methods: Rat pups were separated from their dams either for 15 (MS15) or 180 (MS180) minutes from postnatal (P) days 1 through 14. When the rats reached adulthood (P55), all animals were subjected to a battery of behavioral tests including: novelty-suppressed feeding, open field, elevated plus maze, and social interaction tests to assay anxiety-like behavior. In addition, we utilized forced swim test to measure depressive like behavior. The animals were then implanted with radiotelemetry probes to assess cardiovascular functioning, including arterial pressure, heart rate, and heart rate variability.
Results: Adult MS15 animals exhibited a number of enhanced depressive- and anxiety- like behaviors as compared to their MS180 counterparts, including: diminished rearing frequency in the open field, increased latency to sniff and consume food in the novelty-suppressed feeding test, decreased social interaction, and increased learned helplessness on the forced swim test. Cardiovascular recordings revealed a number of alterations in the MS15 rats that are reminiscent of those observed in major depression. These included: increased baseline heart rate, potentiated stress-induced tachycardia in response to a novel cage presentation, and a decrease in heart rate variability. MS15 rats also manifested an increase in pulse pressure during the active phase, while no differences in activity levels were detected.
Conclusions: Extensive evidence supports the notion that early life experience has significant impact on adult behavior and physiology. The current study is the first one to demonstrate significant differences in depressive- and anxiety- like behaviors along with cardiovascular function in adulthood in rats with an inborn predisposition to stress in response to early-life manipulations. Importantly, these behavioral and physiological differences recapitulate several features of major depressive disorder.
Keywords: heart rate, learned helplessness, variability, autonomic, social interaction.
Disclosures: I. Kerman, Nothing to Disclose; S. Rana, Nothing to Disclose; N. Jackson, Nothing to Disclose; P. Pugh, Nothing to Disclose.

T56. Spontaneous Nicotine Withdrawal Enhanced Anxiety-like Behavior in the Fear-potentiated Startle Procedure in Rats

Xia Li*, Athina Markou, Victoria Risbrough

University of California San Diego, La Jolla, California

Background: Anxiety is a common symptom of nicotine withdrawal in humans, and may predict the inability to abstain from tobacco smoking. Thus, it is important to understand the neuropsychological basis of increased anxiety during nicotine withdrawal in animal models with predictive validity for anxiolytic compounds. Our previous findings indicated that spontaneous nicotine withdrawal selectively potentiated stress responding to anxiogenic stimuli in the light-enhanced startle (LES) procedure in rats that assesses unconditioned fear responding. In the present studies, we tested the hypothesis that nicotine withdrawal would also modulate conditioned fear responses.
Methods: The fear-potentiated startle (FPS) procedure in rats was used to examine the effects of nicotine withdrawal on conditioned fear responses. This procedure was adapted to allow for the detection of both context-elicited and cue-elicited anxiety-like responses. This test was chosen based on its similarity to FPS procedures used in humans where both context-elicited and cue-elicited anxiety responses can be observed.
Results: In rats, we found that the anxiolytic buspirone (1 and 3 mg/kg, subcutaneously), a 5-HT_{1A} receptor partial agonist, significantly inhibited startle reactivity to cued, but not context, fear in the FPS procedure. Conversely, the anxiolytic diazepam (1 and 3 mg/kg, intraperitoneally), a $GABA_A$ allosteric modulator, significantly inhibited startle reactivity to context, but not cued, fear in the FPS in rats. Varenicline (0.3, 1 and 3 mg/kg, intraperitoneally), a beta2 nicotinic acetylcholine receptor partial agonist and an FDA-approved medication used to treat smoking addiction, had no effect on context or cued potentiated startle in nicotine naïve rats. Most interestingly, spontaneous nicotine withdrawal robustly increased startle reactivity during cued fear, but had no effect on context fear.
Conclusions: Our findings indicate that the FPS test has predictive validity for standard anxiolytic compounds and may be a useful test to examine the effects of nicotine withdrawal on anxiety and discover potential new treatments. Like contextual and cued fear potentiated startle in humans, this test may discriminate between anxiolytic/anxiogenic effects across contextual and cued fear constructs, processes that are subserved by different mechanisms.
Keywords: anxiety, nicotine, withdrawal, fear-potentiated startle.
Disclosures: X. Li, Nothing to Disclose; A. Markou, **Part 1:** Consulting fee from AbbVie., **Part 4:** Research contract from Bristol-Myers-Squibb, Forest Laboratories and AstraZeneca.; V. Risbrough, **Part 4:** Research grants at UCSD from Johnson and Johnson, Omerons and Sunovian Pharmaceuticals.

T57. Long-term Modulation of Memory and Emotion after a Systemic Inflammatory Event

Natalie Tronson*, Ian Speirs

University of Michigan, Ann Arbor, Michigan

Background: Activation of the innate immune system by chronic illness or injury is correlated with depression, anxiety and PTSD. In patients of heart attack (myocardial infarction, MI), rates of major depression and PTSD diagnoses are two- and three-fold higher, respectively, than the population at large. In addition, many patients report lasting cognitive impairments after MI or major surgery. The role of systemic inflammation in triggering lasting changes in cytokine expressio, mood and memory remain unknown. Furthermore, the intracellular signaling cascades mediating the effects of cytokine-mediated memory dysregulation and depression-like behavior remains unknown.
Methods: We used a surgical model of heart attack (myocardial infarction, MI) in male and female C57Bl6 mice to trigger systemic inflammation in order to investigate the role of sustained peripheral inflammation on learning, memory, and depression-like behaviors. Mice with MI, Sham, or no surgery underwent fear conditioning, sucrose-preference, or anxiety tests 2, 4 or 8 weeks after surgery. After behavioral testing, we dissected hippocampus and hypothalamus and used immunoassays to determine levels of cytokines, and activation of JAK/STAT pathways.
Results: Male mice exhibited enhanced context fear conditioning 2 weeks after MI, but impaired context fear conditioning 8 weeks after MI. Male sham operated mice were not significantly different from non-operated controls. In contrast, both MI and Sham operated female mice exhibited impaired fear conditioning 8 weeks after MI, but showed no initial enhancement of a fear-associated memory. Interestingly, the increased susceptibility of female mice to the long term consequences of surgery was consistent for depression-like behavior, with decreased sucrose preference observed only in MI male mice, but was observed in both Sham and MI females. Interestingly, JAK/STAT pathway was increased in hippocampus of both male and female mice soon after surgery, but female mice additionally exhibited increased serine phosphorylation of STAT.
Conclusions: Thus we demonstrate bidirectional changes in memory after MI, and differential cytokine expression and JAK/STAT signaling in males and females. Importantly, females seem resiliant to early changes in memory, but more susceptible to the later impairments. These findings suggest that cytokine-dependent signaling in the brain after systemic inflammation is a candidate mechanism for susceptibility to, or development of depression, PTSD, and long lasting cognitive impairments. Furthermore, qualitatively different processes may mediate changes in mood and memory in females and males. These findings identify new potential targets for prevention of post-illness depression and cognitive impairments, and highlight the need for investigations diverging treatment options for males and females.
Keywords: Sex differences, cytokines, fear conditioning, hippocampus, JAK/STAT.
Disclosures: N. Tronson, Nothing to Disclose; I. Speirs, Nothing to Disclose.

T58. Selective Enhancement of Cue-induced Motivation in Obesity Prone *vs* Resistant Rats is Accompanied by Sensitization to Cocaine and Increased Striatal AMPA Receptor Expression

Carrie R Ferrario*, Cameron Nobile, Michael JF Robinson, Kent Berridge

University of Michigan Medical School, Ann Arbor, Michigan

Background: It is well established that while the decision to eat is shaped strongly by hunger, satiety, and energy demand, it is also greatly influenced by external environmental cues associated with food (food-cues). For example, in humans exposure to food-cues, like the smell of brownies or a blinking donuts sign, can increase ratings of desire to eat, and the amount of food consumed (Fedoroff *et al*, 1997; Soussignan *et al*, 2012). Similarly, in rodents food-cues can elicit approach, reinforce operant responding, and increase food consumption (Kelley *et al*, 2005; Holland and Petrovich, 2005; Cardinal *et al*, 2002; Balleine, 2005). Recent studies suggest that obesity prone people are more susceptible to these motivational effects of food-cues. In addition, exposure to food-cues activates the striatum (a brain region important for reward and motivation) more strongly in obese *vs* non-obese people (Rothemund *et al*, 2007; Stoeckel *et al*, 2008), even prior to weight gain (Demos *et al*, 2012). This differential activation may be driven in part by differences in AMPA receptor (AMPAR) levels as AMPARs provide the main source of excitatory transmission in the striatum, play a role in behavioral responses to food-cues, and are increased after exposure to sugar (Crombag *et al*, 2008; Peng *et al*, 2011; Tukey *et al*, 2013). The recent global rise in obesity heightens the need to understand the neurobiological mechanisms governing these processes, as enhanced neurobehavioral sensitivity to food-cues may contribute to weight gain and hamper weight loss, particularly in susceptible individuals. The goals of the work presented here were: (1) to determine whether obesity prone rats show enhanced motivation for cues paired with sugar (2) to examine alterations in mesolimbic function related to obesity *vs* exposure to a sweet and fatty 'junk-food' diet (3) to determine whether striatal AMPAR expression is increased in obesity prone *vs* resistant rats.
Methods: Two models of individual susceptibility to obesity were used: outbred male Sprague-Dawley rats given free access to a sweet and fatty 'junk-food' diet and male rats selective bred for their propensity or resistance to diet induced obesity (Taconic DIO/DR; Levin 1997). The 'junk-food' diet consisted of a mash of standard lab chow, potato chips, chocolate chip cookies, peanut butter and powdered chocolate milk. Prior to any diet manipulation, rats were trained to associate a discrete cue with delivery of a sucrose pellet (45 mg) using established Pavlovian conditioned approach procedures. After prolonged exposure to the 'junk-food' diet in outbred rats or without any diet manipulation in selectively bred rats, we then examined: (1) motivation for the sucrose-paired cue in a test of instrumental conditioned reinforcement (2) the locomotor response to systemic cocaine administration and (3) differences in AMPA receptor surface expression using a BS^3 crosslinking procedure.

Results: Some of the rats fed the 'junk-food' diet readily gained weight and became obese, whereas others remained the same weight as chow fed controls. In selectively bred rats, obesity prone rats were generally heavier than obesity resistant rats even when fed a standard chow diet. We found that both obese 'junk-food' diet fed and selectively bred obesity prone rats were more willing to work for a presentation of the cue paired with sucrose compared to non-obese 'junk-food' diet fed and obesity resistant rats. In addition, obese and obesity-prone rats showed a sensitized locomotor response to cocaine compared to non-obese and obesity-resistant rats. In outbred obese rats, sensitization was seen after 'junk-food' diet was removed and rats were given ad lib access to standard lab chow only for 2 weeks, whereas sensitization was evident in selectively bred obesity prone *vs* resistant rats without any diet manipulation. Finally, surface expression of AMPAR subunit GluA1 was increased in the nucleus accumbens (NAc) of obese *vs* non-obese outbred rats, while GluA2 protein expression remained unchanged.
Conclusions: Recent studies in humans suggest that enhanced neural and behavioral responses to cues that are paired with food may hamper weight loss and promote weight gain in susceptible individuals. Here we show that obesity prone rats are more willing to work for a cue paired with sucrose than obesity resistant rats using two complimentary models of individual susceptibility to obesity. Obesity prone rats were also more sensitive to the locomotor activating effects of cocaine compared to obesity resistant rats, consistent with enhanced reactivity of mesolimbic systems. In addition, prolonged exposure to a 'junk-food' diet followed by a return to standard lab chow for two weeks was associated with a selective increase in surface GluA1 protein expression only in obese rats. The up-regulation of GluA1 in obese rats in the absence of changes in GluA2 may be indicative of calcium-permeable AMPARs, which play a role cue-induced drug craving (Loweth *et al*, 2013; Wolf and Ferrario, 2010). These data will be discussed in light of the potential contribution of enhanced glutamatergic transmission and sensitization of incentive-motivation to obesity.
Keywords: obesity, AMPA receptor, motivation, striatum, sensitization.
Disclosures: C. Ferrario, Nothing to Disclose; C. Nobile, Nothing to Disclose; M. Robinson, Nothing to Disclose; K. Berridge, Nothing to Disclose.

T60. Ultra-high Magnetic Field (9.4 Tesla) Magnetic Resonance Imaging Reveals Neuroanatomical and Neurochemical Homologies between Schizophrenia and the Serine Racemase Knockout Mouse

Matthew D Puhl*, Dionyssios Mintzopoulos, J Eric Jensen, Timothy E Gillis, Marc J Kaufman, Joseph T Coyle

Harvard Medical School, Belmont, Massachusetts

Background: A prominent hypothesis proposes that, in schizophrenia, activity of the *N*-methyl-D-aspartate receptor (NMDAR) is decreased compared to healthy controls. In part, this NMDAR hypofunction may be due to decreased availability of D-serine, an NMDAR co-agonist, as the genes encoding serine racemase (SR; the synthetic enzyme for D-serine), D-amino acid oxidase (DAAO; the degradation enzyme for D-serine), and the DAAO activator G72 are risk genes for schizophrenia. Evidence suggests that one of the consequences of NMDAR hypofunction is down-regulation of the fast-firing, parvalbumin-positive γ-aminobutyric acid (GABA) interneurons, resulting in disinhibition of pyramidal neurons. It is thought that these abnormalitiesdirectly relate to the negative symptoms and cognitive impairments that are hallmarks of the disorder. Furthermore, schizophrenia is characterized by neuroanatomical abnormalities, including cortical atrophy accompanied by ventricular enlargement. Given the heterogeneous symptom domains of schizophrenia, as well as the contribution of complex genetic and epigenetic factors to the presentation of the disease, it is essential that valid animal models are developed so that neural substrates and novel therapeutic targets can be identified.
Methods: Our laboratory has developed a transgenic mouse line with a constitutive deletion of exon 1 of the SR gene, which encodes the catalytic domain of the enzyme. Null mutants (SR−/−) from this line exhibit NMDAR hypofunction. Previously our laboratory demonstrated a decrease in dendritic spine density and complexity that underlies deficits in several molecular pathways mediating neuroplasticity in SR−/− mice *ex vivo*. Using ultra-high magnetic field (9.4 Tesla) *in vivo* magnetic resonance structural imaging (MRI) and ultra-short echo time proton magnetic resonance spectroscopy (MRS), the current study investigated whether the neuroanatomical and neurochemical abnormalities similar to those evident in schizophrenia also occur in SR−/− mice. SR−/− mice and wildtype (WT) controls were anesthetized with isoflurane for MRI (SR−/−: $n=8$, WT: $n=8$) and MRS (SR−/−: $n=8$,WT: $n=8$) scans.
Results: Compared to WT controls, SR−/− mice exhibited a 22% increase in ventricular volume ($p<0.05$). Additionally, in a medial frontal cortex voxel (3.0 mm × 2.5 mm × 2.0 mm), SR−/− mice exhibited significantly increased GABA/Creatine ratios (62%, $t=3.45$, $p<0.01$), similar to findings in untreated humans with schizophrenia.
Conclusions: Collectively, these data demonstrate *in vivo* neuroanatomical and neurochemical homologies between humans with schizophrenia and the SR−/− NMDAR hypofunction mouse model of the disease and suggest that the elevated GABA levels observed with MRS reflect a reduction in GABA turnover.
Keywords: magnetic resonance imaging, magnetic resonance spectroscopy, schizophrenia, serine racemase knockout mouse.
Disclosures: M. Puhl, Nothing to Disclose; D. Mintzopoulos, Nothing to Disclose; J. Jensen, Nothing to Disclose; T. Gillis, Nothing to Disclose; M. Kaufman, **Part 1:** NARSAD (Brain and Behavior Research Fund), PhotoThera, Inc., Michael J. Fox Foundation for Parkinson's Research, Air Products and Chemicals, Inc., **Part 4:** PhotoThera, Inc., Michael J. Fox Foundation for Parkinson's Research, Air Products and Chemicals, Inc.; J. Coyle, **Part 1:** Abbott, Jansen Pharmaceutical, Puretech, En Vivo, A patent owned by Massachusetts General Hospital for the use of D-serine as a treatment for serious mental illness could yield royalties for Dr Coyle.

T61. Loss of GFAP-positive Astrocytes in the Nucleus Accumbens Following Cocaine Self-administration and Extinction Is Associated with Increased IL-6 Expression

Phuong K Tran, Heather A Boger, Sade Spencer, Michael D Scofield, Peter W Kalivas, Kathryn J Reissner*

University of North Carolina Chapel Hill, Chapel Hill, North Carolina

Background: A decrease in expression and function of the high affinity astroglial glutamate transporter GLT-1 in the nucleus accumbens (NAc) has been described following exposure to multiple drugs of abuse, including cocaine, heroin, and nicotine. Further, restored expression of GLT-1 is a necessary mechanistic component of the therapeutic effects against drug seeking of N-acetylcysteine and propentofylline. Because decreased GLT-1 expression is a hallmark feature of reactive astrocytes, we sought to test the hypothesis that astrocytes in the nucleus accumbens exist in a reactive, activated state following cocaine self-administration and extinction.
Methods: Male Sprague-Dawley rats were trained to self-administer cocaine on a limited access (2 h per day) FR1 schedule of reinforcement. Upon meeting a minimal criteria of 10 days of 10 infustions or more of cocaine (0.2 mg/infusion), animals entered extinction training. Following 15 days of extinction training, analysis was performed by either Western blotting, immunohistochemistry, or ELISA assay. Fresh tissue from NAc core or dmPFC was taken for GFAP Western blots, or for cytokine ELISAs; for quantitative immunohistochemistry, animals were perfused with paraformaldehyde, and fixed-frozen brains were sliced 45 υm thick. Following immunohistochemistry for GFAP, astrocytes were blindly counted using Microbrightfield's Stereoinvestigator software. Enzyme linked immunosorbent assays (ELISA) were run to measure reactive cytokine levels for TNFa, IL1b, and IL-6 in the NAc core.
Results: Both Western blots and quantitative immunohistochemistry revealed a significant decrease in GFAP expression in the NAc core following cocaine self-administration and extinction, *vs* saline controls. Moreover, there was no evidence of hypertrophy or change in morphology of GFAP-positive astrocytes in cocaine *vs* yoked saline-administering animals. In contrast, there was no effect on GFAP in the dmPFC. Protein expression of cytokines TNFa and IL1b showed no effect of cocaine *vs* saline administration in the NAc either 24 h after the end of the last self-administration, or 24 h after the last extinction session. In contrast, IL-6 levels were significantly elevated following cocaine self-administration and extinction.
Conclusions: Previous studies have reported an increase in GFAP expression, as well as evidence of astrocyte proliferation in the NAc three weeks after seven days of i.p. cocaine injections (Bowers and Kalivas, 2003). Further, a decrease in astroglial glutamate transporter GLT-1 has been thoroughly described following both non-contingent and self-administration of multiple drugs of abuse. Thus, we tested the hypothesis that the decrease in GLT-1 expression following cocaine self-administration and extinction may represent a feature of activated astrocytes. Astrocyte activation is frequently characterized by decreased GLT-1 expression, increased GFAP expression, astrocyte hypertrophy, and increased inflammatory cytokine expression. However, we report a significant decrease in GFAP, lack of evidence for astrocyte hypertrophy, and no change in expression of cytokines TNFa or IL1b. However, levels are IL-6 are elevated by self-administration and extinction. Ongoing studies are designed to investigate the precise mechanism by which GLT-1 expression is regulated by cocaine self-administration, and the relationship between IL-6, GFAP, and GLT-1 expression.
Keywords: cocaine, astrocytes, GFAP, cytokines, nucleus accumbens.
Disclosures: P. Tran, Nothing to Disclose; H. Boger, Nothing to Disclose; S. Spencer, Nothing to Disclose; M. Scofield, Nothing to Disclose; P. Kalivas, Nothing to Disclose; K. Reissner, Nothing to Disclose.

T62. Evaluation of an Electronic Information System to Enhance Practice at a Medication-assisted Opioid Treatment Program

Lawrence Brown*, Steven Kritz, Melissa Lin, Ben Louie, Roberto Zavala, Charles Madray

START Treatment and Recovery Centers—formerly Addiction Research and Treatment Corporation, Brooklyn, New York

Background: START Treatment & Recovery Centers (formerly Addiction Research and Treatment Corporation)is an outpatient medication-assisted opioid treatment program that also provides primary medical care, including HIV/AIDS care for approximately 3000 predominantly minority adults in New York City. We received National Institute on Drug Abuse R01 funding to evaluate the implementation of an electronic health information system integrating counseling, social services, medical services, case management, HIV services, methadone dispensing, and administrative/fiscal data.
Methods: For the research aspects of this project, four domains (Quality, Satisfaction, Productivity, and Financial Performance) were evaluated utilizing a pre and post-implementation research design. A fifth domain, Risk, was dropped from the analysis due to insufficient numbers for valid statistical comparison. Once the research project was completed, the capabilities of the electronic system were utilized to better evaluate Quality, Regulatory and Contractual Compliance, and Productivity in all disciplines. This was particularly timely, given that OASAS (Office of Alcoholism and Substance Abuse Services) in New York State instituted APGs (Ambulatory Patient Groups) as their payment methodology.
Results: For the research aspects of this project, the following was found: (1) For Quality, pre-implementation annual medical assessments and annual, 30-day, and 90-day multidiscipline assessments were timely for 83 and 70%, 72, and 42% of cases, respectively. Post-implementation, timeliness of annual medical and annual multidiscipline assessments was 97 and 96%, 87, and 70% respectively, all highly statistically significant improvements. Also in the Quality domain, hepatitis C viral load was appropriately performed in 85% of cases pre-implementation and 81% post-implementation; a non-significant difference; (2) for

Satisfaction, there was no change for patients and a non-statistically significant upward trend post-implementation for staff; (3) Productivity tended to decline post-implementation; reaching statistical significance for counselors; and (4) Financial Performance (revenue per capita staff; cost per patient visit) did not change significantly. Post-research study, the ability to present provider staff with monthly reports covering Quality (opiate and cocaine-free status for $>80\%$ of patients; HIV viral load suppression; HgbA1c <7 for diabetes mellitus; and BP $<140/90$ for hypertension); Regulatory and Contractual Compliance (timely completion of behavioral and medical assessments; meeting or exceeding state targets for vocational/educational status); and Productivity (all clinical/administrative disciplines) resulted in improved performance over time.

Conclusions: Despite results that were somewhat less robust than expected in some of the domains examined for the research; our ability to exploit the electronic system capabilities to provide timely feedback in critical processes allowed us to meet the challenges presented by the change in reimbursement and documentation in New York. It will also help us to navigate the changes as a result of the Affordable Care Act.

Keywords: electronic medical record; medication-assisted opioid treatment program; pre and post-implementation design; quality.

Disclosures: L. Brown, Nothing to Disclose; S. Kritz, Nothing to Disclose; M. Lin, Nothing to Disclose; B. Louie, Nothing to Disclose; R. Zavala, Nothing to Disclose; C. Madray, Nothing to Disclose.

T63. Effects of Social Defeat Stress on Anhedonia in the Intracranial Self-stimulation Test

Rachel Donahue*, John Muschamp, Sam Golden, Scott Russo, Eric Nestler, William Carlezon Jr

Harvard Medical School, Belmont, Massachusetts

Background: Chronic social defeat stress (SDS) is a robust model of stress-related illness in mice that exploits the ethological relevance of territorial aggression. It reliably produces persistent behavioral adaptations including social avoidance, increased anxiety, and anhedonia (reduced sensitivity to reward) as assessed by decreased preference for rewarding sucrose solutions. However, it can be difficult to distinguish whether these behavioral adaptations reflect depressive-like signs. The present studies were designed to characterize the effects of chronic SDS on sensitivity to reward, since anhedonia is a core symptom of depressive disorders in humans. We further sought to identify manipulations that can reverse or systems that are involved in regulating SDS-induced depressive-like phenotypes.

Methods: To examine whether chronic SDS induces changes in reward function, we used intracranial self-stimulation (ICSS). Adult male C57BL6/J mice were implanted with lateral hypothalamic (LH) electrodes and trained using a 'rate-frequency' procedure. Once stable baselines were established, the mice were subjected to 10 consecutive days of 10-min social defeat bouts by a novel and more aggressive CD-1 mouse. We assessed ICSS thresholds following each of the defeat sessions, and during a 5-day recovery period. Chronic SDS-induced changes in ICSS were also assessed in mice with inducible ΔFosB overexpression in striatum, which have been shown to exhibit resilience to SDS. We then examined whether ketamine (2.5 or 20 mg/kg, intraperitoneal), an N-methyl-D-aspartate (NMDA) antagonist that produces rapid antidepressant responses in humans, could reverse chronic SDS-induced social avoidance and anhedonia. Finally, we examined the role of the kappa opioid receptor (KOR) system, which has been shown to play an important role in depressive- and anxiogenic-like effects in animal models of stress, in regulating SDS behavioral and molecular adaptations. We used quantitative-PCR to examine the time course of SDS-induced plasticity in KOR or prodynorphin (the endogenous KOR ligand) messenger RNA (mRNA) expression in the nucleus accumbens (NAc), and assessed the effects of SDS on ICSS in mice in which KORs are ablated selectively in dopamine-containing neurons.

Results: We found that chronic SDS significantly increases mean ICSS thresholds, indicating decreases in the rewarding impact of LH stimulation (anhedonia). This anhedonic phenotype persisted post-SDS, although to a lesser extent. Inducible ΔFosB overexpression in striatum blocks the anhedonic effects of chronic SDS in the ICSS test, consistent with a pro-resilient action of this transcription factor. A single high (20 mg/kg) but not low (2.5 mg/kg) dose of ketamine administered at the conclusion of the chronic SDS regimen ($\sim$20 h prior to social interaction testing) attenuated social avoidance in defeated mice, although this effect was transient. Ketamine administered 24 h prior to the first day of chronic SDS was not capable of reversing CSDS-induced social aversion suggesting that a single ketamine dose is not effective as a preventative treatment for chronic stress. Surprisingly, ketamine did not block chronic SDS-induced anhedonia in the ICSS test. Ongoing studies are investigating the role of the KOR system in SDS adaptations. Wild-type mice subjected to acute SDS (1-day) showed significant increases in prodynorphin mRNA expression and nominal increases in KOR mRNA expression within the NAc. In contrast, following a chronic (10-day) SDS regimen, prodynorphin mRNA expression was significantly decreased regardless of whether the mice showed a 'stress-susceptible' or 'stress-resilient' phenotype in social interaction tests. Preliminary data indicate that mice with KORs selectively ablated in dopamine neurons show signs of resilience to SDS, as indicated by delays in the onset of SDS-induced increases in ICSS thresholds across the 10-day regimen.

Conclusions: Our findings complement previous work showing that chronic SDS results in decreased preference for natural rewards, and demonstrates that ICSS is a sensitive, reliable and quantifiable method for detecting anhedonic effects of SDS over time in mice. We further demonstrate that ketamine has rapid antidepressant-like effects on some, but not all, chronic SDS-induced behavioral adaptations. Lastly, our findings raise the possibility that the endogenous KOR system mediates the effects of acute stress, but may be subject to counter-adaptations in response to chronic stress.

Keywords: social defeat stress (SDS), anhedonia, intracranial self-stimulation (ICSS), ketamine, kappa opioid receptor (KOR).

Disclosures: R. Donahue, Nothing to Disclose; J. Muschamp, Nothing to Disclose; S. Golden, Nothing to Disclose; S. Russo, Nothing to Disclose; E. Nestler, Nothing to Disclose; W. Carlezon Jr., **Part 1:** Dr Carlezon and McLean Hospital are co-owners of a patent on the use of KOR antagonists to treat depressive disorders.

T64. Toward a Bidirectional Model Animal of Bipolar Disorder: Genetic Susceptibility to Conditions That Induce Cycling Between Mania and Depression in Mice

Jared W Young*, Davide Dulcis, Jordy van Enkhuizen, Nicholas Spitzer, Andrea Grim, Mark A Geyer

University of California San Diego, La Jolla, California

Background: Bipolar disorder (BD) is a unique neuropsychiatric disorder wherein patients cycle between extreme conditions of mania and depression. Heritability of BD is high but there are also environmental contributing factors. Theories regarding the evolutionary origin of BD postulate that long daylight lengths in the summer gave rise to mania-relevant behaviors, while short daylight lengths in the winter gave rise to depression-like behaviors. This theory was supported by Dulcis *et al* (2013; Science) demonstrating that rats kept in light equivalent to long activity-periods exhibited increased risk-like preferences while rats exposed to short activity-periods exhibited increased depression-like behaviors. A key question remains whether specific genes that appear in manic and depressed patients can confer susceptibility and result in an exaggerated response to such environmental conditions. The present studies examined the effects of day-length changes on mice that exhibit dopamine transporter (DAT) levels [heterozygous (HT) mice] consistent with BD patients ($\sim$50% compared with healthy subjects). We hypothesized that relative to their wildtype (WT) littermates, these DAT HT mice would exhibit exaggerated mania-like behavior in response to long activity-periods but exaggerated depression-like behavior in response to short activity-periods.
Methods: Age- and littermate-matched male DAT HT ($n=30$) and WT ($n=19$) mice were produced from HT breeding pairs that had been backcrossed to C57BL/6J mice for more than 10 generations. At 3 months of age, these mice were housed in long-active photoperiods [LAP; 5Light(L):19Dark(D)], regular photoperiods (12L:12D), or short-active photoperiods (SAP; 19L:5D) for 2 weeks ($n=10$ or 6/7 per genotype by photoperiod). After two-weeks, the mice were tested for: (1) Depression-like behavior via immobility time in the forced swim test (FST); and (2) Risk-like preference via open arm entries in the elevated plus maze (EPM). All procedures were approved by the UCSD Institutional Animal Care and Use Committee. The UCSD animal facility meets all federal and state requirements for animal care and was approved by the American Association for Accreditation of Laboratory Animal Care.
Results: (1) FST: Photoperiod length interacted with genotype affecting immobility ($F_{(2,43)}=4.3$, $p<0.05$). While SAP-housed mice exhibited depression-like behavior (higher immobility time) compared with regular- and LAP-housed mice ($p<0.05$), this effect was exaggerated in HT compared with WT mice ($p<0.05$). (2) EPM: Photoperiod length and genotype tended to interact ($F_{(2,43)}=3.9$, $p=0.06$). While mice housed in LAP exhibited higher risk-like preference (more open arm entries) in the EPM compared with regular- and SAP-housed mice ($p<0.005$), this effect was exaggerated in HT compared with WT mice ($p<0.05$).
Conclusions: Mice with reduced DAT expression at levels comparable to those of BD patients exhibit susceptibility to the environmental impact of altered photoperiod lengths that are hypothesized to contribute to cycling in BD. Importantly, these mice were hypersensitive to environmental conditions that induce both mania- and depression-like behaviors in mice. The genetic polymorphisms of DAT associated with BD are hypothesized to be the source of reduced DAT expression in patients. The studies presented here provide evidence that the reduced DAT expression of BD patients observed using positron emission tomography studies may in fact underlie the cycling in BD, particularly in response to changing photoperiod lengths. These studies therefore provide a model animal of BD that includes cycling between mania and depression. Thus, treatments can be tested targeted specifically at mechanisms relevant to cycling in BD.
Keywords: dopamine transporter, despair, risk-taking, mice, etiology.
Disclosures: J. Young, **Part 1:** Received consultancy fees from Amgen, Received grant support from Lundbeck and Omeros, **Part 4:** Received grant support from Lundbeck and Omeros; D. Dulcis, Nothing to Disclose; J. van Enkhuizen, Nothing to Disclose; N. Spitzer, Nothing to Disclose; A. Grim, Nothing to Disclose; M. Geyer, **Part 1:** Received consulting compensation from Abbott, Acadia, Addex, Cerca, Dart, Lundbeck/Otsuka, Merck, Neurocrine, Omeros, Takeda, and Teva, Received research grant support from Intracellular Therapeutics, Johnson & Johnson, NIDA, NIMH, and the U.S. Veteran's Administration VISN 22 Mental Illness Research, Education, and Clinical Center, **Part 2:** Equity interest in San Diego Instruments, **Part 4:** Received research grant support from Intracellular Therapeutics, Johnson & Johnson, NIDA, NIMH, and the U.S. Veteran's Administration VISN 22 Mental Illness Research, Education, and Clinical Center.

T65. Adolescent Cannabis Exposure Interacts with a Glial Genetic Risk Factor to Produce Cognitive Deficits in Adulthood

Bagrat Abazyan, Sofya Abazyan, Michael Ballinger, Atsushi Kamiya, Mikhail V Pletnikov*

Johns Hopkins University, Baltimore, Maryland

Background: Gene-environment interactions (GEI) contribute to the development of major mental disorders. Adolescent cannabis exposure increases the risk of psychotic disorders and cognitive dysfunction in adulthood. Astrocytes have been implicated in mediating the cognitive effects of cannabis and other adverse environmental effects. Identification of glial expression of Disrupted-In-Schizophrenia (DISC1), a genetic risk factor for mental disorders, allows for elucidating the role of astrocytes in GEI. Thus, we evaluated how selective expression of mutant DISC1 in

astrocytes moderates the cognitive effects of adolescent cannabis exposure in mice.
Methods: Control and mutant DISC1 mice were treated daily with 8 mg/kg of Δ9-tetrahydrocannabinol (THC) for 21 days beginning at postnatal day 30. A separate cohort of control and mutant DISC1 mice was identically treated with THC and was also given doxycycline (DOX)-containing food to shut down expression of mutant DISC1 during adolescence and on. 3 weeks after the cessation of THC treatment, all mice were subjected to a series of cognitive tests followed by still on-going postmortem analyses of CB1 receptors signaling in neurons and astrocytes.
Results: Compared to all other groups, THC-treated DISC1 mice exhibited significant deficits in spatial recognition memory in Y maze, object recognition and new place recognition. Importantly, these deficits were no longer present in THC-DISC1 mice given DOX food that had prevented expression of mutant DISC1 during adolescent exposure to THC.
Conclusions: Our findings indicate that adolescent interactions of astrocytic mutant DISC1 and cannabis exposure synergistically produce cognitive deficits in adult mice. Our results suggest a new role for astrocytes in mediating adverse cognitive effects of adolescent cannabis exposure. Intriguingly, the poster presented by Kamiya's lab shows the different cognitive deficits in mice with neuronal expression of mutant DISC1 following adolescent THC exposure, pointing to cell type-specific interactions between DISC1 and CB1 signaling in inducing cognitive abnormalities resembling those in schizophrenia and related psychiatric disorders.
Keywords: schizophrenia, cannabis, adolescence, DISC1, astrocytes, cognitive deficits.
Disclosures: B. Abazyan, Nothing to Disclose; S. Abazyan, Nothing to Disclose; M. Ballinger, Nothing to Disclose; A. Kamiya, Nothing to Disclose; M. Pletnikov, Nothing to Disclose.

T66. The Interplay of Cannabinoid Signaling and DISC1 During Adolescence: Effects on Prefrontal Cortex Function in Adulthood

Michael Ballinger, Bagrat Abazyan, Yu Taniguchi, Atsushi Saito, Koki Ito, Mikhail Pletnikov, Atsushi Kamiya*

Johns Hopkins University School of Medicine, Baltimore, Maryland

Background: Adolescence is an important developmental period vulnerable to environmental stimuli capable of altering functional and structural organization of the developing brain. It is likely a critical time for the emergence of full-blown schizophrenia onset in early adulthood. Thus, despite etiological complexities for schizophrenia encompassing multiple genetic and environmental risks, exploring the molecular mechanisms of environmental influences and their convergence with genetic insults during adolescence may offer insight in understanding the etiopathophysiologies of schizophrenia. In this respect, the clinical effect of cannabis use during adolescence, which may disturb proper late brain maturation, is worth investigating as an environmental risk factor for schizophrenia. Recently, there has been substantial progress in understanding the mechanisms of endocannabinoid signaling regarding synaptic communication in the central nervous system. Furthermore, the impact of endocannabinoid signaling on brain development has been clarified. Therefore, it now becomes crucial to explore the converging molecular pathways of endocannabinoid signaling and genetic risk factors during adolescence, and to test long term consequences of adolescent cannabis exposure on adult high brain functions to conceive preclinical models of gene-environment interaction.
Methods: To this end, we utilize animal models which express the disease-associated mutant of disrupted in schizophrenia 1 (DISC1) gene, a genetic risk for major mental disorders, including schizophrenia. This mutant's expression is restricted under the control of the αCaMKII promoter in pyramidal neurons of the forebrain, including the prefrontal cortex and hippocampus which are critical brain regions for cognition and emotion. Given that cannabinoid receptor type 1 (CB1R), the most well-characterized cannabinoid receptor, is preferentially expressed in these brain regions, our mutant DISC1 animal model is a good tool to study intra-and intercellular communication of CB1R-mediated signaling and DISC1 pathways in these specific brain areas. We examine the effect of adolescent chronic treatment of delta-9-tetrahydrocannabinol (Δ^9-THC), a major psychoactive ingredient of cannabis, on cognitive behaviors in adulthood in this animal model. In addition, we investigate the effect of adolescent Δ^9-THC exposure on potential convergent mechanisms of endocannabinoid signaling and DISC1 by using molecular and biochemical approaches.
Results: We found that adolescent Δ^9-THC exposure and developmental expression of mutant DISC1 in forebrain regions impair context-dependent fear memory, but not spatial recognition memory in adulthood. Our biochemical data suggest that expression of mutant DISC1 increases synaptic CB1R expression in the prefrontal cortex, but not in the hippocampus, whereas no changes in the total expression of CB1R were observed in these brain regions.
Conclusions: To our knowledge, there are few studies investigating the potential convergence of endocannabinoid signaling and genetic risk factors for schizophrenia and none are linked to DISC1. We hope to further address molecular and physiological mechanisms of how endocannabinoid signaling and DISC1 interact to affect brain maturation and associated behavior in adulthood, which may provide us with clues to find novel targets for therapeutic intervention. Of note, Pletnikov's group observed deficits in spatial recognition memory in astrocyte-specific mutant DISC1 expression mice by adolescent cannabis exposure, which contrasts with our data using pyramidal neuron-specific mutant DISC1 mice. These data suggest that cell-type specific interaction of CB1-mediated signaling and DISC1 pathways may have independent effects on long lasting behaviors relevant to psychiatric conditions.
Keywords: endocannabinoid, adolescence, DISC1, schizophrenia.
Disclosures: M. Ballinger, Nothing to Disclose; B. Abazyan, Nothing to Disclose; Y. Taniguchi, Nothing to Disclose; A.

Saito, Nothing to Disclose; K. Ito, Nothing to Disclose; M. Pletnikov, Nothing to Disclose; A. Kamiya, Nothing to Disclose.

T67. A Zebrafish Model for the Functional Analysis of Genes in Autism

Ellen J Hoffman*, Joseph M Fernandez, Antonio J Giraldez, Matthew State

Yale University, New Haven, Connecticut

Background: Whole-exome sequencing has led to the rapid expansion of the identification of genes in autism spectrum disorders (ASD). Despite considerable clinical and genetic heterogeneity, there is emerging evidence that such risk genes converge on common pathways in nervous system development. One of the central challenges that we currently face is that we are in need of a biologically relevant *in vivo* system that will aid in the identification of these pathways. Zebrafish offer important advantages in this regard. Because of their transparent embryos and ease of genetic manipulation, zebrafish provide a means of visualizing processes in the developing nervous system, such as neuronal migration and axon fasciculation, in real-time. Moreover, with the advent of transgenic lines carrying calcium indicators, such as GCaMPs, one of the key advantages of this system is its ability to elucidate the effect of early structural changes on neuronal activity in circuits that underlie simple behaviors. Further, large progenies allow for the conduct of pharmacological screens, which have the potential to lead to the discovery of novel therapeutic targets. We generated zebrafish knockouts of four genes that are strongly associated with ASD: ***CNTNAP2*** (*Contactin Associated Protein-like 2*), ***SCN2A*** (*sodium channel, voltage-gated, type II, alpha subunit*); ***BCKDK*** (*branched chain ketoacid dehydrogenase kinase*); and ***CHD8*** (*Chromodomain Helicase DNA-binding protein 8*). *CNTNAP2* and *BCKDK* were identified as ASD susceptibility genes by homozygosity mapping in consanguineous families where deleterious mutations resulted in a monogenic syndrome of autism, epilepsy, and intellectual disability. Whole-exome sequencing led to the identification of *SCN2A* and *CHD8* as ASD risk genes. Moreover, all four genes are known to have distinct biological functions: CNTNAP2 is a cell adhesion molecule in the neurexin family; BCKDK is an enzyme involved in the metabolism of branched chain amino acids; SCN2A is a voltage-gated sodium channel; and CHD8 is a chromatin modifier. However, there is considerable evidence supporting each of these genes in ASD, and the first three have been shown to be associated with autism both with and without epilepsy. We anticipate that investigating the function of these genes in zebrafish knockouts will illuminate common biological mechanisms in ASD and provide a means of identifying novel pharmacotherapies.
Methods: We utilized zinc finger- and transcription activator-like effector nucleases (ZFNs, TALENs) to generate zebrafish carrying deleterious insertion-deletion mutations in the zebrafish orthologs of *CNTNAP2*, *BCKDK*, *SCN2A*, and *CHD8*, all of which were found to be expressed in the zebrafish central nervous system during embryonic to larval stages (24–72 h post fertilization). Where two zebrafish paralogs of the human gene were identified, we generated double knockouts by targeting both paralogs. Specifically, we utilized ZFN directed against *CNTNAP2a* (exon 3), *CNTNAP2b* (exons 2 and 4), *BCKDK* (exon 3), *SCN1lab* (exon 4), *SCN1a* (exon 6), and TALENs targeting *CHD8* (exons 8 and 22). We have conducted a battery of morphological and simple behavioral assays in zebrafish double knockouts of *CNTNAP2*. We employed *in situ* hybridization and immunohistochemistry to assess the formation of axon scaffolds, forebrain development, and the establishment of neurotransmitter systems. We used transgenic lines co-labeling excitatory and inhibitory neuron populations to visualize the development of glutamatergic and GABAergic neurons. We conducted assays of larval behavior, including acoustic startle, habituation, and optokinetic response. In addition, we are in the process of conducting large-scale quantitative behavior profiling of all knockouts.
Results: We generated zebrafish founders carrying deleterious mutations in all four genes, including the duplicated paralogs of *CNTNAP2* and *SCN2A*. We focused our initial phenotyping efforts on double knockouts of *CNTNAP2*, which survive to adulthood, are fertile, and lack gross abnormalities in CNS structure. Western blot analysis reveals the loss of protein expression in *CNTNAP2* double knockouts. Immunohistochemistry using anti-acetylated tubulin revealed delays in anterior commissure formation at 28 h post fertilization in double knockouts of *CNTNAP2* compared to wild-type fish, and a trend towards increased defasciculation of the post-optic commissure. In addition, *in situ* hybridization demonstrated decreased expression of the proneural gene, *Neurod*, and *glutamic acid decarboxylase* (*GAD*) in *CNTNAP2* knockouts at 48 hpf. *CNTNAP2* mutants do not display abnormalities in acoustic startle, habituation, or optokinetic response. We are currently conducting large-scale quantitative behavioral profiling of rest-wake cycle behaviors in all zebrafish knockout lines to identify common neural pathways involving these genes.
Conclusions: These studies lay the foundation for utilizing zebrafish to elucidate common biological pathways in genes that are strongly associated with ASD. By generating knockouts of four ASD risk genes with disparate biological functions, we anticipate that our approach will provide new insights into the function of these genes in neural circuits underlying simple behaviors and provide a clear path forward for the development of novel pharmacotherapies.
Keywords: autism, genetics, zebrafish, neurodevelopment, CNTNAP2.
Disclosures: E. Hoffman, Nothing to Disclose; J. Fernandez, Nothing to Disclose; A. Giraldez, Nothing to Disclose; M. State, Nothing to Disclose.

T68. Reduced Motivation to Consume Alcohol after an Extended Access

Eric Augier*, Ruslan Damadzic, Erick Singley, Alexandra Pincus, Markus Heilig

NIH, Bethesda, Maryland

Background: Escalation of drug use, a hallmark stage in the transition from a controlled drug use to compulsion is one of the first features of addiction demonstrated in rats with

extended access to drug. While this phenomenon has been well characterized with different pharmacological classes of drugs of abuse such as cocaine or heroin, the impact of a differential access to alcohol is mostly unknown.
Methods: In this study, we wanted to investigate if an extended access to alcohol could also promote an escalation in ethanol intake. Rats were trained to self-administer 20% EtOH under two different conditions : intermittent long access (12 h per day, 3 days a week) and short access (2 h per day).
Results: Contrary to this hypothesis, our results showed that rats with a short access to a 20% solution of ethanol self-administered significantly more alcohol that rats with an extended access. They also showed a greater motivation to consume the drug as measured using progressive-ratio.
Conclusions: These results can't be explained by a difference in the sensitivity to the hypnotic effects of ethanol as both extended and short access groups showed a similar sleep time in the loss of righting reflex paradigm following an acute injection of ethanol (3 g/kg). Finally, we investigated if these surprising observations could result from a difference in the mediation of alcohol-drinking behavior by the central nucleus of the amygdala.
Keywords: alcohol, addiction, self-administration, extended access, amygdala.
Disclosures: E. Augier, Nothing to Disclose; R. Damadzic, Nothing to Disclose; E. Singley, Nothing to Disclose; A. Pincus, Nothing to Disclose; M. Heilig, Nothing to Disclose.

T69. Effects of Perinatal and Adolescent Oxidative Stress 'Double Hit' on GABAergic Interneurons and Behavior in Mice

Susan B Powell*, Loek deJong, Mary E Kamenski, Jacinta Lucero, Jared W Young, M Margarita Behrens

University of California San Diego, La Jolla, California

Background: Decreased number of GABAergic neurons is a consistent finding in schizophrenia postmortem studies, where the subpopulation of parvalbumin-expressing (PV) fast-spiking inhibitory neurons is specifically affected. Deficits in cortical fast-spiking inhibitory systems may underlie the psychotic features and cognitive deficits associated with schizophrenia-related disorders. Our initial studies indicated that perinatal NMDA-R antagonists decreases PV-expressing GABAergic interneurons through an oxidative stress mechanism. Clinical studies suggest that a 'second hit' may be required for the full manifestation of neuropsychiatric disease. Thus, the current study examined the effects of a second oxidative stress—exposure to high levels of dopamine—during adolescence on GABAergic inhibitory neurons and behavior in mice. In the current studies, we assessed the combined effects of perinatal ketamine and adolescent exposure to the selective dopamine uptake inhibitor, GBR12909, on PV-interneurons and behaviors relevant to schizophrenia (eg, locomotor activity, startle reactivity/plasticity, and probabilistic learning).
Methods: C57BL/6J were bred in our facility. Ketamine (30 mg/kg, SC) or saline was administered on postnatal days 7, 9, and 11 (perinatal ketamine). Half of the mice received subcutaneous GBR12909 (5 mg/kg) injections on 10 consecutive days, starting at PND 35 through 44, encompassing adolescence in mice. PV-expressing neurons were labeled and counted as described in (Dugan *et al*, 2009; PLoS One 4(5):e5518). Activity wasmeasured in six 30.5×61 cm Plexiglas chambers with floor and wall holes by use of photobeams to register holepokes, horizontal activity, and rearing (Halberstadt *et al* 2009; Neuropsychopharmacology. 34(8):1958–67). Startle and PPI testing were performed in SR-LAB startle chambers, using an experimental session that was specified previously (Asp *et al* 2010; Int J Neuropsychopharmacol. 13(4):475–85). Male mice were tested in both between- and within-session probabilistic reversal-learning tasks, similar to human learning tests.
Results: Analysis of PV expression showed a decrease in PV expression associated with both perinatal ketamine and adolescent GBR ($p < 0.01$). The effect of perinatal ketamine, however, was not further decreased with adolescent exposure to GBR. The behavioral characterization of the doublehit model revealed several differences in behavior. Ketamine-treated mice, particularly females, tended to have increased PPI during the varied interstimulus interval block of prepulse trials, (perinatal treatment; $F_{(1,129)} = 7.22$, $p < 0.01$). Adolescent GBR exposure had no consistent effects on startle or prepulse inhibition. In contrast, adolescent GBR increased locomotor activity (Drug[female]:$F_{(1,55)} = 5.81$, $p < 0.05$) and investigatory behavior (Drug: $F_{(1,129)} = 4.88$, $p < 0.05$), particularly in females. In order to examine the effects of perinatal ketamine exposure on cognitive function, we tested male mice in a probabilistic reversal learning task. Mice exposed to adolescent GBR took longer to reach criterion (increased days to criterion) compared to saline-treated mice ($F_{(1,56)} = 4.27$, $p < 0.05$). Mice exposed to the 'double hit' (perinatal ketamine + adolescent GBR) showed increased premature responses in the within-session version of the task.
Conclusions: Taken together, these data support the hypothesis that adolescent exposure to the dopamine uptake inhibitor GBR12909 results in a decrease of PV in GABAergic neurons, similar to that observed with perinatal ketamine. The combination of perinatal ketamine and adolescent GBR did not further exacerbate the effect of perinatal ketamine on PV + interneurons, however. The behavioral effects of perinatal ketamine are subtle and suggest increases in PPI, particularly during the block of prepulse trials with varied interstimulus intervals. Adolescent GBR12909 exposure increased exploratory and investigatory behavior in the mouse behavioral pattern monitor and slowed learning in the between-session probabilistic learning task, indicating a potential learning deficit in this model. The 'double hit' of perinatal ketamine and adolescent GBR12909 resulted in increased premature responses in the within-session probabilistic learning task, perhaps indicating increased impulsivity. Hence, the GABAergic inhibitory system, specifically the PV + interneurons, may be uniquely sensitive to various environmental perturbations during early development. While the two manipulations on their own had some effects on behavior, the combined effects of these two 'hits', onlymanifested on measures of impulsivity.

Keywords: ketamine, animal models, oxidative stress, DAT inhibitor, GBR12909, learning, schizophrenia, GABA, parvalbumin, adolescent, perinatal.
Disclosures: S. Powell, **Part 1:** Service contract with Servier Pharmaceuticals; L. deJong, Nothing to Disclose; M. Kamenski, Nothing to Disclose; J. Lucero, Nothing to Disclose; J. Young, **Part 1:** Consultancy from Amgen, **Part 4:** Grant support from Lundbeck and Omeros; M. Behrens, Nothing to Disclose.

T70. Hyperactivity and Cortical Disinhibition in Mice with Restricted Expression of Mutant Huntingtin to Parvalbumin-positive Cells

Sarah E Dougherty, John J Hollimon, Laura J McMeekin, Andrew S Bohannon, Andrew B West, Mathieu Lesort, John J Hablitz, Rita M Cowell*

University of Alabama Birmingham, Birmingham, Alabama

Background: Recent evidence suggests that interneurons are involved in the pathophysiology of Huntington Disease (HD). Abnormalities in the function of interneurons expressing the calcium buffer parvalbumin (PV) have been observed in multiple mouse models of HD, although it is not clear how PV-positive interneuron dysfunction contributes to behavioral and synaptic deficits.
Methods: We used the cre-lox system to drive expression of mutant huntingtin (mthtt) in PV-positive neurons and evaluated motor behavior, regional and cell-specific location of mutant huntingtin expression, and cortical inhibition using whole cell patch clamp electrophysiology in mice 6–24 months of age.
Results: Mutant mice exhibited diffuse mthtt immunoreactivity in PV-rich areas at 10 months of age and mthtt aggregates in PV-positive processes at 24 months of age. At midlife mutant mice were hyperactive and displayed impaired GABA release in the motor cortex, characterized by reduced miniature inhibitory events and severely blunted responses to gamma frequency stimulation, without a loss of PV-positive interneurons. In contrast, 24 month-old mutant mice showed normalized behavior and responses to gamma frequency stimulation, possibly due to compensatory changes in pyramidal neurons or the formation of inclusions with age.
Conclusions: These data indicate that mthtt expression in PV-positive neurons is sufficient to drive a hyperactive phenotype and suggest that mthtt-mediated dysfunction in PV-positive neuronal populations could be a key factor in the hyperkinetic behavior observed in HD. Further clarification of the roles for specific PV-positive populations in this phenotype is warranted to definitively identify cellular targets for intervention.
Keywords: interneuron, cortex, Huntington disease, GABA, motor function.
Disclosures: S. Dougherty, Nothing to Disclose; J. Hollimon, Nothing to Disclose; L. McMeekin, Nothing to Disclose; A. Bohannon, Nothing to Disclose; A. West, Nothing to Disclose; M. Lesort, Nothing to Disclose; J. Hablitz, Nothing to Disclose; R. Cowell, Nothing to Disclose.

T71. Overexpression of CRF in the Central Nucleus of the Amygdala Diminishes the Dysphoric-like State Associated with Nicotine Withdrawal in Rats

Xiaoli Qi, Zhiying Shan, Yue Ji, Valerie Guerra, Jon C Alexander, Brandi Ormerod, Adrie Bruijnzeel*

University of Florida, Gainesville, Florida

Background: Tobacco addiction is a chronic relapsing disorder that is characterized by dysphoria, anxiety, and cognitive impairments upon smoking cessation. There is extensive evidence that negative affective symptoms contribute to relapse to smoking. In previous studies we showed that corticotropin releasing factor (CRF) and CRF_1 receptor activation plays a critical role in the dysphoric-like state associated with nicotine withdrawal in rats. These studies investigated whether a long-term viral vector-mediated increase in CRF levels in the central nucleus of the amygdala (CeA) affects the dysphoric-like state associated with nicotine withdrawal in rats.
Methods: Male Wistar rats (200–250 g) were prepared with intracranial self-stimulation (ICSS) electrodes in the lateral hypothalamus/medial forebrain bundle in order to study brain reward function. Elevations in ICSS thresholds reflect a dysphoric-like state. When the ICSS thresholds were stable ($<10\%$ variation over 5-day period), an adeno-associated virus (AAV, pseudotype 2/5) vector was used to overexpress CRF or green fluorescent protein (GFP, control group) in the CeA. After the administration of the viral vectors, the rats were tested daily in the ICSS test chambers for 28 days. Then the animals were prepared with minipumps that delivered nicotine (3.16 mg/kg/day, nicotine base) or saline. The nAChR antagonist mecamylamine (0.33–3.0 mg/kg, sc) was used to precipitate nicotine withdrawal. Mecamylamine was administered according to a Latin square design and the rats received the first dose 7 days after the pump implantations. In order to study spontaneous withdrawal, the minipumps were removed at day 28. The rats were tested in the ICSS procedure 6, 12, 24 36, 48, 72, and 96 h after removal of the minipumps. At the end of the experiment, the brains were removed to assess CRF and CRF receptor gene expression levels.
Results: Before the behavioral studies the viral vectors were tested. Immunostainings revealed that GFP was expressed in the CeA 2, 4, and 8 weeks after the AAV2/5-GFP infusions. This indicates that the AAV2/5 vector induces a long-term increase in transgene expression. Furthermore, the transduction efficacy of the AAV2/5-CRF vector was investigated using a primary neuronal cell cultures. The viral vector, AAV2/5-CRF, greatly increased CRF mRNA levels in primary neurons. In the behavioral experiment, the AAV2/5-CRF vector induced a 40% increase in CRF protein and mRNA levels in the CeA. There were no differences in baseline ICSS thresholds between the groups (AAV2/5-GFP-saline, AAV2/5-GFP-nicotine, AAV2/5-CRF-saline, AAV2/5-CRF-nicotine) at any time point during the experiment. The administration of mecamylamine and removal of the nicotine pumps led to elevations in ICSS thresholds, which is indicative of a dysphoric-like state. The overexpression of CRF did not affect baseline ICSS thresholds but diminished

the elevations in ICSS thresholds associated with precipitated and spontaneous nicotine withdrawal. Overexpression of CRF led to a decrease in CRF_1 mRNA levels and an increase in CRF_2 mRNA levels in the CeA.
Conclusions: The present studies show that the overexpression of CRF in the CeA diminishes the dysphoric-like state associated with mecamylamine-precipitated and spontaneous nicotine withdrawal. Furthermore, the overexpression of CRF led to a decrease in CRF_1 receptor gene expression and an increase in CRF_2 receptor gene expression. There is experimental evidence that suggests that CRF_1 receptor activation contributes to negative mood states and that CRF_2 receptor activation opposes these effects. Therefore, it is suggested that the downregulation of CRF_1 receptors and upregulation of CRF_2 receptors may have contributed to the decrease in the dysphoric-like state associated with nicotine withdrawal.
Keywords: nicotine, dysphoria, CRF, central amygdala, AAV2/5, rats.
Disclosures: X. Qi, Nothing to Disclose; Z. Shan, Nothing to Disclose; Y. Ji, Nothing to Disclose; V. Guerra, Nothing to Disclose; J. Alexander, Nothing to Disclose; B. Ormerod, Nothing to Disclose; A. Bruijnzeel, Nothing to Disclose.

T72. G-protein-dependent Signaling in Corticostriatal Afferents Regulates Locomotor Sensitization, Drug-taking and Drug-seeking Behaviors

Kerry Kerstetter, Amanda Wunsch, Tess Donckels, John F Neumaier, Susan Ferguson*

Seattle Children's Research Institute, Seattle, Washington

Background: The cortico-basal ganglia-thalamic circuit is a complex neural network involved in motivation and reward, and dysfunction of this pathway has been implicated in drug addiction. Previous work has demonstrated that the nucleus accumbens (NAc) regulates the development of locomotor sensitization to psychostimulant drugs, as well as drug-taking and drug-seeking behaviors during cocaine self-administration. Although glutamate signaling within the NAc regulates striatal neuron plasticity, the primary source of glutamatergic drive has not been well-established. For example, cortical pyramidal neurons provide a major excitatory input into the NAc, but the NAc also receives glutamatergic input from other brain regions, such as the thalamus, and the cortex projects to other parts of the basal ganglia circuit, as well as to structures that feed into the NAc. How cortical inputs into the NAc in particular regulate behaviors related to addiction has not been well-characterized.
Methods: To address this, we used a Cre recombinase-dependent viral vector based flip-excision (FLEX) switch system to express engineered $G_{i/o}$-coupled DREADD (Designer Receptor Exclusively Activated by a Designer Drug; hM_4Di) receptors selectively in prefrontal cortical (PFC) neurons that project to the NAc. Activation of hM_4Di receptors by the synthetic ligand clozapine-*N*-oxide increases $G_{i/o}$-signaling cascades and leads to a transient reduction in neuronal excitability. The effects of this manipulation were assessed during the development of locomotor sensitization to amphetamine and cocaine self-administration under a progressive ratio schedule of reinforcement and during cocaine prime-induced reinstatement.
Results: We found that increasing $G_{i/o}$ signaling selectively in PFC projections to NAc facilitates the development of locomotor sensitization to amphetamine as well as drug-taking behaviors, but attenuates drug-seeking during reinstatement.
Conclusions: Although these data support the idea that drug exposure leads to a loss of top-down control in cortical inputs that contribute to behaviors that are associated with a transition to addiction, they demonstrate that the impact of reducing excitability of PFC on drug behaviors depends on the phase of drug use.
Keywords: amphetamine cocaine rat DREADD receptors addiction.
Disclosures: K. Kerstetter, Nothing to Disclose; A. Wunsch, Nothing to Disclose; T. Donckels, Nothing to Disclose; J. Neumaier, Nothing to Disclose; S. Ferguson, Nothing to Disclose.

T73. Christianson Syndrome Protein NHE6 Regulates Intra-Endosomal pH, BDNF Signaling and Circuit Development

Eric M Morrow*, Julie Kauer, Qing Ouyang, Sofia Lizarraga

Brown University, Providence, Rhode Island

Background: Autism is a heterogeneous group of disorders caused by abnormal neuronal circuit development. Altered neuronal arborization as a basis for circuit dysfunction appears to be a point of convergence for diverse genetic forms of severe autism. Human null mutations in the gene for the endosomal Na+/H+ exchanger 6 (NHE6, also known as SLC9A6) constitute a novel monogenic, severe autism-related disorder called Christianson syndrome (CS). Also, down-regulation of NHE6 gene expression is evident in postmortem brains in idiopathic autism where these changes are highly correlated with decreased expression synapse genes. Our data are consistent with a model wherein NHE6 permits proton leak from endosomes and functions to prevent acidification within the endosome lumen in neurons which may be required for persistent retrograde neurotrophin signaling from distal neurites. In the absence of NHE6 (as in null mutations in CS or down-regulation in autism), endosomal signaling pathways, including TrkB/BDNF signaling are attenuated which results in abnormal circuit development. This study is important because it supports a model whereby the pathology in a subset of severe autism-related disorders (such as CS and Rett syndrome) may reflect reduced levels of neurotrophin signaling.
Methods: We have established an NHE6 null mouse mutant line and we have analyzed circuit development and physiology by a variety of levels of analysis. We use live-cell fluorescent imaging to quantify the pH within endosomes in developing hippocampal pyramidal neurons. Computer-assisted measures of neuronal arborization and synapse development are collected *in vitro* and *in vivo* using

Neurolucida software in hippocampus and cortex. Hippocampal slice physiology is used to collect a variety of measures related to synapse and circuit function. Biochemical studies are conducted to study TrkB/BDNF signaling in mutant hippocampal tissues.
Results: Our results support an important role for NHE6 in endosomal signaling and circuit development in hippocampus and cortex. Using immunohistochemistry, we observe that NHE6 is localized to endosomes in all the major components of the endosome compartment (ie Rab5 + early endosomes, Rab11 + recycling endosomes, and Rab11 + late endosomes) in hippocampal neurons. We also find that NHE6-associated endosomes are enriched in growing axons and dendrites. Using fluorescent ratio imaging of fluorescein-conjugated (pH sensitive) transferrin to Alexa-Fluor-546-conjugated (pH non-sensitive) transferrin, we discover that intra-endosomal pH is more alkaline (pH = 7.2) in early endosomes along growing neurites as compared to early endosomes in the soma (pH = 6.2). In mutant neurons, endosomes were over-acidified, for examples, pH 5.8 in mutant *vs* 6.2 ($p = 0.0004$) in wt in the soma, or pH = 6.5 in mutant *vs* pH = 7.2 ($p = 5.7E{-}06$) in neurites. In response to BDNF addition, we observed the TrkB co-localized to NHE6-associated endosomes. Also in response to BDNF addition, TrkB receptor level in mutant hippocampal neurons was significantly reduced to 76.5% of the control ($p = 8.35E{-}05$). Induction of phospho-Trk was also significantly reduced in mutant after adding BDNF (induction was 48% compared to control, $p = 2.44E{-}08$). Pre-treatment of cells with protease inhibitors partially rescued these effects on signaling suggesting that attenuated TrkB signaling was due in part due to enhanced receptor degradation. Morphologic analysis of neurons *in vitro* and *in vivo* in hippocampus and cortex demonstrated significant impoverishment of dendritic and axonal branching. For example, analysis of axons *in vivo* from layer III cortical pyramidal neurons demonstrated fewer branch points by Neurolucida computer reconstruction in mutant (0.77 ± 0.15 branches per cell in mutant compared to 3.21 ± 0.28 branches in wild-type, $p = 5.7E{-}09$). Using site-directed mutagenesis and a rescue assay *in vitro*, we demonstrate that the arborization function of the NHE6 protein requires an intact proton exchanger. Also, analysis of synapses revealed fewer SV2 + punctae in the mutant . Spine analysis revealed a 12% reduction in dendritic spines on CA1 pyramidal cells ($p = 0.03$) and more immature forms of spines ($p = 0.0001$) in mutant. Hippocampal slice physiology demonstrated a 23.2% reduction in synaptic field strength ($p < 0.0001$), with normal paired-pulse facilitation, and a 25% reduction in pre-synaptic fiber volley. Taken together these circuit studies are consistent with fewer synaptic connections due to reduced neuronal arborization, although other synapse studies are required to study further synaptic function. Finally, we demonstrate that neuronal arborization defects in the mutant *in vitro* may be rescued by the addition of exogenous BDNF. This experiment suggests that deficits in circuit development in the NHE6 mutant may be the result of a dosage sensitive mechanisms that impair BDNF signaling.
Conclusions: In conclusion, our study dissects a novel mechanism, namely regulation of intra-endosomal pH, for modulating a well-studied and important signaling pathway, ie neurotrophin signaling. We report a mechanism of attenuated endosomal signaling and a cellular phenotype involving impoverished arbors in CS, a new neurogenetic disorder and in related conditions, such as autism wherein NHE6 may be down-regulated. Our study is relevant to comorbidity between neurologic and psychiatric phenotypes. By modeling CS, we pinpoint cellular mechanisms of disease relevant to neuropsychiatric disorders and potential treatments.
Keywords: autism cell biology neurodevelopment BDNF/TrkB neurotrophin signaling.
Disclosures: E. Morrow, Nothing to Disclose; J. Kauer, Nothing to Disclose; Q. Ouyang, Nothing to Disclose; S. Lizarraga, Nothing to Disclose.

T74. Memory Enhancement by Targeting Cdk5 Regulation of the NMDA Receptor Subunit NR2B

Florian Plattner*, Adan Hernandez, Karine Pozo, Gabriel Mettlach, Tanvir Singh, Deena Sajitharan, Chunfeng Tan, James A Bibb

UT Southwestern Medical Center, Dallas, Texas

Background: Many psychiatric and neurological disorders are characterized by learning and memory deficits, for which cognitive enhancement is considered a valid treatment strategy. The N-methyl-D-aspartate receptor (NMDAR) is a prime target for the development of cognitive enhancers due to its fundamental role in mnemonic functions. In particular, the NMDAR subunit NR2B has been found to improve synaptic plasticity and memory when over-expressed in glutamatergic neurons. However, NR2B regulation is not well understood and no therapies potentiating NR2B function have been developed.
Methods: Novel phosphorylation sites of the protein kinase cyclin-dependent kinase 5 (Cdk5) on NR2B were identified employing a PhosphoScan approach. The physiological function of these sites was evaluated using primary hippocampal neuron cultures, acute hippocampal slice pharmacology and electrophysiology. Based on the sequence surrounding the novel Cdk5 sites, small interfering peptides (siP) were developed that selectively disrupt the protein-protein interaction between NR2B and Cdk5. The effect of the siP on synaptic neurotransmission and on contextual fear conditioning, a hippocampus-dependent learning task, was characterized.
Results: Here, we show that NR2B is directly phosphorylated by Cdk5 within its carboxy-terminal tail. Cdk5-dependent phosphorylation of NR2B is regulated by neuronal activity and, in turn, controls the receptor's cell surface expression. Disrupting the interaction between NR2B and Cdk5 with siP increases NR2B surface levels and facilitates synaptic transmission. Accordingly, intra-hippocampal infusion of the siP improved fear memory formation *in vivo*.
Conclusions: Taken together, our results reveal a novel molecular mechanism critically regulating NR2B function via Cdk5. A small molecule targeting this mechanism acted as a cognitive enhancer and hence may serve as the basis for the development of more effective therapeutics for memory impairment as well as age-dependent cognitive decline.

Keywords: NMDA receptor, Cdk5, small interfering peptide, fear conditioning, cognitive enhancer.
Disclosures: F. Plattner, Nothing to Disclose; A. Hernandez, Nothing to Disclose; K. Pozo, Nothing to Disclose; G. Mettlach, Nothing to Disclose; T. Singh, Nothing to Disclose; D. Sajitharan, Nothing to Disclose; C. Tan, Nothing to Disclose; J. Bibb, Nothing to Disclose.

T75. Reduced Somatostatin and Vasoactive Intestinal Peptide mRNAs in the Frontal Cortex of Subjects with Schizophrenia and Bipolar Disorder

Samantha J Fung*, Cynthia S Weickert

Neuroscience Research Australia, Sydney, Australia

Background: A lack of inhibitory signaling by GABAergic interneurons has been proposed to play an important role in the cortical pathophysiology of mental illness. Deficits in mRNAs expressed by GABAergic interneurons have been widely reported in schizophrenia and also suggested in bipolar disorder by postmortem studies. However, the extent to which interneuron subtypes are affected, in particular the extent to which interneuron pathology is distinct or shared between the two diseases, remains unclear.
Methods: We examined mRNA expression of seven interneuron biochemical markers: the calcium binding proteins parvalbumin, calretinin and calbindin; and the neuropeptides somatostatin, neuropeptide Y, cholecystokinin, and vasoactive intestinal peptide in the dorsolateral prefrontal cortex and orbitofrontal cortex of healthy control ($n = 35$), as well as schizophrenia ($n = 35$) and bipolar disorder subjects ($n = 31$) from the Stanley Array Consortium collection.
Results: In the dorsolateral prefrontal cortex, we found that the largest change in mRNA expression relative to controls was a 20.9 and 34.7% reduction in somatostatin in schizophrenia and bipolar disorder subjects, respectively ($F = 4.59$, $df = 92$, $p = 0.013$ covarying for pH, Fisher LSDs $p = 0.011$ and 8.4×10^{-5} respectively). Vasoactive intestinal peptide mRNA was also reduced significantly in both disease groups (overall $F = 9.10$, $df = 92$, $p = 2.5 \times 10^{-4}$) decreased by 17.9% in schizophrenia ($p = 0.018$) and 32.7% in bipolar disorder subjects ($p = 5.2 \times 10^{-5}$) relative to controls. Thus, while the magnitude of the interneuron mRNA deficit was greater in subjects with bipolar disorder than schizophrenia, the direction of expression changes of interneuron markers was strikingly similar in both schizophrenia and bipolar subjects relative to healthy control subjects. We found no change in expression of parvalbumin, neuropeptide Y, cholecystokinin or calretinin mRNA in the dorsolateral prefrontal cortex by one way ANOVA between diagnostic groups. Notably, compared to controls, calbindin mRNA expression was significantly increased (24.1%, $p = 5.4 \times 10^{-4}$) in the dorsolateral prefrontal cortex in schizophrenia, but unaltered in bipolar disorder ($p > 0.05$; overall $F = 7.404$, $df = 89$, $p = 1.1 \times 10^{-3}$, covarying for age), and increased in schizophrenia relative to bipolar disorder (25.9%, $p = 3.3 \times 10^{-4}$). In the orbitofrontal cortex we similarly found a reduction in somatostatin mRNA in schizophrenia (18.2%, $p = 0.029$) and bipolar disorder (26.2%, $p = 2.8 \times 10^{-3}$) relative to controls (overall $F = 2.57$, $df = 89$, $p = 0.082$, covarying for pH and RIN), and VIP mRNA was reduced in bipolar (19.1%, $p = 0.017$) with a trend to reduction in schizophrenia (12.4%, $p = 0.10$; overall $F = 3.11$, $df = 89$, $p = 0.049$). Interestingly, there was a trend to reduction of calbindin mRNA expression in the bipolar orbitofrontal cortex relative to healthy controls (15.4%, $p = 0.095$), but calbindin expression was not significantly changed in schizophrenia relative to controls (10.8% higher, $p = 0.22$). Calbindin mRNA expression in schizophrenia was, however, significantly higher than that in bipolar disorder ($p = 4.4 \times 10^{-3}$; overall $F = 4.26$, $df = 91$, $p = 0.017$).
Conclusions: Our findings suggest that much of the cortical interneuron pathology could be shared between schizophrenia and bipolar disorder, with the largest changes in interneuron mRNAs in both diseases being in somatostatin and vasoactive intestinal peptide, expressed primarily by dendrite targeting interneurons. We did not detect a reduced expression of other interneuron mRNAs, including that of parvalbumin, and this pattern was similar across the two cortical regions and the two major mental illness categories examined in this cohort. Interestingly, however, elevated expression of calbindin mRNA is not evident in bipolar disorder and could be more specific to schizophrenia. Such an upregulation in calbindin mRNA may reflect a compensation for deficits in other interneuron subtypes, or could be associated with altered development/differentiation of interneurons in schizophrenia compared to bipolar.
Keywords: schizophrenia, bipolar disorder, frontal cortex, post-mortem, interneuron.
Disclosures: S. Fung, Nothing to Disclose; C. Weickert, Nothing to Disclose.

T76. Dopamine D1-D2 Receptor Heteromer Activation Induces Place Aversion and Abolishes Cocaine Reward via a Cyclin-dependent Kinase 5 Mechanism

Melissa L Perreault*, Ahmed Hasbi, Maurice Shen, Brian F O'Dowd, Susan R George

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

Background: A number ofstudies have shown that in rat striatum a subset of medium spiny neurons (MSNs) coexpress the dopamine D1 and D2 receptors (D1R, D2R). This finding is consistent with recent studies that utilized BAC transgenic mice, with fluorescent protein expression driven by the promoters of D1R or D2R, which also showed a proportion of striatal MSNs expressed both receptor subtypes. Indeed, together these studies have estimated the highest D1R and D2R coexpression (up to ~38% of MSNs) in a subregion of the nucleus accumbens (NAc) shell (the bundle-shaped region) with very low levels of coexpression (~5–7% of MSNs) in caudate putamen. These D1R/D2R-coexpressing neurons in NAc have additionally been demonstrated to express the dopamine D1-D2 receptor heteromer, as shown by confocal microscopy FRET studies, a receptor complex that couples to Gq/11 to activate PLC and generate intracellular calcium release upon its activa-

tion. The D1-D2 heteromer was additionally shown to increase CaMKII phosphorylation and Cdk5 activity, which in turn promotes the site-specific phosphorylation of DARPP-32 at Thr75 leading to the subsequent inactivation of ERK. Given the integral role of these proteins in mediating the addictive properties of drugs of abuse in NAc, we therefore sought to elucidate a role for the D1-D2 receptor heteromer in the regulation of reward processes.
Methods: The effects of D1-D2 heteromer activation on reward was evaluated in place conditioning tests. In these tests the Gq-coupled dopamine receptor agonist SKF 83959 (SKF) was used to activate the D1-D2 heteromer, and the selectivity of the drug's effects on the heteromer was validated by pretreatment with a highly specific interfering peptide, TAT-D1, to disrupt the heteromeric complex. To test a role for the D1-D2 heteromer in the acquisition of cocaine conditioned place preference (CPP), each animal was administered 3 injections of SKF (1 mg/kg, s.c.), TAT-D1 or scrambled peptide (300 pmoles/4 μl, i.c.v), TAT-D1 + SKF, cocaine (10 mg/kg, i.p.), SKF + cocaine, TAT-D1 + cocaine alternately with vehicle during conditioning and placed in the designated chamber (drug-paired/non-drug paired) for 30 min. 24 h following the final conditioning session, rats were allowed access to the entire apparatus for 30 min and the time spent in each chamber recorded. The effect of pretreatment with the Cdk5 inhibitor roscovitine (200 nmoles/4 μl, i.c.v.) on SKF-mediated changes in place conditioning was also evaluated. Another experiment assessed D1-D2 heteromer-induced effects on the expression of cocaine-induced CPP. In this study, animals were given cocaine (10 mg/kg i/p.) or saline during the conditioning phase as described above. On the test day, SKF (2.5 mg/kg, s.c.) or saline was administered immediately before the session, and time spent in each compartment determined. The effect of SKF on Cdk5 signaling was examined in striatal neuronal culture and *in vivo* in rat NAc using immunohistochemisty and Western Blot analysis.
Results: We demonstrated that systemic D1–D2 heteromer activation by SKF resulted in the development of conditioned place aversion (CPA), an effect abolished by pretreatment with TAT-D1 or roscovitine but not the scrambled peptide. Conversely, TAT-D1 administration alone resulted in the development of CPP. Whereas SKF eliminated the acquisition of cocaine CPP, TAT-D1 moderately enhanced cocaine's effects. A single administration of SKF on the test day to animals previously conditioned with cocaine was sufficient to abolish the expression of cocaine-induced CPP in rats. Consistent with this, SKF effectively eliminated the cocaine-induced increase in ERK phosphorylation in NAc. We were further able to identify that the dopamine D1-D2 receptor heteromer-mediated calcium signaling cascade played a key role in the Cdk5-mediated increase in Thr75-DARPP32 phosphorylation.
Conclusions: The present findings highlight a novel physiological role for the dopamine D1-D2 receptor heteromer, linking activation of the receptor complex to Cdk5 signaling in NAc, aversive behavioural responding, and the inhibition of cocaine-induced conditioned reinforcement. Conversely, disruption of the D1–D2 heteromer induced CPP. Together these findings suggest that the D1-D2 heteromer (1) may exert a tonic inhibitory control over brain reward processes, (2) negatively modulates the perception of cocaine reward, and (3) may influence reward processes via a Cdk5-DARPP-32(Thr75)-ERK mediated mechanism. We therefore suggest that the tonic as well as active aversion mediated through the D1–D2 receptor heteromer may represent the physiological basis for anhedonia, by which the dopamine system could exert a 'brake' on the processes that regulate reward. Thus the D1–D2 receptor heteromer may represent an important novel pharmacological target for pathophysiologies involved in motivational disorders, depression, as well as drug dependence and addiction.
Keywords: dopamine D1–D2 receptor heteromer, conditioned place preference, reward, cyclin-dependent kinase 5, DARPP-32, disrupting peptide.
Disclosures: M. Perreault, Nothing to Disclose; A. Hasbi, Nothing to Disclose; M. Shen, Nothing to Disclose; B. O'Dowd, Nothing to Disclose; S. George, Nothing to Disclose.

T77. Schizophrenia-associated Alterations of Microtubule-associated Protein 2 in Human Auditory Cortex

Micah Shelton, Jason Newman, Kenneth Fish, Matthew L MacDonald, Peter Penzes, David A Lewis, Robert A Sweet*

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: The pathology of schizophrenia (SZ) includes laminar-specific reductions in dendritic spine density in the primary auditory cortex (PAC) and other cortical regions, suggesting functional alterations in the proteins responsible for maintenance of dendritic spine architecture. Microtubule-associated protein 2 (MAP2) is crucial for establishing and maintaining both dendrite and spine structure in response to changes in glutamate signaling. A decrease in MAP2 immunoreactivity (IR) has been reported in several areas in SZ. We therefore hypothesized a role for MAP2 in reduced dendritic spine density in the PAC.
Methods: Using quantitative fluorescence microscopy coupled with immunohistochemical markers for dendritic spines and MAP2, we examined MAP2-IR and dendritic spine density in human post-mortem tissue taken from the PAC of individuals with SZ and matched controls.
Results: MAP2-IR based on an antibody targeting the protein's c-terminal microtubule binding domain was significantly ($p < 0.05$) attenuated in SZ, with 60% of SZ subjects exhibiting fluorescence values near background, below the lowest values observed in controls. Reductions in dendritic spine density were restricted to those SZ subjects with reduced MAP2-IR. Pilot follow-up investigations revealed that restoration of MAP2-IR was achieved through antigen retrieval. As well, MAP2-IR was detected through the use of antibodies targeting alternate epitopes along the protein, and using targeted iquid chromatography-mass spectrometry to measure MAP2 peptides.
Conclusions: Altered MAP2-IR is disease-associated and the changes are correlated with dendritic spine loss in these subjects. Pilot findings suggest the hypothesis that the decrease in MAP2-IR is due to a disease specific post-

translational effect that masks the c-terminal epitope targeted by our antibody. Using a combination of quantitative microscopy, antibody-based epitope mapping, and targeted proteomics, we will generate total and domain specific measures of MAP2 to determine how changes in protein levels and epitope availability contribute to the loss of MAP2-IR in SZ, which may provide leads to how MAP2 may contribute to spine loss in SZ.
Keywords: dendritic spine, microtubule-associated protein 2, postmortem, auditory cortex.
Disclosures: M. Shelton, Nothing to Disclose; J. Newman, Nothing to Disclose; K. Fish, Nothing to Disclose; M. MacDonald, Nothing to Disclose; P. Penzes, Nothing to Disclose; D. Lewis, **Part 1:** Bristol-Myers Squibb and Concert Pharmaceuticals, **Part 5:** Bristol-Myers Squibb, Curridium Ltd and Pfizer; R. Sweet, **Part 1:** Consultant, Lilly, USA.

T78. Novel Replacement Strategy for Dissecting NMDA Receptor Regulation

John Gray*, Roger Nicoll

University of California, San Francisco, San Francisco, California

Background: NMDA-type glutamate receptors (NMDARs) play crucial roles in synaptic plasticity and neuronal development and disturbances in NMDAR function have been implicated in a broad range of neuropsychiatric disorders. The functional and regulatory properties of NMDARs are largely determined by their GluN2 subunit composition, which is tightly regulated at synapses. NMDARs are also found extrasynaptically where their physiological function is not known, though excessive activation of these receptors may contribute to excitotoxicity. Until recently, the dogma in the field was that NMDARs were relatively immobile fixed structures, though it is now apparent that there is a remarkable plasticity of synaptic NMDARs. Indeed, the expression, trafficking, synaptic localization and functioning of different NMDARs subtypes are under dynamic cellular control, though the mechanisms are poorly understood.
Methods: We have developed a novel molecular replacement strategy in hippocampal cultures from mice with conditional knock-out (KO) alleles for both GluN2A and GluN2B. Slices are biolistically co-transfected with Cre recombinase to remove the endogenous subunits and replacement mutant subunits. Simultaneous whole-cell patch clamp recordings are then obtained from transfected and neighboring control cells. These simultaneous recordings allow for rigorous, quantitative analysis of the postsynaptic effects of the genetic manipulation while controlling for presynaptic inputs. After measuring synaptic NMDAR currents, local application of NMDA to the cell pair assesses the whole-cell complement of NMDARs, allowing for a rigorous analysis of both synaptic and extrasynaptic NMDARs.
Results: Cre expression in the conditional KO mice eliminates NMDAR responses and co-expression of wild-type GluN2 subunits precisely and reproducibly recovers both the synaptic and whole cell responses, suggesting tight cellular regulation of NMDAR expression. Preliminary findings include a role for a known GluN2B phosphorylation site in regulating post-endocytic sorting, and a novel non-PDZ domain involved in the synaptic targeting of GluN2A subunits.
Conclusions: Synaptic and extrasynaptic NMDAR pools are independently and tightly regulated, allowing for hypothesis-driven dissection of the mechanisms involved in NMDAR targeting and trafficking. This approach will facilitate the identification of novel trafficking proteins and cellular mechanisms for NMDAR regulation and may yield insights into the pathophysiology of schizophrenia and other neuropsychiatric disorders.
Keywords: glutamate, NMDA, glycine, plasticity, neurodevelopment.
Disclosures: J. Gray, Nothing to Disclose; R. Nicoll, Nothing to Disclose.

T79. Gene Expression Profiling of Stress-induced Changes in CA3 Neurons Using Translating Ribosome Affinity Purification (TRAP)

Jason Gray*, Todd Rubin, Bruce S McEwen

The Rockefeller University, New York, New York

Background: Environmental stress can substantially impact cognitive function and is associated with the onset of mood disorders. Studying how stress-induced changes alter brain function will provide important insight into the development and treatment of mood disorders. Acute and chronic stress have been shown to alter hippocampal morphology and function, and yet the molecular mechanisms underlying these stress-induced changes remain incompletely understood. Previously researchers have relied on the combination of microdissection and high-throughput genomic techniques to analyze global changes in gene expression within subregions of hippocampus. The present research improves upon these techniques by utilizing transgenic mice expressing an EGFP fused to the L10a ribosomal subunit that is under the control of a cell-type specific promoter (Gprin3). This allows the isolation of *in vivo* translating RNA fractions from a genetically homogenous population of CA3 neurons that express the Gprin3 regulated transgene.
Methods: Mice expressing Gprin3-EGFP-L10a were subjected to an acute forced swim test (FST; 6 min) or chronic restraint stress (CRS; 2 h/21 days), followed by rapid decapitation, dissection of hippocampus, and then RNA isolation by Translating Ribosome Affinity Purification (TRAP). Briefly, anti-EGFP antibodies are used to immunoprecipitate GFP-positive ribosomes along with their bound RNA, which is then purified by using commercial methods. Both bound (TRAP) and unbound fractions were subjected to RNA-sequencing using an Illumina Hi-Seq 2000. Results were aligned against the mouse genome (mM9) and the numbers of reads for each transcript were normalized against total reads to obtain relative expression levels (ie integrated read densities). Genespring software was then used to perform statistical analyses of relative expression levels to identify differentially expressed genes.

Results: Cluster analysis demonstrated a distinct separation between bound and unbound fractions, suggesting the TRAP was successful. Further, there was substantial overlap of the differentially expressed genes from each TRAP v. Unbound test (83% FST, 92% CRS, 93% Control), suggesting consistency in isolating the cell population. Comparison of TRAP fractions between stress and control revealed over 7900 entities changed by FST and nearly 5800 changed by CRS. Comparing these gene lists with earlier microarray studies from whole hippocampus using the same stress treatments found many of the same genes known to be altered by acute (cFos, Arc, Egr1) and chronic stress (BDNF, Rock2, Gria3). Specifically, genes of the Nfkb pathway showed stress-induced changes in expression. Nfkbia and RelA were found to be highly expressed in the TRAP fraction, suggesting the stress-induced changes are occurring in CA3 neurons and not surrounding tissue. Splice variant, pathway, and gene ontology (GO) analysis of genes differentially expressed in response to stress are ongoing.
Conclusions: The *in vivo* gene expression profiles generated provide a refined map of stress-induced hippocampal changes that are unique to CA3 neurons and will help unravel the mechanisms underlying the vulnerability of these cells to stress-induced damage. Importantly, future studies will expand this work to genetic and environmental models of mood disorder susceptibility. Early life stress is one model of increased stress-susceptibility that has been shown to decrease CA3 spine density in adulthood, but not CA1, suggesting that this region plays an important role in the persistence of increased susceptibility over the lifespan. Further, CA3 exhibits elevated levels of brain derived neurotrophic factor (BDNF), an essential molecule for adaptive neuroplasticity. Decreased levels of BDNF have been associated with mood disorders and the examination of expression profiles from BDNF transgenic mice will be illustrative of increased genetic susceptibility. Comparison of these profiles with those derived from WT mice will reveal novel mechanisms involved in increased stress susceptibility and potentially lead to new targets in the treatment of mood disorders.
Keywords: hippocampal neurons, stress, gene expression.
Disclosures: J. Gray, Nothing to Disclose; T. Rubin, Nothing to Disclose; B. McEwen, Nothing to Disclose.

T80. Glutamatergic Neurons in the Ventral Tegmental Area: Properties & Physiological Role

Thomas S Hnasko*, Ji Hoon Yoo, Gregory Hjelmstad, Howard Fields, Robert Edwards

University of California, San Diego, La Jolla, California

Background: Although the Ventral Tegmental Area (VTA) is generally regarded as a dopaminergic region, non-dopamine neurons are also present. In addition to the well-established GABAergic population, excitatory glutamatergic neurons have been identified by virtue of vesicular glutamate transporter (VGLUT2) expression. Although a subset of dopamine neurons have been shown to co-release both dopamine and glutamate, others express VGLUT2 without expressing dopaminergic markers. However, the projection targets, electrophysiological properties, and physiological roles of these excitatory neurons have not been defined.
Methods: In the first set of experiments, genetically modified mice were used to label VGLUT2$^+$ neurons with green fluorescent protein (GFP) while dopamine neurons labeled with red fluorescent protein (RFP) so that we could directly visualize these populations in the same animal. Patch-clamp electrophysiological recordings were made to compare their intrinsic membrane properties and pharmacological responses of VTA glutamate and dopamine neurons. In a second set of experiments we used another line of mice that express Cre recombinase in VGLUT2$^+$ neurons. Mice were injected with a conditional viral vector to express Channelrhodopsin-2 fused to mCherry (ChR2:mCherry) in a Cre-dependent fashion. ChR2:mCherry was used first as a cell-type specific anterograde tracer to identify the projection targets of VGLUT2$^+$ VTA glutamate neurons. Optical stimulation of VGLUT2$^+$ terminals was then used to demonstrate functional synapses *in vitro*—and, *in vivo*, to begin defining their physiological role in behavioral reinforcement.
Results: We found that VTA glutamate neurons project alongside their dopamine neighbors to the nucleus accumbens, amygdala, prefrontal cortex, and septum. However, glutamate neurons also projected to the lateral habenula and ventral pallidum, regions that receive little or no dopaminergic input. These projections were found to form functional excitatory synapses as evidenced by optically evoked monosynaptic excitatory postsynaptic currents (EPSCs). Preliminary data has now begun to reveal a functional role for these projections in processes relevant to behavioral reinforcement.
Conclusions: Using sophisticated molecular-genetic approaches, our results have begun to shed light on a novel excitatory circuit emanating from the VTA. Like their dopamine-releasing neighbors, it is likely that this heterogeneous population of neurons will have multiple diverse roles in the control of behavior. Their complexity presents important challenges, but also opportunities as new targets for the prevention and treatment of psychiatric disease.
Keywords: ventral tegmental area, VTA; vesicular glutamate transporter, VGLUT2; dopamine; glutamate; corelease.
Disclosures: T. Hnasko, Nothing to Disclose; J. Yoo, Nothing to Disclose; G. Hjelmstad, Nothing to Disclose; H. Fields, Nothing to Disclose; R. Edwards, Nothing to Disclose.

T81. Locus Specific Epigenetic Reprogramming: Bidirectional Regulation of the FosB Gene Using Synthetic Transcription Factors *In Vivo*

Elizabeth Heller*, Hannah Cates, Haosheng Sun, Catherine Pena, Deveroux Ferguson, Scott Knight, H Steve Zhang, Eric Nestler

Icahn School of Medicine at Mount Sinai, New York, New York

Background: Transcriptional regulation underlies sensitivity to psychostimulant exposure and is associated with altered expression of several chromatin-modifying enzymes in key brain reward regions. Genome-wide assessments of

histone posttranslational modifications (HPTMs) have identified drug and stress regulation at numerous target genes implicated in the associated behavioral abnormalities. However, it has not previously been possible to manipulate the epigenome in order to causally link the chromatin state of a single locus with behavioral and molecular responses to psychostimulant exposure. Engineered transcription factors, such as zinc finger proteins (ZFPs) and transcription activator-like effectors (TALEs), can direct enzymatic moieties to specific genomic loci. We are interested in epigenetic regulation of the immediate early gene, *FosB*, which is both necessary and sufficient for many of the downstream molecular changes mediating stress and reward response. Previous work has demonstrated that chronic cocaine exposure in both humans and rodents leads to a reduction in the levels of the histone methyltransferase, G9a, and the repressive epigenetic mark, histone H3 lysine 9 dimethylation (H3K9me2), at the *FosB* locus. This may be the mechanism by which FosB and its stable splice variant, DFosB, accumulate in reward regions of the brain in order to mediate drug responsiveness. Furthermore, DFosB expression is reduced in the brain reward regions of chronically stressed rodents and depressed human patients; we have recently found an increase in H3K9me2 at the *FosB* promoter in depressed human Nucleus accumbens (NAc) relative to healthy controls. Despite the robustness of these correlations, it has not yet been possibly to directly link a particular HPTM at the *FosB* gene to behavioral response, due to the use of experimental paradigms that affect the entire genome (eg, overexpression of G9a, HPTM ChIP-chip, cocaine or stress exposure). Thus, we have pioneered the use of engineered transcription factors to direct a single epigenetic modification to a single gene of interest, within a single brain region.

Methods: A suite of 65 6-finger cys2/his2 ZFPs were designed in silico to recognize 18bp motifs within −200 to +1000 bp of the *FosB* promoter relative to the transcription start site. These ZFPs were tethered to the transcriptional activation domain, p65, and screened *in vitro* for activation of FosB mRNA expression by qRT-PCR. From this screen several ZFPs were selected for fusion to alternative enzymatic domains, such as the preSET/SET domains of G9a and the viral activation domain, VP64. In addition, three TALE-VP64 constructs were designed to target similar sequences. To confirm specificity of the binding of the ZFP across the genome, ZFP-NFD (no functional domain) constructs were fused to 3xFLAG affinity tag, expressed *in vitro* and subject to chromatin immmunopurification (ChIP)-Sequencing. For *in vivo* analysis, mouse NAc neurons were infected with herpes simplex virus (HSV) expressing each of the constructs, and qRT-PCR and immunohistochemistry was used to determine activation or repression of *FosB* expression. We relied on qChIP using a variety of anti-HPTM antibodies to analyze the chromatin modifications induced by the ZFP-G9a and ZFP-p65 constructs. To determine the role of epigenetic remodeling on behavioral responses to psychotimulant exposure, mice were subject to either cocaine locomotor sensitization or acute social stress following HSV NAc infection with the ZFP constructs.

Results: We have found that FosB-ZFP-p65 and -TALE-VP64 constructs efficiently and robustly activate FosB/DFosB expression in NAc neurons, while FosB-ZFP-G9a represses expression. In addition, immunohistochemistry with an anti-FosB/DFosB antibody demonstrates that FosB-ZFP-G9a blocks cocaine induction of FosB/DFosB in NAc, while basal levels of protein are unchanged. Using qChIP, we have found that the mechanism of this repression is HSV-G9a deposition of H3K9me2 specifically at the *FosB* gene *in vivo*, while FosB-ZFP-p65 activates *FosB* via H3K9/14 acetylation. In addition, this gene-specific epigenetic remodeling is associated with changes in *FosB* promoter binding by additional HPTMs and the transcription factor, pCREB, which is a known regulator of activity dependent activation of *FosB*. Engineered transcription factors are also able to modulate behavior, as FosB-ZFP-p65 expression in the NAc enhances cocaine locomotor sensitization, while FosB-ZFP-G9a expression blocks the cocaine effect on locomotor as well as sensitizes animals to stress.

Conclusions: Engineered transcription factors are effective tools to probe the behavioral and molecular consequences of chromatin remodeling at a single locus *in vivo*. Using this approach, we have identified a direct molecular mechanism for cocaine-mediated activation of the *FosB* gene, and have efficiently manipulated behavioral responses to drugs of abuse and stress. This work opens the field for a more mechanistic and causal analysis of the role of epigenetics in regulating the neurobiological mechanisms that underlie reward pathology.

Keywords: reward pathology engineered transcription factors FosB.

Disclosures: E. Heller, Nothing to Disclose; H. Cates, Nothing to Disclose; H. Sun, Nothing to Disclose; C. Pena, Nothing to Disclose; D. Ferguson, Nothing to Disclose; S. Knight, **Part 5:** Sigma Aldrich; H. Zhang, **Part 5:** Sangamo Biosciences; E. Nestler, Nothing to Disclose.

T82. Revealing Lithium's Molecular Mechanisms in Bipolar Disorder: Using the Circadian Clock

Michael J McCarthy*, Hongbing Wei, Stephen Beesley, Bruce M Cohen, Donna L McPhie, David Welsh

VA San Diego Healthcare System, San Diego, California

Background: Multiple lines of evidence suggest that the circadian rhythms are disrupted in bipolar disorder (BD), and that circadian 'clock genes' are important in the pathology of the illness. We have shown previously that lithium increase the amplitude, and lengthens the period of gene expression rhythms in fibroblast cultures; but that in cultures from BD patients, the increase in amplitude is blunted. Lithium's mechanism of action is complex, and may target inositol metabolism, glycogen synthase kinase 3 (GSK3), and other sites. We sought to identify the mechanisms by which lithium affects gene expression rhythm amplitude and period by genetic and pharmacological manipulation of selected molecular pathways. Identifying the specific molecules perturbed in lithium's effects on BD rhythms may be useful in understanding the pathogenesis of BD and in identifying new drug targets.

Methods: Mouse fibroblast NIH3T3 cells were stably transfected with the Per2::luc reporter gene. Using hygromycin selection, a clonal reporter gene cell line was

developed for use in drug screening studies targeting GSK3, inositol metabolism, and MAP kinase signaling. The impact of drugs on circadian rhythms was determined in a luminometer, measuring gene expression every 10 min over 5 days. For human studies, fibroblast cell lines from control and BD subjects were grown in the presence or absence of lithium (1 mM) for 48 h. Samples were then subject to phospho-protein analysis, examining the activation in response to lithium of 45 different signaling molecules.
Results: As in human cell lines, lithium (10 mM) increased amplitude, and lengthened period in NIH3T3 cells. Use of the selective GSK3B inhibitor CHIR 99021 increased amplitude, but shortened period, suggesting the effects of lithium on circadian rhythms are dissociable into at least two components and are not entirely explained by GSK3B inhibition. Inositol treatment (1 mM) had effects similar to lithium on both period and amplitude. Using 2-APB to block inositol 1,4,5-trisphosphate (IP3) receptors, lithium's effect on period length was attenuated. Using PD-98059 to block ERK1/2 activation, lithium's effects on amplitude were attenuated. In human fibroblasts, ser473 phospho-AKT levels were higher in controls compared to BD. Lithium caused a decrease in ERK1/2 phosphorylation in BD cells, but not in controls. Lithium led to phosphorylation of GSK3β at ser9 in both groups, but this response was attenuated in BD.
Conclusions: Our data suggest that lithium employs at least two additional mechanisms beyond GSK3β to regulate circadian rhythms: IP3 to lengthen period, and ERK1/2 to increase amplitude. In cells from BD patients, the ERK1/2 response to lithium is distinct from controls. As ERK signaling is known at affect circadian rhythms, this pathway could explain the abnormal response to lithium we observed previously in BD cell lines. AKT may also be involved as a regulator of GSK3β and ERK.
Keywords: lithium, bipolar disorder, circadian rhythm, gene expression, glycogen sythnase kinase 3.
Disclosures: M. McCarthy, Nothing to Disclose; H. Wei, Nothing to Disclose; S. Beesley, Nothing to Disclose; B. Cohen, Nothing to Disclose; D. McPhie, Nothing to Disclose; D. Welsh, Nothing to Disclose.

T83. A Cross-sectional Examination of Telomere Length and Telomerase in a Well-Characterized Sample of Individuals with Major Depressive Disorder Compared to Controls

Naomi M Simon*, Zandra Walton, Jennifer Prescott, Elizabeth Hoge, Aparna Keshaviah, TH Eric Bui, Noah Schwarz, Taylor Dryman, Rebecca A Ojserkis, David Mischoulon, John Worthington, Immaculata DeVivo, Maurizio Fava, Kwok-Kin Wong

Massachusetts General Hospital, Boston, Massachusetts

Background: Leukocyte telomeres may be an ideal marker of chronic stress-accelerated aging due to major depressive disorder (MDD) because their length may serve as a marker of cellular turnover, oxidative stress, and telomerase regulation. A number of studies have reported an association of MDD with shorter telomere length, but results have been mixed, particularly with adjustment for medical morbidity. The presence of potentially uncontrolled confounding factors such as medical illness and life stress cannot be ruled out. We thus designed a prospective, well powered study of age and gender matched individuals aged 18–70, selected to minimize confounds, including inclusion of currently depressed participants with at least 5 years since major depressive disorder (MDD) onset to mark chronicity, the absence of current psychiatric medication use, and for both MDD and controls, limited and carefully assessed medical morbidity, age no greater than 70 years to limit survival biases, the absence of regular anti-inflammatory medication use, and a maximum body mass index of 35, with careful assessment of other potential contributions of environmental stressors such as loss, trauma, and cumulative medical illness ratings.
Methods: Leukocyte telomere length was measured by Southern blot and repeated using quantitative polymerase chain reaction (qPCR). Telomerase was assayed from B cells, T cells and peripheral blood mononuclear cells (PBMCs) purified from blood collected in a Ficoll tube, and protein lysates were prepared. Telomerase activity was assayed with a telomerase repeat amplification protocol using a well-established commercial kit (Trapeze by Chemicon). Multivariate linear regression was used to examine whether telomere length and telomerase level varied by diagnosis (MDD *vs* controls) among Caucasians. Telomere length (as measured both by Southern Blot and by PCR) and telomerase levels were natural log transformed to achieve normality. Any demographic or clinical covariates found to significantly differ by diagnosis were controlled for in the multivariate regression models. We also tested the association between age and gender (matching variables) with each outcome, and if significant differences existed, we included these covariates in multivariate models.
Results: The sample comprised 244 Caucasian adults: 130 with MDD (61% women, mean age 42.4 + /14.1, mean BMI 26.4 ± 4.4) and 144 psychiatric controls (68% women, mean age 42.5 + /114.6, mean BMI 25.3 ± 4.1) with telomere length measured via Southern blot. Opposite magnitudes of effect of telomere length by age depending on minority race supported our decision not to combine non-Caucasian subjects into a single category for analysis. Within the MDD sample, mean years of MDD was 21.4 years (SD = 14.0: range 5–61 years). On average, MDD patients had slightly longer telomeres (mean (SD) = 9.1 (2.8)) compared to controls (mean (SD) = 8.7 (2.3)), but differences by diagnosis were non-significant for telomere Southern ($b = 0.022$, SE(b) = 0.035, $t(243) = 0.66$, $p = 0.52$) and qPCR ($b = 0.0098$, SE(b) = 0.041, $t(198) = 0.24$, $p = 0.81$). The relationship between telomere length and MDD did not differ by age but was marginally different by gender ($p = 0.062$ for Diagnosis × Gender interaction) when telomere length was measured by southern blot (but not qPCR): among males, telomeres were shorter for MDD than controls ($p = 0.36$), whereas among females, telomeres length was longer for MDD ($p = 0.078$). Even after adjusting for potential confounders, including detailed assessment of cumulative medical illness, grief, trauma exposure, substance abuse/dependence history, and education level, telomere length did not differ by MDD diagnosis ($b = 0.0098$, SE(b) = 0.053, $t(139) = 0.19$, $p = 0.85$) by Southern or when measured via PCR ($b = -0.0034$, SE(b) = 0.064,

$t(139) = -0.05$, $p = 0.96$). Telomerase level also did not differ by diagnosis. Total duration of mood episodes, years of MDD, or anxiety co-morbidity were not significantly associated with either telomere length or telomerase level.

Conclusions: In contrast to reports of an association of shorter telomeres with MDD, in an age and gender matched sample recruited specifically for this study with careful selection criteria to limit confounding by medical and other factors, and in depth assessment of environmental stressors and cumulative medical illness ratings, we found no cross-sectional difference between MDD and controls for telomere length using two methodologies or for telomerase levels, suggesting prior studies may include residual confounding of non-MDD related factors. Limitations include the cross-sectional nature of the assessments, and the possibility that study requirements intended to limit confounds selected an unrepresentative cohort of depressed patients.

Keywords: stress, aging, major depressive disorder, telomere.

Disclosures: N. Simon, Nothing to Disclose; Z. Walton, Nothing to Disclose; J. Prescott, Nothing to Disclose; E. Hoge, Nothing to Disclose; A. Keshaviah, Nothing to Disclose; T. Bui, Nothing to Disclose; N. Schwarz, Nothing to Disclose; T. Dryman, Nothing to Disclose; R. Ojserkis, Nothing to Disclose; D. Mischoulon, **Part 4:** Bowman Family Foundation, FisherWallace, Ganeden, Nordic Naturals, Methylation Sciences, Inc. (MSI); J. Worthington, Nothing to Disclose; I. DeVivo, Nothing to Disclose; M. Fava, **Part 1:** Advisory/Consulting: Abbott Laboratories; Affectis Pharmaceuticals AG; Alkermes, Inc.; Amarin Pharma Inc.; Aspect Medical Systems; AstraZeneca; Auspex Pharmaceuticals; Bayer AG; Best Practice Project Management, Inc.; BioMarin Pharmaceuticals, Inc.; Biovail Corporation; BrainCells Inc; Bristol-Myers Squibb; CeNeRx BioPharma; Cephalon, Inc.; Cerecor; CNS Response, Inc.; Compellis Pharmaceuticals; Cypress Pharmaceutical, Inc.; DiagnoSearch Life Sciences (P) Ltd.; Dinippon Sumitomo Pharma Co. Inc.; Dov Pharmaceuticals, Inc.; Edgemont Pharmaceuticals, Inc.; Eisai Inc.; Eli Lilly and Company; EnVivo Pharmaceuticals, Inc.; ePharmaSolutions; EPIX Pharmaceuticals, Inc.; Euthymics Bioscience, Inc.; Fabre-Kramer Pharmaceuticals, Inc.; Forest Pharmaceuticals, Inc.; GenOmind, LLC; GlaxoSmithKline; Grunenthal GmbH; i3 Innovus/Ingenis; Janssen Pharmaceutica; Jazz Pharmaceuticals, Inc.; Johnson & Johnson Pharmaceutical Research & Development, LLC; Knoll Pharmaceuticals Corp.; Labopharm Inc.; Lorex Pharmaceuticals; Lundbeck Inc.; MedAvante, Inc.; Merck & Co., Inc.; MSI Methylation Sciences, Inc.; Naurex, Inc.; Neuralstem, Inc.; Neuronetics, Inc.; NextWave Pharmaceuticals; Novartis AG; NuPathe;-Nutrition 21; Orexigen Therapeutics, Inc.; Organon Pharmaceuticals; Otsuka Pharmaceuticals; Pamlab, LLC.; Pfizer Inc.; PharmaStar; Pharmavite® LLC.; PharmoRx Therapeutics; Precision Human Biolaboratory; Prexa Pharmaceuticals, Inc.; Puretech Ventures; PsychoGenics; Psylin Neurosciences, Inc.; Rexahn Pharmaceuticals, Inc.; Ridge Diagnostics, Inc.; Roche; Sanofi-Aventis US LLC.; Sepracor Inc.; Servier Laboratories; Schering-Plough Corporation; Solvay Pharmaceuticals, Inc.; Somaxon Pharmaceuticals, Inc.; Somerset Pharmaceuticals, Inc.; Sunovion Pharmaceuticals; Supernus Pharmaceuticals, Inc.; Synthelabo; Takeda Pharmaceutical Company Limited; Tal Medical, Inc.; Tetragenex Pharmaceuticals, Inc.; Teva; TransForm Pharmaceuticals, Inc.; Transcept Pharmaceuticals, Inc.; Vanda Pharmaceuticals, Inc., Speaking/Publishing: Adamed, Co; Advanced Meeting Partners; American Psychiatric Association; American Society of Clinical Psychopharmacology; AstraZeneca; Belvoir Media Group; Boehringer Ingelheim GmbH; Bristol-Myers Squibb; Cephalon, Inc.; CME Institute/Physicians Postgraduate Press, Inc.; Eli Lilly and Company; Forest Pharmaceuticals, Inc.; GlaxoSmithKline; Imedex, LLC; MGH Psychiatry Academy/Primedia; MGH Psychiatry Academy/Reed Elsevier; Novartis AG; Organon Pharmaceuticals; Pfizer Inc.; PharmaStar; United BioSource, Corp.; Wyeth-Ayerst Laboratories, Equity Holdings: Compellis; PsyBrain, Inc., Royalty/patent, other income: Patent for Sequential Parallel Comparison Design (SPCD), which are licensed by MGH to RCT Logic, LLC; and patent application for a combination of Scopolamine and Ketamine in Major Depressive Disorder (MDD). Copyright for the MGH Cognitive & Physical Functioning Questionnaire (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, **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, ; K. Wong, Nothing to Disclose.

T84. Effects of Early Life Stress on Adulthood Stress Reactivity and Its Mechanisms

Li Li*

University of Alabama Birmingham, Birmingham, Alabama

Background: Animal studies have shown that adverse early life stress (ELS) influences later-life stress reactivity. This may be paralleled by early childhood abuse in humans, and there are approximately 3 million reports of child maltreatment annually in the U.S. alone. Stressful life events

also increase vulnerability to a variety of psychiatric and medical disorders.
Methods: To determine the effects of ELS on the response to acute stress in adulthood, individuals with a history of ELS and normal controls without ELS were exposed to a standardized laboratory psychological stressor, the Trier Social Stress Test (TSST) including a free speech and mental arithmetic in front of 2 examiners. Blood samples were collected 10 min before and 1, 45 and 90 min after the TSST for the measurements of cytokines and cortisol levels. The ELS was measured by the Childhood Trauma Questionnaire (CTQ).
Results: Compared with controls, ELS subjects exhibited significantly greater subjective irritation and anxiety, and lower pleasantness during the TSST. The CTQ scores were correlated with stressful perception during the TSST in ELS subjects. Interestingly,the impact of ELS on stress reactivity to the TSST was independent of a diagnosis of major depression or post-traumatic stress disorder in subjects. Elevated levels of different cytokines were observed in different subscales of ELS following the TSST, in which interleukin-10 was elevated in subjects with emotional abuse, or physical and sexual abuse. In contrast, interleukin-12 was elevated in physical neglect subjects. Moreover, a relative dampened cortisol response to the TSST challenge was observed in ELS subjects during and after TSST.
Conclusions: The present findings suggest that an exposure of ELS impacts the reactivity to acute psychosocial stress in adulthood, which may be explained by the dysregulation of the HPA axis activity and activated innate immune factors. Such pathway could partly be responsible for the increased vulnerability for acute psychosocial stress-related diseases in individuals suffering from ELS. Our study also indicates that dissection of the heterogeneous pathophysiology in ELS subjects will assist in developing more specific interventions for different subgroups of patients.
Keywords: early life stress; acute stress; HPA axis; cytokines, depression.
Disclosures: L. Li, Nothing to Disclose.

T85. Brain Region-specific Changes in Extracellular Signal-regulated Kinase (ERK)-5 Signaling in Suicide Subjects

Yogesh Dwivedi*, Ghanshyam Pandey, Hui Zhang

University of Illnois at Chicago, Chicago, Illinois

Background: Mitogen-activated protein (MAP) kinases are serine/threonine protein kinases that play a central role in transduction of external stimuli to the nucleus and thereby regulate expression of genes involved in cell survival, proliferation, and plasticity. The newest member of the MAP kinase family is ERK5, which is activated by stress and growth factors. ERK5 has been implicated in neurogenesis during early developmental stages, and in neuronal survival and synaptic functions. Our aim was to examine whether suicide brain is associated with changes in ERK5 signaling, since we earlier reported changes in expression of growth factors and in activation of ERK1/2, another MAP kinase signaling in brain of these subjects.
Methods: Expression and activation of ERK5, its upstream activator, MEK5, and functional status of MEF2C, one of the major substrates of ERK5, were determined in prefrontal cortex (PFC) and hippocampus of well-matched suicide subjects ($n = 20$) and non-psychiatric normal control subjects ($n = 20$). Postmortem brain samples were obtained from the Maryland Psychiatric Research Center, Baltimore as well as from McGill Suicide Brain Bank. Diagnosis was performed using DSM-IV criteria. mRNA, protein, and catalytic activities of ERK5 and MEK5 were determined by qRT-PCR, Western blot, and enzyme assays, respectively, whereas functional status of MEF2C was determined by DNA-binding activity. Data analysis was performed using SPSS. ANOVA was performed to test differences between groups and $p < 0.05$ was used for significance level, with *post hoc* test and Bonferroni correction. Covariates, such as age, sex, and postmortem interval were added to the analysis.
Results: It was observed that catalytic activity of ERK5 was significantly decreased in the cytosol and nuclear fractions of hippocampus of suicide subjects, whereas, protein level of ERK5 was decreased only in the nuclear fraction. We also observed significantly decreased mRNA level of ERK5 in hippocampus of suicide subjects. Catalytic activity of MEK was decreased significantly without any change in its protein level in hippocampus. DNA-binding activity of MEF2C was also decreased in hippocampus of suicide subjects. None of the measures showed any change in PFC of suicide subjects.
Conclusions: Our findings of altered ERK5 signaling in hippocampus suggest brain region-specific changes in ERK5 signaling in suicidesubjects and implicate that ERK5 signaling may play an important role in the pathophysiology of suicide.
Keywords: suicide, human postmortem brain, hippocampus, MAP kinase, cellular signaling.
Disclosures: Y. Dwivedi, Nothing to Disclose; G. Pandey, Nothing to Disclose; H. Zhang, Nothing to Disclose.

T86. The Genome in Three Dimensions: A New Frontier in Human Brain Research

Amanda Mitchell*, Rahul Bharadwaj, Catheryne Whittle, Karoly Mirnics, Yasmin Hurd, Schahram Akbarian

Icahn School of Medicine at Mount Sinai, New York, New York

Background: Less than 1.5% of the human genome encodes protein. However, vast portions of the human genome are transcriptionally and epigenetically regulated. Many non-coding regulatory DNA elements are thought to regulate the spatial organization of interphase chromosomes. For example chromosomal 'loopings' are pivotal for the orderly process of gene expression. They enable distal regulatory enhancer and silencer elements to interact directly with proximal promoter and transcription start sites potentially bypassing many kilobases of interspersed linear genomic sequence. To date, however, epigenetic studies in the human brain are primarily focused on the exploration of developmental and disease associated modification in DNA methylation, DNA hydroxymethylation, and posttranslational modifications of the nucleosome core histones. In

contrast, very little is known about the regulation of supranucleosomal structures in brain nuclei.
Methods: We introduce chromosome conformation capture (3C) protocols for brain and compare higher-order chromatin structures at the chromosome 6p22.2–22.1 schizophrenia and bipolar disorder susceptibility locus, and additional neurodevelopmental risk genes (DPP10, MCPH1) in adult prefrontal cortex and various cell culture systems, including neurons derived from reprogrammed skin cells.
Results: We show that chromosome conformation capture, a widely used approach to study higher-order chromatin, is applicable to tissue collected postmortem, thereby informing about genome organization in the human brain. Comparative studies in freshly harvested and autolytic mouse brain show that not all chromosomal loopings are stable postmortem.
Conclusions: We predict that the exploration of three-dimensional genome architectures and function will open up new frontiers in human brain research and psychiatric genetics and provide novel insights into the epigenetic risk architectures of regulatory noncoding DNA.
Acknowledgements: Funded by the Brain and Behavior Research Foundation and the National Institutes of Health.
Keywords: chromatin, postmortem, schizophrenia, epigenetics.
Disclosures: A. Mitchell, Nothing to Disclose; R. Bharadwaj, Nothing to Disclose; C. Whittle, Nothing to Disclose; K. Mirnics, Nothing to Disclose; Y. Hurd, Nothing to Disclose; S. Akbarian, Nothing to Disclose.

T87. Acute but Not Chronic Psychosocial Stress Alters the Density and Immune-phenotype of Microglia in Mouse Stress-responsive Brain Regions

Michael Lehmann*, Miles Herkenham

NIMH, Bethesda, Maryland

Background: Microglia are the predominant innate immune cells in the brain parenchyma and are functionally and morphologically dynamic. Under normal physiological conditions, the ramified microglial cell is highly motile, as its processes undergo continuous cycles of extension and withdrawal, which enables it to scan for signals that would indicate a potential threat to CNS homeostasis. Upon the appearance of such 'activating' signals (in infection, trauma or cell impairment) or the loss of constitutive 'calming' signals, microglia rapidly alter both their structure and function. During these alerted or reactive states, microglia retract their processes and produce an array of pro-inflammatory and cytotoxic molecules that under excessive and/or prolonged activation have been associated with various pathologies.
Presently, the studies showing the extent to which chronic psychosocial stress is capable of altering microglial status have been inconsistent and difficult to interpret. Typically the classification of microglia activity has been determined by the relative increase in the area of Iba-1 immunostaining, but what that means in terms of microglial activation is not known. It is also not known how acute and chronic stress contributes to altered microglial function. Here we examined the effects of acute and chronic stress on microglial density and phenotype in specific stress-responsive regions of brain. Because glucocorticoids are known to sensitize CNS innate immune responses to subsequent immune challenges, we hypothesized that acute and chronic stress will prime microglial responses to pro-inflammatory stimuli.
Methods: In the first set of studies, we mapped changes in microglial density after acute and chronic stress exposure using CX3CR1-GFP reporter male mice that display strong GFP reporting confined to microglia. Mice were exposed to either two (acute) or fourteen (chronic) days of social defeat stress. Both treatments are known to potently elevate glucocorticoids. Mice were then phenotyped for social and sexual behaviors, perfused, and brains examined for changes in microglia number and proliferation. In a second set of studies, we used fluorescence-activated cell sorting of microglia cell surface markers, CD11b, CD45, and CD68 to examine changes in microglia activation after acute and chronic stress. Lastly, an *ex vivo* approach was used to address if acute or chronic stress alters the responses of microglia to pro-inflammatory stimuli. Here, male C57BL6/J mice were exposed to acute or chronic social defeat stress, and microglia in the prefrontal cortex were isolated and challenged with LPS to probe for stress-induced sensitization of pro-inflammatory cytokines.
Results: Both acute and chronic stressed mice showed reduced social and hedonic behaviors indicative of depressive-like phenotype. The amount of anhedonic behavioral change was stress-duration dependent. Acute but not chronic stress selectively increased microglia density in stress-responsive brain regions, including the medial prefrontal cortex. These regions also exhibited a greater density of proliferating microglia after acute stress only. Acute stress also enhanced surface expression of CD11b on microglia from the prefrontal cortex. Lastly, when stimulated with LPS, peritoneal macrophages from mice exposed to acute stress expressed higher levels of pro-inflammatory cytokines than those from mice exposed to chronic stress or homecage mice.
Conclusions: Stress has a biphasic effect of microglia activation. Acute stress increased the density, proliferation, and activation of microglia within the prefrontal cortex. After 14 days of stress exposure, microglia appear to have adapted—both the density and levels of activation were comparable to non-stressed mice. Whether adaptation in microglia leads to mal-adaptive changes in other cell types is currently under investigation.
Keywords: microglia, mouse, psychosocial-stress, immune, cytokines, prefrontal cortex.
Disclosures: M. Lehmann, Nothing to Disclose; M. Herkenham, Nothing to Disclose.

T88. The Neuron-specific Chromatin Regulatory Subunit BAF53b is Necessary for Epigenetic Regulation of Synaptic Plasticity and Memory

Annie Vogel-Ciernia*, Dina Matheos, Ruth Barrett, Marcelo A Wood

University of California, Irvine, California

Background: Gene expression is considered a key step for long-term memory processes. Transcription does not occur

on naked DNA, but rather in the context of chromatin, the protein complex that condenses and organizes genomic DNA. The repeating unit of chromatin is called a nucleosome, which consists of DNA wrapped around pairs of the core histone proteins. Histone modification, nucleosome remodeling, and DNA methylation are three main epigenetic mechanisms by which chromatin structure is regulated in order to access DNA and express specific gene profiles. Both histone modification and DNA methylation have been shown to serve as critical regulators of transcription subserving long-term memory formation. However, to date, not a single study has examined the role of nucleosome remodeling in regulating gene expression required for long-term memory processes. Nucleosome remodeling is carried out by ATP-dependent enzymatic complexes, which use the energy of ATP hydrolysis to disrupt nucleosome-DNA contacts, move nucleosomes along DNA, and remove or exchange nucleosomes. Recent human exome sequencing studies have implicated polymorphic Brg1-Associated Factor (BAF) complexes (mammalian SWI/SNF chromatin remodeling complexes) in several intellectual disabilities and cognitive disorders, including autism. However, it is currently unknown how mutations in BAF complexes result in impaired cognitive function. Postmitotic neurons express a neuron-specific assembly, nBAF, characterized by the neuron-specific subunit BAF53b.

Methods: To examine the role of nBAF in neuron-specific nucleosome remodeling, we examined traditional BAF53b heterozyogous knockout mice and transgenic mice that over-express a dominant negative mutant form of BAF53b. We tested these mice in several memory paradigms for both long and short-term memory. Rescue experiments were performed with reintroduction of BAF53b using adeno-associated virus delivered into the hippocampus of adult BAF53b mutant mice. We also examined theta burst long-term potentiation in acute hippocampal slices and alterations in gene expression following a learning event using high density RNA sequencing.

Results: Mice harboring selective genetic manipulations of BAF53b show severe defects in long-term memory and long-lasting forms of hippocampal synaptic plasticity. Reintroducing BAF53b in the adult hippocampus rescues memory impairments in BAF53b mutant mice, suggesting a role for BAF53b beyond neuronal development. The defects in BAF53b mutant mice appear to derive from alterations in gene expression that produce abnormal postsynaptic components, such as spine structure and function, and ultimately lead to deficits in synaptic plasticity.

Conclusions: Our findings indicate a critical role for BAF53b, and consequently nBAF mediated nucleosome remodeling, in regulating long-term memory formation, and provide new insight into the role of BAF complexes in human intellectual and cognitive disorders.

Keywords: long-term memory; epigenetics; synaptic plasticity; chromatin remodeling; gene expression.

Disclosures: A. Vogel-Ciernia, Nothing to Disclose; D. Matheos, Nothing to Disclose; R. Barrett, Nothing to Disclose; M. Wood, Nothing to Disclose.

T89. Nucleus Accumbens Medium Spiny Neuron Subtypes Differentially Mediate Susceptibility and Resilience to Social Defeat Stress

T Chase Francis, Ramesh Chandra, Julie Brooks, Genesis Dayrit, Eric Finkel, Jeffrey D Lenz, Sergio Iñiguez, Patricio O'Donnell, Mary Kay Lobo*

Johns Hopkins University, Baltimore, Maryland

Background: The nucleus accumbens (NAc) is a major integration site of salient information important in mediating emotional behaviors. Previous studies implicate altered neuronal activity in the NAc after social defeat stress, a stress paradigm that results in two distinct mouse phenotypes: mice susceptible to social defeat stress (ie, displaying depression-like behaviors) and mice unsusceptible to social defeat stress (ie, displaying resilient behaviors). However, it is unclear which neurons in the NAc mediate these behavioral and cellular responses to social defeat stress. The principle neurons of the NAc, the medium spiny neurons (MSN), are sub-divided into two anatomically inseparable but functionally distinct neurons, which are differentially enriched in dopamine 1 (D1-MSN) and dopamine 2 (D2-MSN) receptors.

Methods: To investigate the selective roles of the D1-MSNs and D2-MSNs in the behavioral outcomes resulting from social defeat stress, we examined neuronal activity and also manipulated neuronal firing in each MSN subtype. First, we examined c-Fos induction, a marker for neuronal activity, and intrinsic excitability by whole-cell slice electrophysiology in MSN subtypes of the NAc in D1-GFP and D2-GFP mice after social defeat stress. Next, we used optogenetic and pharmacogenetic approaches to alter neuronal firing in MSN subtypes. We used D1-Cre and D2-Cre BAC transgenic mice combined with conditional adeno-associated viruses (AAVs) to selectively express the blue light (473 nm) activated ChETA-A opsin and hM4(Gi), the inhibitory Designer Receptor Exclusively Activated by a Designer Drug (DREADD), in MSN subtypes. Social interaction and the sucrose preference tests were used as behavioral assays for these experiments.

Results: We found c-Fos induction in D1-MSNs after exposure to a novel social target in mice resilient to social defeat stress. In contrast, c-Fos induction was primarily seen in D2-MSNs in susceptible mice following the exposure to the novel social target. Repeated blue light stimulation of ChETA-A expressing NAc D1-MSNs, but not D2-MSNs, produced an anti-depressive effect in mice previously showing a susceptible phenotype. Conversely, stimulation of D2-MSNs, but not D1-MSNs, promotes a susceptible phenotype in mice. Furthermore, inhibition of D1-MSNs by activating the hM4(Gi) DREADD with its ligand, clozapine-N oxide, produced a similar effect on previously resilient mice.

Conclusions: Our data demonstrate that direct optogenetic and pharmacogenetic manipulations of the MSN subtypes mediate opposing outcomes to social defeat stress. Differential activation or suppression of the NAc MSN subtypes is thus implicated in the modulation of depression-related behaviors.

Keywords: nucleus accumbens, depression, optogenetics, pharmacogenetics, medium spiny neurons, social defeat stress.
Disclosures: T. Francis, Nothing to Disclose; R. Chandra, Nothing to Disclose; J. Brooks, Nothing to Disclose; G. Dayrit, Nothing to Disclose; E. Finkel, Nothing to Disclose; J. Lenz, Nothing to Disclose; S. Iñiguez, Nothing to Disclose; P. O'Donnell, **Part 5:** Pfizer; M. Lobo, Nothing to Disclose.

T90. Genetic Background Regulates the Effect of Antidepressant Treatment on Behavioral Despair and Hippocampal Neurogenesis in Mice

Brooke H Miller*, Thomas A Lanz, Zane Zeier, Miguel Lopez-Teledono, Robin Kleiman, Mathew Pletcher, Claes Wahlestedt

University of Florida College of Medicine, Gainesville, Florida

Background: There is strong evidence that chronic treatment with antidepressants such as fluoxetine induces an increase in adult hippocampal cell proliferation and neuronal differentiation, and that this effect may be associated with the behavioral response to antidepressants.
Methods: In order to test the association between antidepressant efficacy and hippocampal neurogenesis, we treated mice from 30 inbred strains with chronic oral fluoxetine and measured the effect of drug treatment on behavioral despair and hippocampal gene expression. The effect of fluoxetine on neurogenesis (BrdU labeling) was measured in a subset of the 30 strains.
Results: We found that approximately 60% of the strains showed a positive behavioral response to fluoxetine treatment, similar to the percent response observed in human cohorts. Gene expression analysis identified a set of approximately 100 genes, many of which have been associated with neurogenesis, that clustered based on the strain-specific behavioral response to fluoxetine. This gene set was found to reliably predict the effect of fluoxetine on cell proliferation (as measured by BrdU labeling) in the dentate gyrus of a subset of the inbred strains. Subsequent genome-wide association mapping (GWAS) identified several genetic loci associated with both the behavioral and neurogenic response to fluoxetine.
Conclusions: These results suggest that the behavioral response to fluoxetine is under genetic regulation and associated with hippocampal neurogenesis: strains that show a positive behavioral response to fluoxetine also show an increase in hippocampal neurogenesis, whereas no change in neurogenesis is observed in strains that do not show a behavioral response. Additional genetic and genomic analysis was used to identify gene networks and genomic loci that may regulate antidepressant efficacy.
Keywords: antidepressant, genetics, mouse, behavior, neurogenesis.
Disclosures: B. Miller, Nothing to Disclose; T. Lanz, **Part 1:** Employee of Pfizer, **Part 3:** Employee of Pfizer, **Part 5:** Pfizer; Z. Zeier, Nothing to Disclose; M. Lopez-Teledono, Nothing to Disclose; R. Kleiman, Nothing to Disclose; M. Pletcher, **Part 1:** Employee of Pfizer, **Part 5:** Pfizer; C. Wahlestedt, **Part 1:** Pfizer, OPKO.

T91. Genetic Modulation of Neuronal Competition Homeostasis in the Adult Dentate Gyrus to Enhance Hippocampal Functions

Amar Sahay*, Kathleen McAvoy, Kimberly Scobie, Stefan Berger, Nannan Guo, Sreyan Choudhry, Sam Miake-Lye, Rene Hen, mark nelson

Center for Regenerative Medicine, Boston, Massachusetts

Background: Adult hippocampal neurogenesis is a unique form of plasticity that generates new neurons in the dentate gyrus (DG) throughout life. Convergent lines of evidence have suggested a role for adult-born neurons in disambiguating or distinguishing between similar representations, a process known as pattern separation. Whereas, pattern separation is essential to distinguish between similar experiences by minimizing interference, pattern completion facilitates the retrieval of memories based on partial cues in the environment. An imbalance in pattern separation-pattern completion may result in the inappropriate retrieval of previous stored aversive memories and activation of circuits subserving fear and stress responses. Thus, development of strategies to rejuvenate the DG with adult-born neurons may have therapeutic potential for reversing pattern separation impairments in depression, anxiety disorders such as post-traumatic stress disorder (PTSD) and also during aging. The dentate gyrus-CA3 circuit is continuously modified throughout life by the integration of adult-born dentate granule neurons that establish new synaptic connections with entorhinal cortical inputs, hilar cells and CA3 pyramidal neurons. Electron microscopy studies and retroviral silencing of NMDA receptor signaling in adult-born neurons have suggested a role for activity-dependent competition between adult-born neurons and pre-existing dentate granule neurons for synaptic inputs. These observations suggest that modulation of synaptic inputs onto pre-existing mature neurons may dictate neuronal competition and integration of adult-born neurons into the DG-CA3 circuit.
Methods: Here, we employ a novel genetic system by which we reversibly eliminate a subset of dendritic spines of mature dentate granule neurons while sparing the dendritic spines of young adult-born neurons. Using genetic reporters, immediate early gene-circuit mapping and confocal microscopy, we analyzed the neural stem cell and progenitor compartment, connectivity of mature and adult-born dentate granule neurons and activation of hippocampal circuitry following our genetic manipulation. Genetically modified mice harboring expanded reservoirs of adult-born neurons of defined ages were examined in behavioral paradigms that probe anxiety and depression-like behaviors and hippocampal dependent learning, specifically, pattern separation and pattern completion.
Results: Partial elimination of dendritic spines of mature dentate granule neurons results in a robust increase in young neuron survival and rapid expansion of the integrating population of young adult-born neurons. Remarkably, complete reversal of elimination of dendritic spines (thin, stubby and mushroom) in mature dentate granule neurons restores neuronal competition-homeostasis and returns the numbers of integrating adult-born

neurons to baseline levels. Genetic expansion of the reservoir of 6–8 weeks old adult-born neurons does not affect anxiety or depression-like behaviors, but enhances pattern separation.

Conclusions: Our studies suggest that modulation of synaptic competition between mature dentate granule neurons and adult-born neurons is sufficient to bias integration in favour of adult-born neurons of a defined age. Furthermore, leveraging the reversibility of our genetic approach, we found that levels of adult hippocampal neurogenesis are maintained at steady state levels by as yet-unidentified homeostatic mechanisms that operate within the lineage. Finally, our studies have begun to define a temporal window for which expansion of adult-born neurons may have maximal impact on encoding functions of the DG such as pattern separation.

Funding support: US National Institute of Mental Health grant 4R00MH086615-03, the Ellison Medical Foundation New Scholar in Aging and the Whitehall Foundation.

Keywords: neurogenesis, hippocampus, anxiety disorders, PTSD, pattern separation.

Disclosures: A. Sahay, Nothing to Disclose; K. McAvoy, Nothing to Disclose; K. Scobie, Nothing to Disclose; S. Berger, Nothing to Disclose; N. Guo, Nothing to Disclose; S. Choudhry, Nothing to Disclose; S. Miake-Lye, Nothing to Disclose; R. Hen, **Part 1:** Consultant for Roche, Lundbeck and Servier Companies; m. nelson, **Part 5:** echelon biosciences.

T92. Disrupting AMPA Receptor Endocytosis Restores the Ability to Form New, and Enables the Recovery of Old, Memories in Mice Genetically Designed to Mimic Alzheimer's Disease

Sheena Josselyn*, Adelaide Yiu, Valentina Mercaldo, Derya Sargin, Paul Frankland

Hospital for Sick Children/University of Toronto, Toronto, Ontario, Canada

Background: The clinical hallmark of Alzheimer's disease (AD) is a progressive decline in cognitive function. The sequence of this decline often follows a stereotyped course; patients first show difficulty forming new memories, then deficits in retrieving older memories followed by deficits in other cognitive domains, and a loss of overall bodily functions. The ultimate outcome of AD is death. B-amyloid (A-beta) is widely implicated in the neuropathology underlying AD and chronically high levels of A-beta may induce cell death. This neurodegeneration readily accounts for the memory and cognitive impairment observed in the later stages of AD. In the early stages of AD, however, patients show deficits in forming new memories and high levels of A-beta but no detectable cell death. This suggests that high levels of A-beta may directly interfere with the synaptic plasticity required for normal memory formation. How A-beta impairs memory is unknown. *In vitro*, high levels of A-beta have been shown to decrease synaptic strength by promoting the internalization of postsynaptic AMPA-type glutamate receptors (AMPARs), suggesting the some of the memory deficits in AD may be due to excessive AMPAR internalization. Here we investigated the role of AMPAR internalization in the memory deficits observed in several types of mice genetically designed to recapitulate important aspects of AD.

Methods: We examined the effects of acutely or chronically increasing A-beta levels on the ability of mice to form stable long-term spatial and contextual fear memories. To transiently increase A-beta levels in wild-type (WT) mice we used replication-defective herpes simplex viral (HSV) vectors expressing human amyloid precursor protein (APP) containing both the Swedish and Indiana familial AD mutations. To chronically increase A-beta levels we used TgCRND8 mice, transgenic mice that chronically express the same mutated APP construct. We used several methods to interfere with AMPAR internalization. First we used viral vectors to express a peptide designed to specifically interfere with GluA2-containing AMPAR endocytosis (GluA2-3Y peptide). Second, we use TAT (Trans-Activator of Transcription) proteins to systemically deliver this small interfering GluA2-3Y peptide. Importantly, the use of this non-toxic method of delivering this peptide via systemic administration may facilitate the translation of our results to the clinic. As a third method to disrupt GluA2-containing AMPAR endocytosis, we targeted Arf6 (ADP-ribosylation factor 6) expression, which is thought to be critical for this form of clathrin-mediated endocytosis.

Results: We observed that either acute or chronic increases in A-beta levels impaired the ability of mice to form stable long-term spatial or a contextual fear memory without inducing cell death. These memory consolidation deficits were accompanied by a decrease in the surface levels of GluA2-containing AMPAR, suggesting that the loss of GluA2-containing AMPAR may be responsible for the memory deficits. Consistent with this interpretation, we observed a strikingly similar phenotype when we used viral vectors to express a constitutively active from of Arf6 (Arf6-Q67L) to induce endocytosis of GluA2-containing AMPAR only in the hippocampus of WT mice. Moreover, the memory deficits induced by increasing A-beta levels were occluded by directly activating Arf6, suggesting that A-beta was acting through this pathway to produce the memory deficits. These results are consistent with the interpretation that high levels of A-beta produce memory deficits by facilitating GluA2-containing AMPAR endocytosis. To examine this more directly, we tested whether it was possible to reverse the memory deficits produced by high levels of A-beta by disrupting AMPAR trafficking. Importantly, transiently interfering with AMPAR internalization (by either a specific interfering peptide or interfering with Arf6 function) was sufficient to reverse the memory deficits produced by either acute or chronic overexpression of A-beta. Together, these data are consistent with the interpretation that high levels of A-beta impair memory by inducing the loss of surface GluA2-containing AMPAR. It is now well-appreciated that memories, even after consolidation, are modifiable. The process of remembering is thought to reactivate memory representations in the brain. The reactivated memory is re-stored in a second wave of consolidation referred to as reconsolidation. Strikingly, similarly disrupting AMPAR endocytosis during a memory reminder enabled the recovery of an otherwise inaccessible memory in mice with chronically high A-beta levels. This result suggests that memory reactivation and subsequent

reconsolidation may open a 'window of plasticity' in which otherwise 'lost' memories may be successfully reconsolidated (and consequently 'recovered') by disrupting AMPAR internalization at the time of the reminder. Our findings raise the possibility that targeting AMPAR trafficking could restore both the ability to form new memories as well as enable recovery of lost past memories in AD patients.
Conclusions: The results from these studies may not only lead to a better understanding of how A-beta disrupts memory but may help identify a novel therapeutic strategy to allow AD patients to form new memories as well as recover 'lost' memories.
Keywords: memory mouse consolidation reconsolidation Alzheimer's disease.
Disclosures: S. Josselyn, Nothing to Disclose; A. Yiu, Nothing to Disclose; V. Mercaldo, Nothing to Disclose; D. Sargin, Nothing to Disclose; P. Frankland, Nothing to Disclose.

T93. Fragile X Mental Retardation Protein (FMRP) – Metabotropic Glutamate Receptor 5 (mGluR5) Signaling in Schizophrenia and Autism

S Hossein Fatemi*, Timothy Folsom

University of Minnesota, Minneapolis, Minnesota

Background: FMRP is an RNA binding protein with 842 target mRNAs in human brain and normally functions as a translational repressor. Loss of FMRP is due to gene silencing of fragile X mental retardation 1 (FMR1) which leads to unregulated activity of group 1 metabotropic glutamate receptors (mGluRs), particularly mGluR5, ultimately leading to the cognitive and behavioral deficits of fragile X syndrome. Our laboratory has previously shown reduction in FMRP and increase in mGluR5 in cerebellar vermis and superior frontal cortex (BA9) of individuals with autism; reductions in FMRP and mGluR5 in lateral cerebella of subjects with schizophrenia, bipolar disorder, and major depression; and reductions for both proteins in frontal cortex of subjects with schizophrenia and bipolar disorder. These novel findings suggest dysregulation of FMRP-mGluR5 signaling in major mental disorders. In the current study we expand upon these results to investigate expression of targets of FMRP-mGluR5 signaling in subjects with schizophrenia, bipolar disorder, and major depression.
Methods: Postmortem, frontal cortex samples were derived from subjects with schizophrenia ($N=20$), bipolar disorder ($N=19$), and matched controls ($N=29$) and postmortem, lateral cerebellum samples were derived from subjects with schizophrenia ($N=15$), bipolar disorder ($N=15$), major depression ($N=15$), and matched controls ($N=15$). We will investigate expression of four targets of FMRP and mGluR5 signaling—homer 1, amyloid beta A4 precursor protein (APP), ras-related C3 botulinum toxin substrate 1 (RAC1), and striatal-enriched protein tyrosine phosphatase (STEP)—that we have previously demonstrated to have altered expression in brains of subjects with autism, in subjects with schizophrenia and mood disorders. SDS-PAGE and western blotting will be performed. All protein measurements for subjects with schizophrenia, bipolar disorder, major depression, and control subjects will be normalized against neuronal specific enolase (NSE) and β-actin. Group differences will be analyzed statistically using student's t-test. Significant differences will be defined as those with a p value < 0.05. Confound effects (ie, age and PMI) will be examined using Pearson's correlation test.
Results: Experiments are currently underway and we hypothesize to find altered expression of some or all of the four targets of FMRP-mGluR5 signaling in schizophrenia or mood disorders.
Conclusions: Our results in subjects with autism are the first to demonstrate altered expression of FMRP targets in the cerebellar vermis and superior frontal cortex of subjects with autism. It is likely, due to our previous findings that FMRP and mGluR5 are altered in subjects with schizophrenia and mood disorders, that FMRP targets will similarly be altered in these disorders.
Keywords: schizophrenia, autism, FMRP, mGluR5, mood disorder.
Disclosures: S. Fatemi; T. Folsom.

T94. Orbitofrontal Cortical Dendritic Spines: Markers of Adolescent (Stressor) Experience and Determinants of Habit Formation

Elizabeth A Hinton, Andrew M Swanson, Shannon L Gourley*

Emory University, Atanta, Georgia

Background: My group has dedicated considerable attention to the modulatory influence of adolescent experience on dendritic spine density and structure. Here we will summarize evidence using two unique models of adolescent adversity, in both male and female mice, that deep-layer excitatory orbitofrontal cortical neurons remodel considerably in response to pathological stimuli during adolescence. Remarkably, orbitofrontal cortical neurons fail to recover by adulthood, providing evidence that adverse experience during adolescence fundamentally derails the developmental trajectory of this critical neuron population.
Methods: Male mice were exposed to corticosterone via the drinking water from postnatal day (P) 35–56. Female mice were socially isolated from P31–56, then socially reintegrated in adulthood. Throughout, transgenic GFP-expressing mice were used, allowing us to image and reconstruct deep-layer orbitofrontal cortical neurons, a neuron population that is largely spared by Golgi-Cox impregnation.
Results: Adolescent corticosteroid exposure eliminated orbitofrontal cortical dendritic spines, and unlike in the infralimbic cortex, reduced spine density in this region persisted into adulthood. Social isolation, by contrast, impaired dendritic spine pruning during adolescence, resulting in a persistently proliferative phenotype. Dendritic spine profiles were associated with chronic anhedonic-like behavior and a propensity to develop stimulus-response habits at the expense of engagement in goal-directed response strategies in adulthood.
Conclusions: We argue that both adolescent stress hormone exposure and social isolation commonly derail the normal developmental trajectory of deep-layer orbitofrontal cortical spines. We will discuss implications for orbitofrontal cortical coordination of action and habits.

Keywords: orbital, prefrontal, actions, habits, spine.
Disclosures: E. Hinton, Nothing to Disclose; A. Swanson, Nothing to Disclose; S. Gourley, Nothing to Disclose.

T95. The Methyltransferase PRDM2 Regulates Escalated Alcohol Consumption

Estelle Barbier*, Jenica Tapocik, Andrea L Johnstone, Jesse Schank, Zhifeng Zhou, Qiaoping Yuan, David Goldman, Claes Wahlestedt, Markus Heilig

NIH, Bethesda, Maryland

Background: Rats subjected to repeated cycles of alcohol intoxication and withdrawal develop a marked and long lasting increase in voluntary alcohol intake. However, the underlying neural adaptations are not well understood. Recent data suggest that epigenetic alterations such as histone modifications and DNA methylation may play a role in several diseases including alcoholism. In this study, we investigated whether long-term and intermittent alcohol exposure modify the expression of 'epigenetic enzymes' and whether these modifications could play a role in the escalated alcohol consumption observed in our alcohol post-dependent rats model.
Methods: Alcohol post-dependent Rats were exposed to alcohol vapor for 7 weeks, 14 h/day to induce alcohol dependence. To identify persistent neuroadaptations rather than transient changes associated with acute intoxication or withdrawal, behavioral and molecular tests were performed 3 weeks after completion of alcohol exposure. Total RNA from the dorsal medial prefrontal cortex (dmPFC) was extracted and run through the whole transcriptome sequencing protocol ($n = 4$/group). The raw sequencing data was Log2 transformed and normalized using the Limma package from Bioconductor. The normalized reads were mapped to rat genomic sequences (UCSC rn4) using Bowtie. shRNA lentiviruses specific for prdm2 and kdm6b were injected in the dmPFC of naïve rats and oral alcohol self-administration behavior was assessed under a fixed ratio 1 in 30 min sessions.
Results: Whole transcriptome sequencing generated a total of 30 million reads. 783 genes were differentially regulated between post-dependent and control rats. Among those genes we found significant and sustained decrease in expression of epigenetic-related enzymes in the dmPFC after chronic alcohol vapor exposure. More specifically, chronic alcohol vapor exposure decreased the expression of the histone lysine 9 methyltransferase 'PRDM2' and the histone lysine 27 demethylase 'KDM6B'. Specific inhibition of prdm2 in the dmPFC of alcohol naïve rats significantly increased alcohol self-administration. However; inhibition of kdm6b in the dmPFC had no effect on self-administration behavior.
Conclusions: Our results show a significant decrease of prdm2 and kdm6b in the dmPFC following a history of alcohol dependence. Inhibition of prdm2 but not kdm6b specifically in the dmPFC of alcohol naïve rats increased alcohol self-administration suggesting that prdm2 may play a critical role in escalated alcohol self-administration observed in our alcohol post-dependent rat model.

Keywords: alcohol dependence, epigenetic, histone methyltransferase.
Disclosures: E. Barbier, Nothing to Disclose; J. Tapocik, Nothing to Disclose; A. Johnstone, Nothing to Disclose; J. Schank, Nothing to Disclose; Z. Zhou, Nothing to Disclose; Q. Yuan, Nothing to Disclose; D. Goldman, Nothing to Disclose; C. Wahlestedt, Nothing to Disclose; M. Heilig, Nothing to Disclose.

T96. Alterations in Telencephalic Neuronal Fate, Neuronal Calcium Signaling and Neurotransmitter Release in iPSC Models of Bipolar Disorder

Melvin McInnis*, Monica Bame, Haiming Chen, Cynthia J DeLong, Todd J Herron, Omar Mabrouk, Robert Kennedy, K Sue O'Shea

University of Michigan, Ann Arbor, Michigan

Background: A major challenge in studying complex, human neuropsychiatric disorders such as Bipolar Disorder (BPD) has been the lack of reliable cell models. Patient-derived induced pluripotent stem cells (iPSC) now offer the opportunity to study a full range of neural tissues and the exciting prospect of identifying novel disease mechanisms in BPD. We have derived fibroblast cell lines from15 patients with BPD and 6 Controls, reprogrammed them into iPSC, which have been extensively characterized.
Methods: The iPSC were differentiated into neurons by growth in suspension culture in medium containing nodal and BMP inhibitors, followed by plating on polyornithine/laminin-coated coverslips. With reprogramming, pluripotency factors were induced, while levels of fibroblast-restricted genes were down-regulated. With neuronal differentiation, expression of transcripts for membrane receptors and ion channels were significantly increased in BPD-derived neurons compared to controls, and expression of transcription factors involved in the specification of neuronal identity altered. Control neurons expressed transcripts that confer dorsal telencephalic fate, while neurons derived from BPD iPSC expressed genes involved in the differentiation of ventral (LGE) regions. To simultaneously measure action potential (Vm) and calcium wave propagation, we employed Fluo-4 AM optical mapping of BPD and C neurons after four and eight weeks of differentiation + 24 h pre-treatment with 1 mM lithium chloride, the most common and effective treatment for BPD.
Results: Exposure of BPD neurons to lithium significantly decreased their calcium transient ($p < 0.007$) and wave amplitude ($p < 0.018$) following stimulation with 50 mM KCl, compared with lithium-exposed Control neurons. To determine what neurotransmitters the neurons released, we carried out mass spec analysis of 21 analytes in supernatants from the calcium imaging experiments, as well as from pooled culture medium from BPD or C neurons collected over a 12 week period. Levels of serotonin and the dopamine metabolite DOPAC were significantly higher in media from unstimulated BPD *vs* C neurons, while both adenosine and glutamine were significantly increased in stimulated BPD neurons following lithium treatment.
Conclusions: Since current evidence suggests that subtle alterations in neurodevelopmental pathways can produce

consequences that only become apparent much later in life, iPSC lines provide a unique opportunity to develop models of neuroaffective disorders such as BPD, with the long term goals of identifying alterations in their differentiation and thereby novel treatment approaches. This functional model of neuronal behavior in BPD holds the potential for testing novel molecules for their effect in this specific model with implications for a basis for later clinical testing in the clinical disorder.
Keywords: bipolar disorder iPSC calcium.
Disclosures: M. McInnis, **Part 1:** Merck Pharmaceuticals, **Part 2:** Merck Pharmaceuticals; M. Bame, Nothing to Disclose; H. Chen, Nothing to Disclose; C. DeLong, Nothing to Disclose; T. Herron, Nothing to Disclose; O. Mabrouk, Nothing to Disclose; R. Kennedy, Nothing to Disclose; K. O'Shea, Nothing to Disclose.

T97. Modulation of Dopamine Transporter by DISC1 Assemblies: A Novel Pharmacological Target

Verian Bader, Svenja Trossbach, Ingrid Prikulis, Sandra Schäble, Angelica de Souza, Zoe A Hughes, Nicholas Brandon, Joseph Huston, Carsten Korth*

University of Düsseldorf, Düsseldorf, Germany

Background: Disrupted-in-schizophrenia 1 (DISC1) is a major gene of behavioral control that has been genetically linked ans associated to mental illnesses of various chronic behavioral disorders like schizophrenia across ethnicities. Schizophrenia, is a so far exclusively clinically defined, chronic, multifactorial psychiatric disease, characterized by a disturbed dopaminergic balance between the striatum and the medial prefrontal cortex. In previous studies we demonstrated insoluble, mutlimeric DISC1 protein in a subgroup of patients with chronic mental illnesses with different clinical phenotypes. Here, we investigated whether DISC1 assembly to insoluble multimers was related to dopamine metabolism.
Methods: To address this interplay *in vitro*, we generated a tetracyclin-inducible, full length non-mutant human DISC1 neuroblastoma cell line. We performed a detailed biochemical and cell biological analysis and measured dopamine kinetics by HPLC and a reporter assay. To model human DISC1 aggregation and its role in DA homeostasis *in vivo* we generated a transgenic rat model where full length, non-mutant DISC1 is slightly overexpressed. We also performed immunohistochemical stainings of human brains with mental illnesses and normal controls.
Results: Upon exposure to high DA, DISC1 formed distinct perinuclear and reversible aggresomes and led to a characteristic high molecular weight immunoreactive band in Western blots. DISC1 induction suppressed clearance of extracellular DA clearance, an effect that was potentiated by the formation of DISC1 aggregates. These effects were mediated through a functional interaction with the dopamine transporter (DAT). A DISC1 transgenic rat with abundant DISC1 aggregates and wild type rats inoculated with exogenous, cell-invasive DISC1 aggregates were hypersensitive to amphetamine, indicating of a DA dysregulation phenotype *in vivo*. Corroborating our earlier data on biochemical purification of insoluble DISC1 from human brains we demonstrate perinuclear DISC1 assemblies in human cases with mental illness but not normal controls.
Conclusions: We propose a biological function for DISC1 multimeric assemblies in regulating the clearance of the metabolite and neurotransmitter dopamine through an interaction with DAT. DISC1 assemblies are a novel target for dopamine-modulating pharmaceuticals.
Keywords: dopamine homeostasis protein assemblies protein aggregates dopamine transporter Disrupted-in-schizophrenia 1.
Disclosures: V. Bader, Nothing to Disclose; S. Trossbach, Nothing to Disclose; I. Prikulis, Nothing to Disclose; S. Schäble, Nothing to Disclose; A. de Souza, Nothing to Disclose; Z. Hughes, **Part 5:** Pfizer Inc.; N. Brandon, **Part 5:** Astra Zeneca; J. Huston, Nothing to Disclose; C. Korth, Nothing to Disclose.

T98. Role of Hippocampal ΔFosB in Associations of Cocaine with Environment

Andrew Eagle, Paula Gajewski, Pamela Kennedy, Alfred Jay Robison*

Michigan State University, East Lansing, Michigan

Background: The hippocampus underlies the learning of associations between the environmental context and salient stimuli including exposure to drugs of abuse. In addition, stimulation of the hippocampus causes enhanced release of dopamine into the nucleus accumbens, a mechanism for the rewarding properties of drugs, suggesting that the hippocampus may play a role in both determining a mechanism for drug acquisition and in drug reward. Indeed, stimulation of the hippocampus elicits cocaine-seeking behavior in rodent models of addiction. However, it is unknown how chronic exposure to drugs alters hippocampal function, and whether such alterations may underlie the aberrant valuation of drugs and the strong associations of drugs with environmental cues that comprise the addiction phenotype. Although the transcription factor ΔFosB is induced in the hippocampus by chronic drug exposure, and its expression in other brain regions regulates drug reward, the roles of ΔFosB in hippocampal function in general and in the ability of the hippocampus to mediate the strong association of drug with environment remain unknown. Therefore, we explored whether formation of associations between drugs and spatial context alter gene expression in the hippocampus, and sought to determine the role of ΔFosB in hippocampal neuronal gene expression and morphology and the whether hippocampal ΔFosB expression can control formation of spatial memories and the association of drugs with specific environments.
Methods: All mice used in this study were male C57Bl6/J strain from Jackson Labs; all rats were male Sprague-Dawley strain from Charles River Labs; and all experiments were performed in accordance with Michigan State University and Mount Sinai School of Medicine IACUC-approved protocols. The water maze was a white round water pool (diameter: 1 m, height: 0.6 m) in which a platform (8 cm^2) was located 1 cm below the water surface in the center of one of the four quadrants. The pool was filled with water (22–24°C, depth: 25 cm), and a curtain was used to cover

spatial cues during visible platform control experiments. The novel environment was a 50×30 cm plexiglass case with multiple fixed objects and spatial cues. Mice were exposed to the novel environment for 45 min per day for 7 days. Conditioned spatial preference was performed in a round white chamber 1 m in diameter divided into 4 quadrants by internal walls. Pairing with cocaine (15 mg/kg) or saline was 25 min for 5 days, and pre- and post-tests were 15 min in the open maze. Human hippocampal samples were provided by the Bell Canada Brain Bank. ΔFosB immunohistochemistry was performed using the SC-48 antibody (Santa Cruz) and visualized using DAB staining, and ΔFosB Western blotting used the 5G4 antibody (Cell Signaling). Viral vectors were purchased from the Viral Gene Transfer Core at MIT, injected into the hippocampus by stereotaxic surgery (coordinates from Bregma (mm): A/P −2.0, M/L +1.9, D/V −1.6), visualized by confocal microscopy and analyzed using Neuron Studio software.

Results: We find that ΔFosB is induced in the hippocampus, but not the nucleus accumbens, by exposure to novel environments and spatial learning, and is induced in both brain regions by formation of associations between the environment and cocaine exposure. We also demonstrate that this induction of ΔFosB by spatial learning is specific to the CA1 region of the hippocampus. In addition, we find global changes in covalent modification of histones in animals exposed to cocaine in the conditioned spatial preference maze, indicating that patterns of hippocampal gene expression are regulated by associating cocaine exposure with a novel environment. We further support this finding by observing similar global changes in covalent modification of histones in the hippocampus of human cocaine addicts. We also show that overexpression of ΔFosB can control dendritic spine formation in the CA1 but not the CA3 region of the hippocampus, correlating with our expression data. Finally, we explore whether hippocampal ΔFosB expression can regulate performance in the conditioned spatial preference task.

Conclusions: ΔFosB is a unique candidate factor for spatial memory associations because, unlike other immediate early genes, it possesses remarkable stability (weeks in a living brain) and its known gene targets in NAc include proteins involved in synaptic function. Here, we demonstrate that ΔFosB is induced in the mouse hippocampus specifically by spatial learning and by the association of a salient cue (cocaine) with a spatial location. Because gene targets of ΔFosB previously identified in other brain regions are known to be key players in hippocampal learning and synaptic function (like CaMKII and subunits of the AMPA glutamate receptor), we hypothesize that ΔFosB could regulate hippocampal synaptic funtion. Indeed, we find that ΔFosB overexpression increases dendritic spines specifically in the CA1 region, and future studies will focus on ΔFosB regulation of hippocampal physiological properties. Overall, these studies suggest that hippocampal ΔFosB may be important for the associations of drug and environment that underlie location-induced drug craving and relapse, and thus that hippocampal ΔFosB and its downstream targets may represent novel therapeutic inroads for the treatment or prevention of drug addiction.

Keywords: hippocampus, cocaine, DeltaFosB, learning, transcription.

Disclosures: A. Eagle, Nothing to Disclose; P. Gajewski, Nothing to Disclose; P. Kennedy, Nothing to Disclose; A. Robison, Nothing to Disclose.

T99. Overexpression of the Steroidogenic Enzyme Cytochrome P450 Side Chain Cleavage in the Ventral Tegmental Area Increases 3α,5α-THP and Reduces Long-term Operant Ethanol Self-administration in Alcohol Preferring Rats

A Leslie Morrow*, Jason B Cook, David F Werner, Antoniette M Maldonado-Devincci, Maggie N Leonard, Kristen R Fisher, Todd K O'Buckley, Patrizia Porcu, Thomas J McCown, Clyde Hodge, Joyce Besheer

University of North Carolina Chapel Hill, Chapel Hill, North Carolina

Background: Neuroactive steroids are neuromodulators synthesized in the brain that influence emotional and addictive behaviors. Some neuroactive steroids alter ethanol consumption when administered systemically. Pregnenolone reduces operant ethanol self-administration in alcohol-preferring (P) rats (Besheer *et al*, 2010). The GABAergic neuroactive steroid (3α,5α)-3-hydroxypregnan-20-one (3α,5α-THP or allopregnanolone) produces biphasic effects on ethanol consumption, with low doses increasing and high doses decreasing consumption (Janak *et al*, 1998; Ford *et al*, 2005). To optimize the therapeutic potential for neuroactive steroids, we have employed viral vector-mediated gene delivery to identify sites in brain where neurosteroids modulate ethanol drinking. We developed an adeno-associated viral vector (rAAV) to drive neurosteroidogenesis by delivery of cytochrome P450 side chain cleavage (P450scc) in the ventral tegmental area (VTA) and nucleus accumbens (NAc). P450scc converts cholesterol to the neuroactive steroid pregnenolone, which is the rate-limiting enzymatic reaction in steroidogenesis. However, the subsequent production of GABAergic neuroactive steroids occurs only in principal neurons that express the requisite biosynthetic enzymes.

Methods: First, we determined that the P450scc expressing vector (rAAV2-P450scc) increases P450scc mRNA in cultured cells and pregnenolone levels in the cell media. Next, we determined that rAAV2-P450scc vector infusion increases P450scc mRNA and protein levels *in vivo*. The effect of rAAV2-P450scc vector delivery to VTA and NAc on ethanol reinforcement and consumption was investigated in alcohol-preferring (P) rats. rAAV2-P450scc or control green fluorescent protein (GFP) expressing vector (rAAV2-GFP) was injected bilaterally into the VTA (2 μl/hemisphere, $n = 14$–15/group) or NAc (3 μl/hemisphere, $n = 7$/group) of alcohol preferring (P) rats previously trained to self-administer ethanol. Following viral vector injection, the animals were given 1 week to recover from surgery before operant self-administration sessions resumed for 2–3 weeks. Subsequently, the rats were tested for effects on locomotor activity and thigmotaxis. Finally, immunostaining for the GABAergic steroid 3α,5α-THP was conducted in free-floating brain sections. Co-localization with tyrosine hydroxylase (TH) was also evaluated in these regions.

Results: rAAV2-P450scc vector delivery to the VTA reduced ethanol responding by 20% (two-way ANOVA, $p < 0.005$) and ethanol intake by 14% (two-way ANOVA, $p < 0.01$)

compared to control rats over the test period. There was no effect on water intake. In contrast, P450scc overexpression in the NAc did not alter ethanol or water self-administration. General locomotor activity and thigmotaxis were not altered by rAAV2-P450scc vector administration in the VTA or NAc. P450scc overexpression in in the VTA produced a 36% increase in 3α,5α-THP positive cells in the VTA, however, transduction of NAc did not increase local 3α,5α-THP immunoreactivity. Some VTA neurons were co-labeled with 3α,5α-THP and TH, while others were solely labeled by 3α,5α-THP or TH.

Conclusions: These results provide evidence that P450scc gene delivery was associated with an increase of 3α,5α-THP positive cells in the VTA and a reduction of ethanol reinforcement and consumption. It is noteworthy that reduced responding was persistent across 3 weeks of testing. Additional studies are needed to determine the dose effect of vector injection, since the changes in operant responding were modest. Nonetheless, these data provide evidence that P450scc gene delivery in the VTA reduces ethanol reinforcement with no evidence of sedation or changes in water responding. The ability of 3α,5α-THP to modulate ethanol drinking is likely due to modulation of neural circuitry via $GABA_A$ receptor-mediated neuronal inhibition. Cellular localization of 3α,5α-THP was found in projecting principal GABAergic and glutamatergic neurons, but not in interneurons or glial cells (Saalmann *et al*, 2007). This same pattern of expression has also been observed for the biosynthetic enzymes 5α-reductase and 3a-hydroxysteroid dehydrogenase, which are needed for 3α,5α-THP synthesis (Agis-Balboa *et al*, 2006). The lack of change in ethanol responding following rAAV2-P450scc vector injection in NAc may relate to the lack of an increase in local 3α,5α-THP in this region. Nevertheless, we show that 3α,5α-THP is located in multiple cell types, including dopaminergic cells in the VTA. Therefore, 3α,5α-THP may endogenously regulate activity of mesocorticolimbic cells, which are involved in drug reinforcement and motivation. Vector-mediated gene delivery is a useful tool for studying the role of neuroactive steroids in ethanol reinforcement and consumption, and may lead to the development of new therapeutic strategies for the treatment of alcoholism.

Keywords: neuroactive steroids, gene delivery, P450scc, allopregnanolone, 3α,5α-THP.

Disclosures: A. Morrow, Nothing to Disclose; J. Cook, Nothing to Disclose; D. Werner, Nothing to Disclose; A. Maldonado-Devincci, Nothing to Disclose; M. Leonard, Nothing to Disclose; K. Fisher, Nothing to Disclose; T. O'Buckley, Nothing to Disclose; P. Porcu, Nothing to Disclose; T. McCown, Nothing to Disclose; C. Hodge, Nothing to Disclose; J. Besheer, Nothing to Disclose.

T100. Increased Rage, TLRs, and HMGB1 Expression in the Human Alcoholic Orbitofrontal Cortex Is Linked to Adolescent Drinking

Ryan P Vetreno*, Liya Qin, Fulton T Crews

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Background: We hypothesized that adolescent binge drinking induces long-lasting changes in the expression of receptor for advanced glycation end-products (RAGE) and Toll-like receptors (TLRs) as well as their endogenous agonist, high-mobility group box 1 (HMGB1). We have previously found that adolescent binge ethanol exposure in rats persistently upregulates expression of these proteins in the brain. We measured the expression of these signaling molecules in the human post-mortem brain to determine if they are upregulated in alcoholics and whether their expression is related to age of drinking onset.

Methods: Immunohistochemistry and Western blot analysis was used to compare levels of RAGE, TLRs, and HMGB1 in the post-mortem human alcoholic orbitofrontal cortex with moderate drinking controls. Expression of RAGE, TLRs, and HMGB1 was correlated with age of drinking onset and lifetime alcohol consumption as assessed by the New South Wales Tissue Resource Centre at the University of Sydney using patient and family interviews.

Results: In the post-mortem human alcoholic orbitofrontal cortex, we found increased RAGE (196%, $p<0.05$), TLR2 (208%, $p<0.01$), TLR3 (259%, $p<0.01$), and TLR4 (356%, $p<0.01$) immunoreactivity, relative to moderate drinking controls. Similarly, Western blot analysis found elevated protein levels of RAGE (176%, $p<0.05$), TLR2 (147%, $p<0.05$), TLR3 (180%, $p<0.05$), and TLR4 (184%, $p<0.01$), relative to moderate drinking controls. We also found increased expression of HMGB1-immunoreactive cells (213%, $p<0.05$) and Western blot HMGB1 protein (151%, $p<0.01$) in the alcoholic orbitofrontal cortex. Confocal microscopy revealed that the majority of RAGE (78%), TLR2 (90%), TLR3 (89%), TLR4 (80%), and HMGB1 (83%) colocalized with NeuN-positive neurons. Across subjects, HMGB1 positively correlated with RAGE and TLR expression ($r=0.85$, $p<0.01$), which is consistent with neuroimmune loops of amplification. We also found that age of drinking onset ($r=-0.63$, $p<0.01$) and lifetime alcohol consumption ($r=0.78$, $p<0.01$) correlated with RAGE/TLR-HMGB1 signaling.

Conclusions: These studies suggest that alcohol upregulates RAGE/TLR-HMGB1 signaling in the OFC, which appear to be dependent on age of drinking onset.

Keywords: neuroimmune, prefrontal cortex, RAGE, HMGB1.

Disclosures: R. Vetreno, Nothing to Disclose; L. Qin, Nothing to Disclose; F. Crews, Nothing to Disclose.

T101. The Extent of the Incorporation of the G Protein, Gsα, in Lipid Raft Membrane Fractions from Erythrocyte Membranes May Provide a Biomarker for Major Depressive Disorder

Mark M Rasenick*, Robert Donati, Cynthia Fu, Sergi Costafreda, Peng Liu, Lauren Marangell

University of Illinois at Chicago, Chicago, Illinois

Background: Lipid rafts are specialized membrane domains rich in cholesterol and intimately associated with cytoskeletal components. G protein signaling is influenced by lipid rafts, but, depending upon the receptor, G protein, and effector enzyme, signaling components are either brought into close association or driven into separate membrane microdomains (Allen *et al*, 2007; Allen *et al*, 2009). The former facilitates signaling and the latter attenuates

signaling. We have demonstrated that, for Gs and Gs-coupled receptors (b-adrenergic, VPAC and 5HT-4, -6, -7), lipid rafts attenuate signaling by sequestering Gsa and adenylyl cyclase in separate membrane compartments. Experiments from several different laboratories suggest a post-synaptic effect of chronic antidepressants and a possible postsynaptic target for these drugs. Data from rats, cultured neural and glial cells, all suggest that the localization of the G protein, Gsa, in lipid rafts is modified by chronic treatment with a number of antidepressant compounds (Donati and Rasenick, 2005; Zhang and Rasenick, 2010). Antidepressants facilitate translocation of Gsa from lipid rafts while post mortem studies show increased Gsain raft fractions from several brain regions of depressed suicide subjects relative to controls (Donati *et al*, 2008). In this study, we sought to determine whether raft fractions prepared from platelet and erythrocyte membranes of depressed subjects showed enrichment of Gsa in lipid raft fractions.

Methods: Subjects were patients with a DSM-IVR diagnosis of major depressive disorder (MDD) ($n = 32$) in an acute episode and age, gender, and IQ matched healthy controls ($n = 25$). All MDD patients were medication-free at baseline. Subjects had blood samples taken after giving informed consent. Membrane, cytosol and lipid raft fractions of platelets and RBC were prepared, coded, and assayed by persons blind to clinical status or treatment. Gsa was extracted, sequentially with Triton X-100 (non- raft fraction) and Triton X 114 (raft fraction) and Gsa was identified and quantified by immunoblotting. Other G proteins and cytoskeletal proteins were similarly assayed in these membrane fractions.

Results: The mean ratio between Gsa in the non-lipid raft fraction (TX-100) *vs* Gsa in the raft (TX-114) fraction was numerically greater in RBC prepared from depressed subjects than those from healthy controls (1.36 *vs* 0.80, $p = 0.051$).

Conclusions: These data suggest the possible development of a blood test to indicate the presence of depression. Combining biochemical data with fMRI and other biochemical measures may contribute to a more powerful biosignature in MDD. This study was sponsored by Eli Lilly and Company, Indianapolis, IN, USA.

References:

Allen J, Halverson-Tamboli R, Rasenick MM (2007). Lipid raft microdomains and neurotransmitter signaling. *Nature Reviews Neuroscience* **8**: 128–140.

Allen JA, Yu J-Z, Dave RH, *et al* (2009) Caveolin-1 and lipid microdomains regulate Gs trafficking and attenuate Gs/adenylyl cyclase signaling. *Mol Pharmacol* **76**: 1082–10893.

Donati RJ, Rasenick MM (2005) Chronic Antidepressant Treatment Prevents Accumulation of Gsalpha in Cholesterol-Rich, Cytoskeletal-Associated, Plasma Membrane Domains (Lipid Rafts). *Neuropsychopharmacology* **30**: 1238–1245.

Donati RJ, Dwivedi Y, Roberts RC, *et al* (2008) Post-mortem Brain Tissue of Depressed Suicides Reveals Increased Gs Localization in Lipid Raft Domains Where it is Less Likely to Activate Adenylyl Cyclase. *J Neuroscience* **28**: 3042–3050.

Zhang L, Rasenick MM (2010) Chronic treatment with escitalopram but not R-citalopram translocates Galpha s from lipid raft domains and potentiates adenylyl cyclase: a 5-hydroxytryptamine transporter-independent action of this antidepressant compound. *J Pharmacol Exp Ther* **332**: 977–984.

Keywords: major depressive disorder, G protein, depression, lipid rafts, duloxetine.

Disclosures: M. Rasenick, **Part 4:** Eli Lilly and Company; R. Donati, **Part 1:** Pax Neuroscience; C. Fu, **Part 1:** Eli Lilly and Company, **Part 4:** Eli Lilly and Company; S. Costafreda, **Part 1:** Eli Lilly and Company, **Part 4:** Eli Lilly and Company; P. Liu, **Part 1:** Eli Lilly and Company, **Part 2:** Eli Lilly and Company, **Part 3:** Employment, Eli Lilly and Company, **Part 5:** Eli Lilly and Company; L. Marangell, **Part 1:** Eli Lilly and Company, **Part 2:** Eli Lilly and Company, **Part 3:** Employee, Eli Lilly and Company, **Part 5:** Eli Lilly and Company.

T102. Epigenetic Enzyme Expression Changes Associated with Alcohol Dependence

Andrea L Johnstone*, Christopher A Rienas, Estelle Barbier, Jenica Tapocik, Markus Meinhardt, Shaun P Brothers, Wolfgang H Sommer, Markus Heilig, Claes Wahlestedt

University of Miami Miller School of Medicine, Miami, Florida

Background: Epigenetic signaling pathways modify DNA methylation and post-translational histone modifications to encode transcriptomic changes in response to environmental cues. Enzymes that oversee these modifications are increasingly recognized to mediate behaviors associated with drug and alcohol dependence. We hypothesize that alcohol exposure influences long-term gene expression and behavioral abnormalities through changes in epigenetic enzyme activity.

Methods: We used a post-dependent rat model in order to characterize epigenetic enzyme expression changes in the brain that are induced by alcohol exposure. In this model, rats are chronically exposed to increasingly intoxicating concentrations of ethanol vapors over a period of seven weeks. Previous studies have shown that these rats exhibit behavioral and molecular changes reminiscent of human alcoholics, including (1) symptoms of tolerance and withdrawal; (2) long-term voluntary increases in alcohol consumption over rats exposed to control vapors; (3) increased sensitivity to stress; and (4) chronic transcriptional changes in pathways implicated in alcohol dependence. Three weeks after ethanol exposure, Nanostring nCounter analysis was used to quantify mRNA expression levels of over 100 epigenetic enzymes in the nucleus accumbens (NAc) of post-dependent and control rats. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was used to validate significantly altered genes in both the NAc and the prefrontal cortex (PFC). Several enzymes of interest were also quantified using qRT-PCR of PFC tissue from human alcoholics. Finally, Western blot analysis was used to quantify the protein levels and histone target modification of one histone demethylase that was dysregulated at the RNA level.

Results: RNA levels of a known histone demethylase, KDM6B, were downregulated in both the PFC and NAc of post-dependent rats. In postmortem brain tissue from

human alcoholics, KDM6B RNA was upregulated within a region of the PFC of alcoholics compared to controls. Examination of protein levels showed that KDM6B protein is increased in the NAc of post-dependent rats. In contract, levels of histone H3 lysine 27 trimethylation (H3K27me3), a known target modification of KDM6B, were unchanged.
Conclusions: KDM6B is dysregulated in key brain regions involved in reward perception in a post-dependent rodent model of alcoholism as well as in human alcoholics. Ongoing experiments aim to (1) identify genomic regions regulated by KDM6B; (2) to study alcohol-seeking behavior in response to KDM6B knockdown; and (3) examine functions of KDM6B outside of H3K27 demethylation. These studies may elucidate how an epigenetic mechanism translates alcohol exposure into the chronic transcriptional and behavioral changes that underlie alcohol dependence.
Keywords: alcoholism, alcohol dependence, post-dependent, prefrontal cortex, nucleus accumbens, epigenetics, histone demethylase, KDM6B.
Disclosures: A. Johnstone, Nothing to Disclose; C. Rienas, Nothing to Disclose; E. Barbier, Nothing to Disclose; J. Tapocik, Nothing to Disclose; M. Meinhardt, Nothing to Disclose; S. Brothers, Nothing to Disclose; W. Sommer, Nothing to Disclose; M. Heilig, Nothing to Disclose; C. Wahlestedt, Nothing to Disclose.

T103. Mechanisms of Focal Thalamic Degeneration in Thiamine Deficiency Induced Wernicke's Encephalopathy-Korsakoff Syndrome (WE-KS)

Fulton T Crews*, Liya Qin

University of North Carolina Chapel Hill, Chapel Hill, North Carolina

Background: Wernicke's encephalopathy-Korsakoff syndrome (WE-KS) is common in alcoholics, caused by thiamine deficiency (TD, vitamin B1) and associated with focal lesions to the thalamus (THAL) and a few other brain regions. TD disrupts glucose metabolism, important for brain as well as other tissues, confounding the focal nature of the TD insult. Further, the high incidence of alcoholism in WE-KS patients suggests that TD and ethanol interact. TD blocks glucose metabolism more than acetate metabolism. Our findings support the hypothesis that the high incidence of WE-KS in alcoholism is due to the protective effect of acetate derived from ethanol metabolism against the TD induced metabolic insult and that the focal THAL lesion is due to reduced ability to use acetate for cellular energy.
Methods: Male C57BL/6 mice were divided into normal chow control, ethanol (EtOH, 5 g/kg, i.g., 25% ethanol w/v), thiamine deficiency (TD, thiamine-deficient diet + pyrithiamine), and ethanol plus thiamine deficiency (EtOH-TD) groups and treated for 5 days (TD5) and 10 days (TD10). Average blood alcohol at 1 h after the first and last ethanol treatments was 290 ± 11 mg/dl (w/v, $n = 10$) and 296 ± 15 mg/dl (w/v, $n = 10$), respectively. Mice were sacrificed 24 h following the last dose of ethanol, and their brains and sera were used for either morphological or biochemical analyses, eg mRNA and protein using western blots, immunohistochemistry and RTPCR. Glycerin triacetate, (GTA, 2 g/kg, i.g. twice daily), treatment was used to increase acetate modeling ethanol derived acetate.
Results: Combined EtOH-TD treatment for 5 days (EtOH-TD5) showed greater THAL neurodegeneration, activated microglia, and proinflammatory gene induction than that found with TD alone (TD5), whereas TD for 10 days (TD10) resulted in marked THAL degeneration and microglial-neuroimmune activation in both groups. In contrast, 10 days of TD did not cause ENT degeneration. Interestingly, in ENT, TD10 alone activated microglia and astrocytes more than EtOH-TD10. In THAL, TD caused the loss of multiple astrocytic markers and neurons consistent with both neuronal and glial cell loss. TD insults pyruvate dehydrogenase that is essential for citric acid cycle metabolism of glucose metabolized to pyruvate. Acetate derived from hepatic ethanol metabolism is transported by monocarboxylic acid transporters (MCT) into cells that use acetyl-CoA synthetase (AcCoAS) to derive citric acid cycle energy from acetate. MCT and AcCoAS expression in THAL is lower than ENT suggesting that focal THAL degeneration is related to insufficient MCT and AcCoAS in THAL. To test this hypothesis we administered glycerin triacetate (GTA) to increase blood acetate and found it protected the THAL from TD induced degeneration.
Conclusions: Our findings suggest that EtOH potentiates TD induced THAL degeneration through neuroimmune gene induction. The findings support the hypothesis that TD deficiency inhibits global glucose metabolism and that a reduced ability to process acetate for cellular energy results in THAL focal degeneration in alcoholics contributing to the high incidence of WE-KS in alcoholism. The ability of acetate to provide brain energy may be useful for WE-KS and other neurodegenerative diseases.
Keywords: thalamus neurodegeneration, ethanol, thiamine deficiency, Wernicke's encephalopathy.
Disclosures: F. Crews, Nothing to Disclose; L. Qin, Nothing to Disclose.

T104. Expression of VEGF Receptor Is Higher with SSRI Treatment in Depressed Individuals and Correlates with Number of Cells, Capillaries and Dendrite Length in the Hippocampal Neurogenic Niche

Adrienne N Santiago, Yan Liu, Mihran J Bakalian, Andrew J Dwork, Gorazd B Rosoklija, René Hen, Victoria Arango, J John Mann, Maura Boldrini*

Columbia University, New York, New York

Background: Adult neurogenesis is hypothesized to be impaired in major depressive disorder (MDD). There is atrophy of the hippocampal formation in MDD *in vivo*, and reduced size and/or density of neurons and shorter dendrites postmortem. Stress-induced depression in animal models is associated with cell death and dendrite shrinkage in the hippocampus, and can be reversed by both antidepressants and neurotrophins. Angiogenesis and neurogenesis in the hippocampal dentate gyrus (DG) are co-regulated by growth factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), brain-derived neurotrophic factor (BDNF) and neuropeptide Y, which stimulate both neurogenesis and angiogenesis.

VEGF is a mediator of increased cell proliferation in rodents following electroconvulsive seizure and antidepressant treatment (fluoxetine, desipramine). It is not known whether antidepressants increase VEGF or BDNF in the human brain, or if this is a mechanism by which antidepressants increase neurogenesis. It is also not known whether there is a deficit of growth factors in MDD, which could affect cell proliferation, survival and cell morphology. A leading hypothesis, supported by rodent studies, describes the role of a neurogenic niche in the DG, where angiogenic and neurogenic factors, including VEGF and its R2 receptor (VEGFR2), are depleted by stress and enriched by antidepressant treatment. Our recent studies support a role for neurogenesis in MDD psychopathology: we found an increase in both neuronal progenitor cells (NPCs) and granule neurons among MDD patients who have received antidepressant treatment as compared to untreated MDD subjects. We therefore sought to examine whether these findings correlate with changes in the quantity of VEGFR2-immunoreactive (IR) cells, and explore the relationship between VEGFR2, dendrite length, and capillaries as well.

Methods: We analyzed the right DG of five controls, nine untreated MDD subjects, and seven subjects treated with SSRI (MDD*SSRI). Groups were matched for age, sex, and postmortem interval. The hippocampus was dissected from 2-cm coronal blocks of the right hemisphere, sectioned at 50 μm, and the volume of the DG was used as the region of interest. We assayed sections every 2 mm along the anterior-posterior axis of the DG. Brains had drug toxicology, neuropathological examination, and psychological autopsies assessing psychiatric history. We used double-immunohistochemistry for VEGFR2 and Nestin, to identify NPCs and capillaries expressing VEGFR2, and immunohistochemistry for neurofilament to label dendrites. Mature granule neurons were identified by neuronal-specific nuclear protein (NeuN). Using stereological methods (StereoInvestigator software, MBF Biosciences Inc., Williston, VT), we quantified the total number of VEGFR2-IR cells within and outside of vessel walls, vessel area and volume, as well as dendrite length for each group. Using double-immunofluorescence, we added a qualitative description of the localization of VEGFR2 in cells of the neurogenic niche. ANOVA tested the main hypotheses.

Results: We found between-group differences in total number of VEGFR2-IR cells in anterior ($F = 6.880$; $df = 15$; $p = 0.009$) and mid ($F = 16.880$; $df = 14$; $p < 0.001$), but not posterior DG. *Post hoc* analyses revealed that MDD*SSRI had a greater number of VEGFR2-IR cells than untreated MDD in anterior DG ($p = 0.008$) and more VEGFR2-IR cells than controls and untreated MDD in mid DG ($p = 0.004$; $p < 0.001$, respectively). VEGFR2 correlated positively with NPCs in mid DG ($r = 0.656$; $p = 0.040$) and granule neurons in anterior DG ($r = 0.687$; $p = 0.002$). Total DG vessel area correlated with VEGFR2-IR cell number in anterior ($r = 0.673$; $p = 0.023$), mid ($r = 0.666$; $p = 0.025$), and posterior ($r = 0.654$; $p = 0.011$) DG, and correlated with in-vessel VEGFR2 cells in anterior ($r = 0.680$; $p = 0.021$), mid ($r = 0.707$; $p = 0.015$), and posterior ($r = 0.633$; $p = 0.015$) DG. In anterior DG, average vessel length correlated with in-vessel VEGFR2 ($r = 0.717$; $p = 0.020$). MDD*SSRI have shorter dendrites ($F = 7.367$, df:2,18 $p = 0.005$) than controls ($p = 0.006$) and untreated MDD ($p = 0.022$). Total dendritic fiber length correlates with mid DG VEGFR2-IR cells within ($r = 0.716$; $p = 0.046$) and outside of vessels ($r = 0.742$; $p = 0.035$). The total number of DG dendrite fibers correlates with VEGFR2-IR cell number in the posterior DG ($r = -0.745$; $p = 0.009$).

Conclusions: Higher VEGFR2 in SSRI-treated MDD subjects indicates that VEGFR2 could be a major contributor to the neurochemical effect of SSRIs. We previously showed that SSRI-treated MDD have more NPCs and granule neurons and here they also show longer dendrites in the DG *vs* untreated MDD and controls. Our results support the hypothesis that VEGFR2 has a role in MDD pathogenesis and treatment. This conclusion is supported by rodent studies, which show that behavioral effects of SSRIs are diminished by VEGFR2 inhibitors. Correlations between VEGFR2 cell number and dendrite length, vessel volume and area, indicate that VEGFR2-induced angiogenesis is involved in the neurogenic role of VEGFR2.

Keywords: VEGF, hippocampus, postmortem, neural progenitor cell, capillaries.

Disclosures: A. Santiago, Nothing to Disclose; Y. Liu, Nothing to Disclose; M. Bakalian, Nothing to Disclose; A. Dwork, Nothing to Disclose; G. Rosoklija, Nothing to Disclose; R. Hen, Nothing to Disclose; V. Arango, Nothing to Disclose; J. Mann, Nothing to Disclose; M. Boldrini, Nothing to Disclose.

T105. Stress-context Detecting Function of the Mesolimbic Reward Circuit: The Role of CRF in Gating BDNF Signaling

Jessica Walsh, Allyson Friedman, Haosheng Sun, Stacy Ku, Elizabeth Heller, Barbara Juarez, Veronica Burnham, Michelle Mazei-Robison, Deveroux Ferguson, Sam Golden, Ja Wook Koo, Dipesh Chaudhury, Daniel J Christoffel, Scott Russo, Eric Nestler, Ming-Hu Han*

Mount Sinai School of Medicine, New York, New York

Background: Neurotransmitters and neuromodulators are often co-localized in single neurons, and their release is regulated by neuronal firing activity. Reward-induced phasic firing is known to increase dopamine release from the mesolimbic reward circuit, which consists of ventral tegmental area (VTA) dopamine neurons projecting to nucleus accumbens (NAc). In these mesolimbic neurons, brain-derived neurotrophic factor (BDNF) is co-localized with dopamine. Our previous work reports that BDNF in this projection pathway is a key determinant for susceptibility *vs* resilience in a chronic social defeat stress model of depression (Krishnan V *et al*, *Cell*, 2007). We recently demonstrated that optogenetically induced phasic firing of the VTA-NAc pathway promotes a susceptible phenotype, evidenced by decreased social behaviors and anhedonia (Chaudhury D *et al*, *Nature*, 2013). Here we investigated if BDNF mediates the optogenetically induced susceptibility, and more interestingly, how BDNF release is regulated by aversive social stress in this reward circuitry.

Methods: Utilizing multiple viral, circuit-probing optogenetic approaches, we specifically expressed channelrhodopsin-2 (ChR2) in VTA neurons projecting to the NAc (VTA-NAc neurons) by injecting retrograde pseudorabies

virus-Cre into the NAc and Cre-inducible AAV-DIO-ChR2-eYFP into the VTA. Following a well-established subthreshold social defeat stress paradigm, we optically activated ChR2-expressing VTA-NAc cell bodies with either tonic or phasic stimulation pattern during a social interaction test. For many experiments, mice were subjected to local drug infusions prior to behavioral testing. 24-h following social interaction tests with optical stimulation, NAc tissue was collected for BDNF protein quantification.
Results: First, we replicated that phasic but not tonic optical activation of VTA-NAc neurons during social interaction induced social avoidant behaviors following the subthreshold social defeat. Consistent with these optically induced social avoidant behaviors, we observed that only phasic stimulation increased BDNF levels in the NAc after the social defeat. Using $BDNF^{fl/fl}$ mice, we selectively deleted BDNF in the VTA-NAc pathway and subjected these mice to subthreshold defeat followed by a social interaction test with phasic activation of VTA-NAc neurons. We saw a block of the optically induced avoidance behavior as well as a loss of increased BDNF levels in the NAc. Additionally, infusion of TrkB antagonist (1 μg, ANA-12) completely blocked the ability of phasic stimulation to induce social avoidance, without affecting elevated levels of BDNF. These data provide direct evidence that BDNF in the NAc mediates the optogenetically induced susceptibility following social stress. Interestingly, and consistent with our previous work, acute optical stimulation of VTA-NAc neurons in stress-naïve mice had no effect on social interaction behavior. Such stimulation produced no change in BDNF levels in the NAc in these stress-naïve mice. Moreover, stronger, repeated stimulation of these neurons (20 min each day for 5 days) also induced no elevation of BDNF levels in the NAc as well as no changes in social interaction. Therefore, we hypothesized that there is a second mediator that works in action with phasic firing to create the stress-induced susceptibility and BDNF increase in this neural pathway. We demonstrate that neuropeptide corticotropin-releasing factor (CRF) is necessary for the elevation of BDNF in the NAc. Following the same stress paradigm, we infused non-selective CRF receptor antagonist (1 μg, alpha-helical CRF) into the NAc 1 h prior to phasic stimulation and behavioral testing. Infusion of the CRF antagonist into the NAc effectively blocked the phasic-firing induced social avoidance and BDNF levels in socially stressed mice. More importantly, phasic stimulation was able to cause a significant increase in NAc BDNF levels in the CRF-infused, stress-naïve mice. However, without phasic stimulation, CRF infusion into the NAc of stress-naïve mice had no effects on social behavior and NAc BDNF levels.
Conclusions: In this *in vivo* study, taking advantage of the temporal precision of optogenetics, we demonstrate the firing pattern-specific upregulation of BDNF in the NAc in socially stressed mice, a phenomenon that is blocked by an intra-NAc infusion of CRF antagonist. However, neither CRF infusion into the NAc nor phasic activation of VTA-NAc neurons is able to induce a significant increase in NAc BDNF levels in stress-naïve mice, suggesting that both contextual stress and phasic activation of the VTA-NAc pathway are required to induce BDNF signaling in the NAc. These data unravel a logic AND function of the brain's mesolimbic reward circuitry in producing algorithmic responses to environmental stimuli (Schnitzer MJ, *Nature*, 2002), which detects aversive stress context in the reward pathway.
Keywords: social defeat stress, mesolimbic circuit, phasic firing, BDNF and CRF.
Disclosures: J. Walsh, Nothing to Disclose; A. Friedman, Nothing to Disclose; H. Sun, Nothing to Disclose; S. Ku, Nothing to Disclose; E. Heller, Nothing to Disclose; B. Juarez, Nothing to Disclose; V. Burnham, Nothing to Disclose; M. Mazei-Robison, Nothing to Disclose; D. Ferguson, Nothing to Disclose; S. Golden, Nothing to Disclose; J. Koo, Nothing to Disclose; D. Chaudhury, Nothing to Disclose; D. Christoffel, Nothing to Disclose; S. Russo, Nothing to Disclose; E. Nestler, Nothing to Disclose; M. Han, Nothing to Disclose.

T106. Altered Synaptic Protein Expression and Co-expression Network Topology Linked to Spine Loss in the Auditory Cortex of Schizophrenia

Matthew L MacDonald*, Ying Ding, Jason Newman, Nathan Yates, David A Lewis, Robert A Sweet

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: Schizophrenia patients have reduced grey matter volume of the auditory cortex and display deficits in auditory processing. Genetic and pharmacologic studies have repeatedly implicated the glutamatergic signaling cascades that mediate synapse formation and maintenance in schizophrenia. Dovetailing with these genetic findings, we have observed decreased dendritic spine density in layer III of the auditory cortex. The molecular processes that orchestrate spine formation and maintenance are highly ordered and complex, involving hundreds if not thousands of proteins. Thus, a global view of synaptic protein activity in schizophrenia is essential to understanding the molecular pathology of spine loss. The goal of this study is to begin assessing this complexity by identifying altered protein expression and protein co-expression network features linked to spine loss in postmortem auditory cortex tissue.
Methods: Whole tissue homogenates were prepared from auditory cortex grey matter of 22 schizophrenia and matched control subjects. 155 selected synaptic proteins were quantified therein by a liquid chromatography—selected reaction monitoring/mass spectrometry approach, recently validated for use in human postmortem brain tissue. Spine density measurements were determined in a subset of this cohort by immunohistochemistry and quantitative fluorescence confocal microscopy. Protein co-expression networks were constructed with WGCNA.
Results: Comparison of average protein amounts and functional pathway analysis revealed significant differences in the expression of glutamatergic signaling proteins (eg glutamate receptors and their signaling partners). The expression of some of these proteins, such as GRIA3, correlated with spine density across all subjects($r = 0.4$, $p = 0.024$). Protein co-expression network node weighted connectivity and node clustering were significantly reduced in schizophrenia ($p_{permuted} = 0.001$ and < 0.012). Four proteins, ANK1, ANK2, SPTA1, and SYNJ1, were the exception to this effect, comprising a unique module that

was present in the schizophrenia network but not control. Both ANK1 and ANK2 have higher connectivity (node degree, $p_{permuted} < 0.02$) in schizophrenia and their levels were significantly inversely correlated with spine density in schizophrenia (both $r = -0.66$, $p = 0.006$), while showing no correlation in controls.

Conclusions: Decreased GRIA3 levels are unlikely to be the simple result of concurrent spine—receptor loss, as other spine-associated proteins, including SYNGAP, GRIN1, CAMKIIA, and PSD95 were unaltered in schizophrenia and not correlated with spine density. Thus, GRIA3 levels may be linked to the biology of spine loss in SCZ. Experiments are currently underway to localize this alteration to a specific neuronal/synaptic population within the auditory cortex. The schizophrenia specific module that inversely correlates with spine density could represent a pathological process linked to spine pathology, eg dysfunctional proteolytic processes, or conversely, a coordinated response to spine loss. At this time the results of this study must be interpreted cautiously until additional experiments are performed. However, this study clearly shows the complexity of schizophrenia molecular pathology at the protein level and the value of orthogonal approaches within subjects.

Keywords: schizophrenia, spine, proteomics, glutamate, postmortem.

Disclosures: M. MacDonald, Nothing to Disclose; Y. Ding, Nothing to Disclose; J. Newman, Nothing to Disclose; N. Yates, Nothing to Disclose; D. Lewis, Nothing to Disclose; R. Sweet, Nothing to Disclose.

T107. Neurotrophin Receptor TrkB Expression in Dentate Gyrus and Hilus of Treated and Untreated Subjects with Major Depression Correlates with Number of Neural Progenitor Cells and Neurons

Giulia Bracci, Mihran J Bakalian, Andrew J Dwork, René Hen, Gorazd B Rosoklija, Victoria Arango, J John Mann, Maura Boldrini*

Columbia University, New York, New York

Background: Adult neurogenesis occurs in the subgranular zone of the dentate gyrus (DG) and in subventricular layer and is increased by selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) in mammals, including humans. Neural progenitor cells (NPCs) grow and differentiate in neurogenic niches in tight clusters around small capillaries. We reported that antidepressants increase neural progenitor (NPCs) and dividing cells in the DG of subjects with major depressive disorder (MDD). Evidence in rodents shows that SSRIs, TCAs, monoamine oxidase inhibitors and noradrenaline reuptake inhibitors administered chronically, increase cell proliferation. Blocking neurogenesis inhibits some antidepressant effects. Tropomyosin-related kinase B (TrkB) is a protein tyrosine kinase receptor expressed in the brain and mediates the brain-derived neurotrophic factor (BDNF) action. BDNF is involved in activity-dependent neuronal plasticity, survival and differentiation of peripheral and central neurons. BDNF signaling is required for the long-term survival of newborn neurons in mouse DG. Antidepressants increase the expression of several molecules associated with neuronal plasticity, in particular BDNF and its receptor TrkB. The lack of TrkB in NPCs of mice hippocampus leads to impaired proliferation and neurogenesis, and poor survival. Both TrkB +/− and BDNF +/− mice are resistant to antidepressants and DG neurogenesis is reduced in BDNF +/− mice. BDNF +/− mice show deficits of the survival of newly formed cells after chronic antidepressant treatment compared with wild-type mice. Post mortem human brain studies have displayed higher TrkB mRNA in the cerebellum associated with antidepressant treatment in patients with depression. Abnormal TrkB signaling was also shown in the prefrontal cortex and hippocampus of patients with schizophrenia, bipolar disorders and depression and in suicide victims.

Methods: We sought to quantify by stereology (Stereo-Investigator software, MBF Biosciences Inc., Williston, VT) cells expressing BDNF receptor TrkB in the human DG of matched subjects with untreated MDD, MDD treated with SSRIs (MDD*SSRI) and non-psychiatric controls ($n = 5$ each). All groups underwent psychological autopsy and neuropathological examination. Brain and blood toxicological screening was performed. Hippocampi were dissected from 2-cm coronal blocks of the right hemisphere, fixed in 4% paraformaldehyde, cryoprotected in 30% sucrose, and sectioned at 50 μm on a freezing microtome (Microm HM 440E, Walldorf, Germany). We assayed sections every 2 mm along the anterior-posterior axis of the DG. We used immunohistochemistry (TrkB rabbit polyclonal IgG) to detect TrkB immunoreactive (IR) cells of the DG and hilus. Tissue was immunostained with nuclear red. NPCs and mature neurons were already identified in the same subjects using immunohistochemistry for nestin and NeuN, and quantified. Pearson Correlation (PCo) analysis tested correlations between cell numbers.

Results: We found a correlation between number of NPCs and TrkB-IR cells in mid hilus ($PCo = 0.762$, $p = 0.028$) in treated and untreated subjects with MDD and normal controls. There was a correlation between mature granule neurons (NeuN) and TrkB-IR cells in anterior DG ($PCo = 0.718$, $p = 0.008$), in anterior hilus ($PCo = 0.758$, $p = 0.011$), and posterior hilus ($PCo = 0.962$, $p = 0.002$). We did not find significant differences in TrkB-IR cell number between groups, possibly due to the small sample size.

Conclusions: Our data show a relationship between TrkB receptor expression and number of NPCs and mature neurons in the human hippocampus. Results suggest the role of this neurotrophin receptor in supporting the NPC pool and neuronal maturation or survival. We previously showed that SSRI-treated MDD have more NPCs and granule neurons. Since more NPCs and granule neurons are found with more TrkB-IR cells in the human brain, TrkB may mediate the effect of antidepressants on cell viability in the human hippocampus.

Keywords: BDNF, TrkB, neurogenesis, depression, hippocampus.

Disclosures: G. Bracci, Nothing to Disclose; M. Bakalian, Nothing to Disclose; A. Dwork, Nothing to Disclose; R. Hen, Nothing to Disclose; G. Rosoklija, Nothing to Disclose; V. Arango, Nothing to Disclose; J. Mann, Nothing to Disclose; M. Boldrini, Nothing to Disclose.

T108. DNA Methylation and Dysregulation of the GABAergic Phenotype in Post-mortem Human Hippocampus in Schizophrenia and Bipolar Disorder

W Brad Ruzicka*, Francine M Benes

McLean Hospital, Belmont, Massachusetts

Background: Among the most robust and widely replicated findings in molecular analysis of schizophrenia (SZ) is the downregulation of the GAD1 gene, with resultant decreased expression of glutamic acid decarboxylase 67 (GAD_{67}), the rate limiting enzyme in GABA synthesis, and abnormal GABAergic neurotransmission. These changes have been observed in multiple brain regions, including the hippocampus, but occur selectively in specific neuronal subpopulations and subregional loci. Expression of the GAD1 gene is regulated by a network of genes coding for proteins that include transcription factors, epigenetic factors, as well as components of TGFβ and Wnt signaling, cell cycle regulation, and DNA repair. Transcriptional activity of genes within this network is altered distinctly in SZ and bipolar disorder (BD) (Benes *et al* 2007), and the differences between diagnoses are specific to discrete locations within the hippocampus. Epigenetic mechanisms are attractive candidates for mediators of cell and regionally specific changes in gene expression such as these, with mounting evidence of their central role in this phenomenon. The current work measured levels of methylation at CpG dinucleotides of genes within the GAD1 regulatory network to assess the role of DNA methylation in the dysregulation of the GABAergic phenotype observed in SZ and BD at specific locations within the neural circuitry of the human hippocampus.
Methods: Tissue was sampled from stratum oriens of region CA1 or region CA3/2 from 8 SZ, 8 BD, and 8 control cases (CON) using laser-assisted microdissection, for a total of 48 samples. DNA was extracted from sampled tissues and modified with sodium bisulfite. Modified DNA was assessed with the Illumina HumanMethylation450 Beadarray to measure levels of cytosine methylation at greater than 480 000 sites across the genome. Data was normalized by subset-quantile within array normalization, and approximately 900 CpG sites associated with our genes of interest designated prior to study initiation were investigated further. Significance of diagnosis and anatomic location associated DNA methylation changes was determined by linear regression analysis with subject age, post mortem interval, sex, and medication exposures as covariates.
Results: Beadarrays yielded high quality data with successful measurement of DNA methylation at between 99.5 and 99.9% of the more than 480 000 sites interrogated by the assay. Comparison across groups and anatomic locations showed a complex pattern of differences. For example, in region CA3/2 the CCND1 gene contains 7 assayed CpG loci that were hypermethylated in SZ *vs* CON (p values between 0.0007 and 0.04), and this data is robust as these sites are strikingly similar in behavior (with regard to baseline methylation level and size and direction of disease effect) and are clustered along the length of the gene. In region CA1 of the same cases, there are no CCND1 gene associated sites with significantly altered methylation in SZ *vs* CON. There are also numerous instances of contrast between diagnoses as in the SMURF1 gene, with 5 sites robustly changed in methylation status in BD region CA3/2 *vs* CON (p values between 0.007 and 0.02), and only one site of significant but minimal change in SZ region CA3/2. There are similar interesting patterns of change in sites associated with multiple GAD1 regulatory genes, most notably CCND1, CCND2, SMURF1, RUNX2, DAXX, and CTNNB1.
Conclusions: Animal models have demonstrated the ability of early life experience to modulate gene expression and complex behavior through changes in DNA methylation, with effects lasting into adulthood. While the power of this study is limited by its small sample size, the results demonstrate robust changes in methylation status at multiple CpG sites associated with genes important in regulation of the GABAergic phenotype at specific anatomic locations within the neural circuitry of the human hippocampus in psychotic disorders. The specificity of these changes reflects the discrete environment and pattern of input experienced by cells at these locations, and reinforces both the need for direct analysis of human brain tissue in investigation of these uniquely human disorders, as well as for more refined methods for sampling the extraordinarily complex tissue of the brain. Pyrosequencing experiments are currently being designed for the purpose of validating the results of the beadarray work, and ongoing analysis will correlate methylation changes with previously reported differences in gene expression and copy number variation at these same anatomic locations in psychotic disorders. Investigation of changes such as those described above will be vital to understanding the pathophysiology of psychotic illness, with the ultimate goal of finding targets for intervention towards improving treatments, and possibly prevention or cure.
Keywords: methylation hippocampus psychosis epigenetics post-mortem.
Disclosures: W. Ruzicka, Nothing to Disclose; F. Benes, **Part 4:** Takeda Pharmaceutical Company 2012–2013.

T109. Using the Olfactory Epithelium as a Surrogate Tissue to Explore Dynamic Molecular Signatures for Brain Diseases

Soumya Narayan*, Koko Ishizuka, Narayan Rai, Charlee McLean, Pearl K Kim, Maria Hipolito, Youjin Chung, Sandra Lin, John Nurnberger, Nicola Cascella, Akira Sawa, Evaristus Nwulia

Johns Hopkins School of Medicine, Baltimore, Maryland

Background: The olfactory epithelium contains receptor neurons (both mature and regenerating populations) and supporting cells, providing a unique opportunity to accessibly study the central nervous system (CNS) through tissue obtained via nasal biopsies. Previously, we reported the advantage of this biopsied tissue as a surrogate tissue to explore dynamic molecular marker for CNS therapy development (Sattler *et al*, Exp Neurol, 2011). However, a drawback of using this tissue is the substantial contamination of neuronal cells with non-neuronal cells. To overcome this issue, we have developed a novel approach to obtain enriched neuronal cell populations by combining nasal biopsies with laser-captured microdissection (LCM)

(Tajinda *et al*, Mol Psychiatry, 2010). Lithium is a classic mood stabilizer important in the treatment of bipolar disorder and related disorders. Yet, our understanding of the pathophysiology of bipolar disorder and the actions of lithium which result in positive clinical outcomes remains limited. Several animal and human genetic studies have indicated that lithium affects molecular targets that are involved in neuronal growth, survival and maturation. Many of these targets are a part of the Wnt signaling and inositol cascades. In our current study, we validate how this new approach using olfactory tissues with LCM can be a platform for exploring disease-associated and/or treatment-related biomarkers in mental disorders. More specifically, we are using this to address alterations in molecular signatures associated with lithium treatment in bipolar disorder.

Methods: *Patient recruitment:* We have recruited 15 non-smoking patients with bipolar disorder. These patients underwent two nasal biopsies. The first biopsy occurred after a washout period in which patients were drug naïve. Patients then underwent 6 weeks of lithium therapy after which a second biopsy was performed. We also recruited 15 non-smoking controls without psychiatric conditions for biopsy. *Neuronal cell enrichment by LCM*: After biopsies, neurons were enriched from the olfactory epithelium tissue via LCM. This procedure allows us to identify neurons from other supporting cells under microscopic visualization and extract these neurons using laser guided cutting to obtain histologically pure enriched populations. *Gene expression analysis*: The total RNA was extracted from each neuronal sample. After the quality and quantity of RNA were assessed via Bioanalyzer and Nanodrop analysis, only high quality RNA samples were converted into cDNA. Then, for each cDNA sample, *OMP* (olfactory marker protein) gene expression levels were compared with those in undissected samples by quantitative PCR (qPCR) to confirm enrichment of neurons. Finally, only neuronal enriched samples were processed for qPCR analysis with target molecules. *Study approval*: This study was approved by IRB at Johns Hopkins University and from IRB at Howard University.

Results: 1. We have optimized the protocol and trouble shooting strategy in the study of molecular signatures, which includes standardization of the criteria for RNA quality [RNA Integrity Number (RIN)] and neuronal enrichment (fold change of *OMP* expression levels).

2. We have found that the baseline expression levels of *AKT*, *GSK-3β* and *CRMP1* were increased in olfactory neurons from bipolar disorder patients compared with controls. Interestingly, a 6-week period of lithium treatment normalized expression levels of *AKT*, *GSK-3β* and *CRMP1* in bipolar disorder patients.

Conclusions: Our findings illustrate the utility of olfactory epithelium as a model to study the effects of lithium treatment in bipolar disorder and as a means to identify biomarkers for treatment response. Also, outcomes of this study will advance our understanding of the pathophysiology of bipolar disorder and facilitate biomarker exploration and drug discovery. More importantly we anticipate that this new platform for obtaining enriched olfactory neuronal cells by combining nasal biopsy and LCM, which has been validated in this study, can have many implications. It can be applied towards biomarker studies, including those for treatment response, and drug discovery efforts for other neuropsychiatric conditions, making a broader impact in the field. We will distinguish the differences between responders and non-responders for CNS therapies at molecular levels by using this system.

Keywords: olfactory epethelium, molecular signatures, brain disorders, laser capture microdissection.

Disclosures: S. Narayan, Nothing to Disclose; K. Ishizuka, **Part 1:** Otsuka, **Part 4:** Otsuka; N. Rai, Nothing to Disclose; C. McLean, Nothing to Disclose; P. Kim, Nothing to Disclose; M. Hipolito, Nothing to Disclose; Y. Chung, Nothing to Disclose; S. Lin, Nothing to Disclose; J. Nurnberger, Nothing to Disclose; N. Cascella, Nothing to Disclose; A. Sawa, **Part 1:** Astellas, Takeda, Tanabe-Mitsubishi, Dainippon-Sumitomo, Johnson and Johnson, Sucampo, Pfizer, Asubio, Eli Lilly, Taisho, Amgen, Afraxis, SanofiAventis, and Astrazeneca, **Part 4:** Astellas, Takeda, Tanabe-Mitsubishi, Dainippon-Sumitomo, Johnson and Johnson, and Sucampo; E. Nwulia, Nothing to Disclose.

T110. Neurobiological Basis of Augmentation Strategy of Serotonin Specific Reuptake Inhibitor by Compounds Able to Limit High Affinity Nicotinic Acetylcholine Receptors

Yann S Mineur*, Emily Einstein, Mattis Wigenstrand, Sam Blakeman, Gianna Fote, Marina Picciotto

Yale University School of Medicine, New Haven, Connecticut

Background: The majority of classical antidepressants target monoamine systems (such as serotonin and norepinephrine), but a significant proportion of patients does not respond fully to existing treatments. Another system that has been explored for development of novel antidepressant drugs is the cholinergic system. Blocking nicotinic and muscarinic acetylcholine receptors results in antidepressant-like effects in animal models, and has been effective in some human clinical trials. Despite the recent failure of a clinical trial, smaller controlled studies have suggested that nicotinic blockers alleviate depressive symptoms, particularly in patients refractory to classical antidepressants such as selective serotonin reuptake inhibitors (SSRIs). These effects are in line with pioneering reports suggesting that increasing cholinergic signaling by blocking acetylcholinesterase can induce symptoms of depression and anxiety, and that these symptoms can be significantly blunted by classical antidepressants. These results in patients and animal models have suggested that there may be cross-talk between cholinergic- and monoamine-based antidepressants. To date, however, the nature of the interactions that may exist between monoamines and acetylcholine in the etiology of depression and in the efficacy of antidepressants is largely unknown. We therefore investigated whether we could alter the behavioral response to nicotinic drugs in tests of antidepressant efficacy by manipulating the monoaminergic system using pharmacological and molecular techniques in specific brain loci.

Methods: First, we determined whether the effect of cytisine, a nicotinic partial agonist with antidepressant-like properties in mice, was altered in tests of antidepressant efficacy when specific monoamines (norepinephrine or serotonin) were depleted. Second, we co-administered subthreshold doses of cytisine and the SSRI fluoxetine to determine whether these two

compounds could interact to induce significant antidepressant-like effects when combined. To determine whether the combined effects of cytisine and fluoxetine were generalized to other serotonergic agents, we combined a subthreshold dose of cytisine with a subthreshold dose of the 5HT1A receptor agonist 8OHDPAT (which can also induce antidepressant-like effects in several mouse models of antidepressant efficacy on its own). Finally, we used viral-mediated delivery of a small hairpin RNA (shRNA) to selectively knockdown 5HT1A in the dorsal raphe where it acts as an inhibitory autoreceptor, and also in the hippocampus where it is highly expressed and acts as a post-synaptic excitatory receptor and determined whether knock down of this serotonin receptor altered the effects of cytisine in a model of antidepressant efficacy.
Results: Serotonin or norepinephrine depletion abolished the antidepressant-like effects of cytisine, contrary to what was observed in control animals. Combination of a subthreshold dose of cytisine with a subthreshold dose of fluoxetine resulted in a significant antidepressant-like effect that was not observed when the compounds were administered separately. A similar pattern of results was observed when 8OHDPAT was combined with a subthreshold dose of cytisine. Finally, the antidepressant-like effect of cytisine was significantly decreased by knock down of the 5HT1A receptor in the hippocampus, but there was no effect of knocking down 5HT1A autoreceptors in the dorsal raphe.
Conclusions: These results indicate that intact monoamine neurotransmission is required for a nicotinic drug to induce antidepressant-like effects. This also suggests that nicotinic receptor activity normally modulates monoamine signaling, and that the combined effects of serotonergic and cholinergic drugs results from interactions in monoaminergic circuits that control mood. The results of the knockdown experiments further indicate that postsynaptic hippocampal 5HT1A receptors are required for the antidepressant-like effects induced by nicotinic receptor blockade, suggesting that subsets of patients with impaired serotonergic transmission may not benefit from nicotinic-based antidepressants. These observations might also explain why nicotinic-based treatments have had varying clinical efficacy in a large cohort, while subsets of SSRI-resistant patients have showed significant improvement in smaller trials. Overall, these data identify neurobiological substrates underlying an augmentation strategy for classical antidepressant therapy, but also highlight specific limitations and etiological factors that are required for successful outcomes of this approach.
Keywords: monoamine, acetylcholine, antidepressants, animal model, knowdown.
Disclosures: Y. Mineur, **Part 1:** Targacept, **Part 4:** Targacept; E. Einstein, Nothing to Disclose; M. Wigenstrand, Nothing to Disclose; S. Blakeman, Nothing to Disclose; G. Fote, Nothing to Disclose; M. Picciotto, **Part 1:** Targacept, **Part 4:** Targacept.

T111. Actin Cytoskeleton Dysregulation in Schizophrenia and Bipolar Disorder: Relevance to Dendritic Spine Pathology

Glenn Konopaske*, Sivan Subburaju, Joseph T Coyle, Francine M Benes

McLean Hospital, Belmont, Massachusetts

Background: Schizophrenia is a severe and persistent mental illness affecting 1% of the population worldwide. Previously, dendritic spine density associated with pyramidal cells was found to be reduced in the deep half of layer III in the dorsolateral prefrontal cortex (DLPFC) in schizophrenia. Since the actin cytoskeleton plays a central role in the development, maintenance, and function of dendritic spines, we sought to determine if dysregulation of the actin cytoskeleton might account for the observed dendritic spine pathology.
Methods: By analyzing microarray data obtained previously using DLPFC tissue from subjects with schizophrenia ($n = 19$), bipolar disorder ($n = 18$), and unaffected control subjects ($n = 25$), we identified 5 candidate genes (IFG1R, MARCKS, PPP1R9A, PTPRF, and ARHGEF2) that regulate both the actin cytoskeleton and the formation or maintenance of dendritic spines. We utilized quantitative real time PCR to validate the microarray findings in post-mortem DLPFC tissue from a new cohort of subjects with schizophrenia ($n = 19$), bipolar disorder ($n = 17$), and unaffected control subjects ($n = 18$).
Results: In the DLPFC, the expression of IGF1R and MARCKS was significantly increased in both the schizophrenia and bipolar disorder subjects relative to the controls. In addition, PPP1R9A expression was significantly increased in bipolar disorder subjects relative to both schizophrenia and unaffected control subjects. The expression of PPP1R9A did not differ between schizophrenia and control subjects. Moreover, PTPRF and ARHGEF2 expression levels did not differ among the groups. *Post hoc* analyses correlating gene expression with dendritic spine density measurements from the same subjects will also be presented.
Conclusions: Overall, the regulation of the actin cytoskeleton and dendritic spines appears to be altered in both schizophrenia and bipolar disorder and may possibly reflect neuropathological changes in layer III of the DLPFC.
Keywords: dendritic spine, actin cytoskeleton, schizophrenia, bipolar disorder, PCR.
Disclosures: G. Konopaske, Nothing to Disclose; S. Subburaju, Nothing to Disclose; J. Coyle, Nothing to Disclose; F. Benes, Nothing to Disclose.

T112. Depression Decreases CD4 and Chemokine Receptor Expression in T-lymphocytes and Macrophages

Tami D Benton*, Kevin Lynch, Steven D Douglas, Benoit Dubé, David Gettes, Nancy Tustin, David S Metzger, Sergei Spitsin, Dwight L Evans

The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

Background: Depression is a common comorbidity of medical illness and is a risk factor for disease progression for many medical conditions. Although the biological basis underlying the relationship remains unknown, evidence derived from pre-clinical and clinical research has linked immune system dysregulation to depression. Furthermore, recent findings of both impaired cellular immunity and inflammation in depressed individuals suggest that depression is a disorder of both immune activation and immune suppression. To further understand the biology underlying

these associations, we investigated whether T-lymphocyte and macrophage CD4 and chemokine receptors were altered by the presence of depression.
Methods: 158 depressed ($n=97$) and non-depressed ($N=61$) participants ages 18–58 completed structured diagnostic assessments for the presence of depression, Hamilton depression rating scales (17 item) for depression severity and medical evaluations. Women comprised 59% of the sample ($n=93$) and 65% were African American ($n=103$). Subjects were excluded for medical comorbidities, current substance abuse, or use of psychotropic medications or immunomodulatory drugs in the four weeks prior to assessment. CD4 receptor, and CCR5 and CXCR4 chemokine receptor mRNA was measured by real-time RT PCR in monocyte depleted peripheral blood mononuclear cells (PBMCs) treated for 3 days with phytohemagglutinin to stimulate T-lymphocytes and in monocyte derived macrophages (MDMs) cultured for 7 days isolated from depressed and non-depressed subjects.
Results: In analyses controlling for age, race, and gender, depression diagnosis was associated with significant decreases in CD4 ($p=0.02$) and CXCR4 ($p=0.03$) in T-lymphocytes, and in CD4 ($p=0.03$) in MDMs. Non-significant decreases were observed in T-lymphocytes for CCR5 ($p=0.08$), and in MDMs for CCR5 ($p=0.20$) and CXCR4 ($p=0.41$). A similar pattern of results was observed for effects of increases in Hamilton score, with significant decreases observed in T-lymphocytes for CD4 ($p=0.05$), CCR5 ($p=0.02$), and CXCR4 ($p<0.001$), and in MDMs for CD4 ($p=0.02$); non-significant decreases were observed for CCR5 ($p=0.48$) and CXCR4 ($p=0.07$) in MDMs. Interaction tests showed no significant heterogeneity of the effects of depression diagnosis or Hamilton score across age, race, or gender, although effects tended to be larger in the African-American subsample, particularly for the CD4 responses.
Conclusions: Depression was associated with decreased CD4 and chemokine receptor expression in T-lymphocytes and CD4 expression in MDMs. Furthermore, depression severity as measured by the Hamilton was associated with significant decreases in T-lymphocyte CD4, CCR5, and CXCR4; higher Hamilton scores were also significantly associated with decreased MDM CD4. Thus, these findings demonstrate reductions in MDM CD4 and T-lymphocyte CD4 and chemokine receptors with depression. To our knowledge, this is the first study to demonstrate a relationship of reduced expression of CD4 immune cell markers and chemokine receptors in depression. The current findings extend our previous reports of impaired immunity in depression and may offer a unique biomarker of depression. Further study will be necessary to determine if this observed down regulation of T-lymphocyte and macrophage receptors reflects immune suppression or if in fact it is a consequence of immune activation (eg elevated circulating inflammatory cytokines). Confirmatory studies of these findings could determine their clinical relevance and help to clarify the biology underlying the relationship between depression, immunity, and medical illness.
Keywords: depression, immunology.
Disclosures: T. Benton, Nothing to Disclose; K. Lynch, Nothing to Disclose; S. Douglas, Nothing to Disclose; B. Dubé, Nothing to Disclose; D. Gettes, Nothing to Disclose; N. Tustin, Nothing to Disclose; D. Metzger, Nothing to Disclose; S. Spitsin, Nothing to Disclose; D. Evans, Nothing to Disclose.

T113. Nicotinic Modulators in Subtype-specific Cortical GABAergic Neurons: Implication for Critical Period Development

Michael Demars, Noreen Bukhari, Poromendro Burman, Ayan Hussein, Hirofumi Morishita*

Mount Sinani School of Medicine, New York, New York

Background: Schizophrenia is a devastating disorder with typical onset during adolescence. It has been suggested that developmental GABAergic interneuron dysfunction (Lewis *et al* 2005) and deficits in the nicotinic acetylcholine receptor (nAChR) system (Freedman *et al* 1994) are major underlying pathophysiological changes. However, to what extent the GABA and nAChR system converge at the molecular and circuit level across development is not well known. Our recent study showed that Lynx1, an endogenous negative modulator of nAChRs with a structure similar to alpha-bungarotoxin in snake venom, is preferentially expressed in parvalbumin positive GABAergic neurons. We further showed that Lynx1 acts as a brake to limit experience-dependent cortical plasticity following the critical period via increased expression (Morishita *et al* 2010). As Lynx1 belongs to a larger Lynx family of proteins with high sequence homology, here we aimed to extend our study to other family members. We sought to examine whether the Lynx family may collectively provide diversity and specificity to nicotinic signaling in different subtypes of GABAergic neurons to regulate critical period plasticity.
Methods: We employed mouse visual cortex as a well-established model of critical period development. Brain samples from different ages across critical period were collected and compared. Expression of Lynx family was detected by: real-time PCR, and *in situ* hybridization. In addition to Lynx1, we focused on another member of the Lynx family, Lypd6, which has also been shown to bind and modulate nAChRs. Interestingly, in contrast to Lynx1, Lypd6 binding potentiates calcium currents through nAChRs (Darvas *et al* 2009). The co-localization of the Lynx family with three major GABAergic neuron markers-parvalbumin, somatostatin, 5HT3AReceptor- (Rudy *et al* 2010) was examined by double *in situ* hybridization. Further, genetically engineered mice with deletion or overexpression of Lynx family genes were analyzed by combining biochemical, anatomical, and electrophysiological techniques.
Results: Developmental expression analysis revealed, in contrast to Lynx1 which elevates its expression, the expression of Lypd6 rather declines across developmental critical period in visual cortex. Among cortical GABAergic interneurons, the expression of Lynx1 mRNA was specific to parvalbumin positive interneurons, but not observed in somatostatin or 5-HT3A receptor positive interneurons. Consistent with this expression pattern, genetic deletion of Lynx1 revealed experience-dependent reduction of perineuronal nets, which specifically accumulate around parvalbumin interneurons to close critical period plasticity.

In contrast to the specific link between Lynx1 and parvalbumin neurons, Lypd6 was detected only in somatostain interneurons, but not in parvalbumin or 5-HT3A receptor positive neurons. Among somatostatin interneurons, Lypd6 mRNA was further preferentially detected in deep cortical layers, which are known to be involved in both local feedback as well as long-range cortico-cortical circuits. Interestingly, highly nicotinic 5HT3A receptor positive interneurons did not show expression of either Lynx1 or Lypd6.
Conclusions: Our findings highlight the diversity and specificity of nicotinic regulation by Lynx family members. The Lynx family may provide a molecular basis to modulate the GABAergic system through regulation of nicotinic signaling in a highly specific way. As somatostatin positive GABAergic neurons are major inhibitory inputs to parvalbumin interneurons, Lynx1 and Lypd6 may act in concert at the local cortical network level to regulate cortical plasticity. Considering the reported deficits of both parvalbumin and somatostiatin GABAergic neurons in the brains of Schizophrenic patients, the Lynx family could provide a promising therapeutic target for modulating key neurodevelopmental events during adolescence in psychiatric disorders.
Keywords: nicotinic, GABA, parvalbumin, somatostatin, plasticity.
Disclosures: M. Demars, Nothing to Disclose; N. Bukhari, Nothing to Disclose; P. Burman, Nothing to Disclose; A. Hussein, Nothing to Disclose; H. Morishita, Nothing to Disclose.

T114. Regulation of Primary Cilia Morphology in Striatum by 5 HT6 Receptor Signaling

John F Neumaier*, Matthew Brodsky, Jane Sullivan

University of Washington, Seattle, Washington

Background: The primary cilium is a sensory organelle stemming from the cell body of most mammalian neurons. This antennae-like microtubule based structure receives both chemical and mechanical signals from other cells and the surrounding environment. These signals are transduced by a discrete set of membrane-bound receptors localized to the primary cilium. Recently, neuronal primary cilia have become a major target of research due to their crucial implication in a variety of disorders, known as ciliopathies. However, the role of primary cilia in normal cognitive functions is not complete understood. Receptors that localize to primary cilia must be selectively trafficked to cilia by specialized cellular machinery. One such receptor is the serotonin-6 receptor (5-HT_6). The 5-HT_6 receptor is an excitatory G_S-coupled metabotropic receptor that is found in the brain and is most prevalent in striatum. 5-HT_6 receptors have substantial impacts on learning and memory, and are linked to a range of cognitive processes and neuropsychiatric syndromes including: anxiety, depression, and addiction.
Methods: Striatal neurons were dissected from P0-P1 wild-type and 5-HT_6 mouse neonates and maintained in culture for 10–12 days. We used a variety of pharmacological and gene transfection strategies to examine the role of 5-HT_6 signaling on primary cilia length and cAMP signaling.
Results: Utilizing immunohistochemistry we confirmed that 5-HT_6 receptors co-localize with adenylyl cyclase III, a selective marker for primary cilia, as has been previously reported. We next investigated the effects of blocking 5-HT_6 signaling by incubating cultures with the selective 5-HT_6 receptor antagonist SB258585 (1 μM). This drug significantly reduced primary cilia length by ~30% ($p = 0.008$) in neurons from wild-type mice but had no effect in neurons from 5-HT_6 receptor knockouts. Experiments to measure cilia length after 5-HT_6 overexpression in wild-type and knockout neurons are underway. A dose-response experiment revealed that this antagonist had a very similar IC_{50} to the Ki in radioligand binding assays. We are currently completing a time course experiment to assess the rate of cilia shortening, and the effects of selective agonists on cilia length. 5-HT_6 knockout neurons have shorter cilia and no effect of SB258585. We are currently assessing the effects of reintroducing mutant and wild-type receptors into 5-HT_6 knockout neurons, and the effects of these receptors on cAMP production.
Conclusions: These studies should elucidate the specific function of the unique localization of endogenous 5-HT_6 receptors on neuronal primary cilia.
Keywords: cilia, striatum, mouse, adenylate cyclase.
Disclosures: J. Neumaier, Nothing to Disclose; M. Brodsky, Nothing to Disclose; J. Sullivan, Nothing to Disclose.

T115. PET/CT *vs* PET/MR for the Clinical Evaluation of Patients with Dementia

Yu-Shin Ding*, Timothy Shepherd, Fernando Boada, Kent Friedman

New York University School of Medicine, New York, New York

Background: Simultaneous PET/MR is a new technology that may be used in the evaluation of dementia patients. There are few data in the literature regarding quantitative differences between PET data obtained at PET/CT *vs* PET/MR and how this may impact image interpretation. This study compared the PET interpretation of PET/CT *vs* PET/MR by two independent experienced nuclear medicine physicians.
Methods: Forty-five minutes following injection of 10 mCi of FDG, 19 patients with clinically-suspected dementia underwent a 15-min clinical brain PET/CT. Simultaneous PET/MR scanning was subsequently performed (60 min list-mode) at approximately 90 min post-injection. Two experienced nuclear medicine physicians blindly interpreted the PET portion of all PET/CT scans, attributing a specific diagnosis (normal, AD, FTD, LBD, other dementia, mixed phenotype or unspecified disease) and severity scale (mild, moderate or severe abnormality). The readers then blindly interpreted the PET data obtained from PET/MR. Concordance between PET/CT (reference standard) and PET/MR with respect to diagnosis and disease severity was assessed for each reader.
Results: Reader A classified 12 PET/CT scans as AD, 5 as unspecified dementia, 1 as LBD and 1 as normal with a mean severity score of 2.0. Reader B classified 10 PET/CT scans as AD, 3 as unspecified, 1 as LBD and 5 as normal

with mean severity score of 2.1. PET/MR interpretations with comparison to PET/CT yielded an 84% (16/19) intra-reader concordance of diagnosis, with 95% (18/19) of severity scores varying by one point or less. Reader B exhibited 84% intra-reader concordance of dementia pattern diagnosis, with 89% (17/19) of all scores varying by one point or less.
Conclusions: Our preliminary analysis in clinically-suspected dementia patients showed a relatively high concordance of intra-reader assignment of diagnosis and severity of findings between PET/CT and PET/MR when evaluated by two blinded experienced nuclear medicine physicians. These results suggest PET/MR brain scans acquired on hybrid PET/MR are of diagnostic quality and interpretation results compare favourably to PET/CT.
Keywords: PET/MR, PET/CT, dementia, FDG.
Disclosures: Y. Ding, Nothing to Disclose; T. Shepherd, Nothing to Disclose; F. Boada, Nothing to Disclose; K. Friedman, Nothing to Disclose.

T116. Neuroimaging Predictors of Clinical Response and Potential Markers of Treatment with Duloxetine in Major Depressive Disorder

Cynthia Fu*, Sergi Costafreda, Mark M Rasenick, Robert Donati, Peng Liu, Lauren Marangell

Kings College London, London, United Kingdom

Background: Functional as well as structural neuroimaging correlates have demonstrated the potential for the prediction of clinical response to the pharmacological and psychological therapies that are commonly used in clinical practice (reviewed in meta-analysis: Fu *et al*, 2013). There is also an emerging pattern of effects of pharmacological therapies on regional cerebral activity (reviewed in meta-analysis: Sankar *et al*, 2013). However, pharmacological treatment studies to date have generally been in the class of selective serotonin reuptake inhibitors (SSRI), while data on neuroimaging predictors of response to serotonin-norepinephrine reuptake inhibitors (SNRIs) have been more limited. Moreover, while anxiety symptoms are common in major depressive disorder (MDD), they have not been well-investigated in neuroimaging studies of MDD treatment. We sought to investigate the neural correlates of depression and anxiety in MDD patients in a prospective, longitudinal treatment study with the SNRI duloxetine. In our primary hypothesis, we expected to observe a 'normalisation' of mean amygdala activation in response to implicit sad facial affect processing in MDD patients following 12 weeks of treatment.
Methods: Subjects were patients in an acute episode of MDD (DSM-IVTR) ($n=32$) and age, gender, and IQ matched healthy controls (HC, $n=25$). All MDD patients were medication-free at baseline and then treated with open duloxetine 60–120 mg. Both groups participated in serial functional (BOLD) and structural (volumetric) magnetic resonance imaging (MRI) scans at weeks 1, 8, and 12 following the baseline visit. Regions of interest were the amygdala, anterior cingulate (AC), and the hippocampus. Affective processing was investigated with the functional MRI task of sad faces. Depressive and anxiety symptoms were measured by Hamilton Depression Rating Scale (HAM-D) and Hamilton Anxiety Rating Scale (HAM-A), respectively. Response was defined as a $>=50\%$ improvement in the HAM-D score and remission was defined as a HAM-D $<=7$. Post-baseline changes in neuroimaging correlates were analysed and compared between MDD patients and HC using a mixed-effect model repeated measure analysis. Among patients, logistic regression was used to examine the association between endpoint remission outcome and changes in neural correlates. Correlation coefficients between changes in clinical scales and neural correlates were also calculated and tested.
Results: Contrary to our hypothesis, there were no significantly different mean changes from baseline to week 12 in BOLD amygdala activation between patients treated with duloxetine and HC. However, significant differences between patients and HC were observed in mean changes in the right amygdala volume and the AC volume. Baseline BOLD AC activation showed a negative correlation with endpoint HAMA change, while the post-baseline change at weeks 8 and 12 in AC activation had a positive correlation with endpoint HAMA change. In logistic regression analysis the change in volume of the left hippocampus from baseline to week 1 was a potential predictor of subsequent remission, as was the change in left hippocampal volume from baseline to week 12.
Conclusions: response, particularly for comorbid anxiety. Structural neural correlates, such as right amygdala volume, showed significant changes following treatment with duloxetine. However, this study did not find an association between anterior cingulate activation at baseline in response to sad facial processing and a higher likelihood of a clinical response. Given the lack of an active control group, it is unknown whether the disparity reflects pharmacological differences, variation due to limited sample sizes, or perhaps differences in comorbid anxiety. The association between a change in left hippocampal volume at week 1 and good clinical outcome may shed light on antidepressant mechanisms of action, but this finding should be evaluated with circumspection due to the limitations inherent with multiple comparisons.
Keywords: duloxetine, major depressive disorder, neuroimaging, biomarkers, anterior cingulate.
Disclosures: C. Fu, **Part 1:** Eli Lilly and Company, **Part 4:** Eli Lilly and Company; S. Costafreda, **Part 1:** Eli Lilly and Company, **Part 4:** Eli Lilly and Company; M. Rasenick, **Part 4:** Eli Lilly and Company; R. Donati, **Part 1:** Pax Neuroscience; P. Liu, **Part 1:** Employment, Eli Lilly and Company, **Part 2:** Employment, Eli Lilly and Company, **Part 3:** Employment, Eli Lilly and Company, **Part 5:** Eli Lilly and Company; L. Marangell, **Part 1:** Employment, Eli Lilly and Company, **Part 2:** Employment, Eli Lilly and Company, **Part 3:** Employment, Eli Lilly and Company, **Part 5:** Eli Lilly and Company.

T117. Whole Genome DNA Cytosine Methylation in a Rat Model of Fetal Alcohol Syndrome

Kornel Schuebel*, Kevin Blackistone, Isioma Mordi, Qiaoping Yuan, Jennifer Thomas, David Goldman

Laboratory of Neurogenetics, Rockville, Maryland

Background: Fetal Alcohol Syndrome (FAS) is a leading cause of childhood cognitive and developmental disorders.

In a rat model of FAS, these effects can be reduced by treatment with choline, a precursor in the one-carbon metabolic cycle that generates s-adenosyl-methionine (SAM). DNA methyltransferases require SAM as a methyl donor for *de novo* cytosine methylation, and choline supplementation alters local and global DNA cytosine methylation patterns. To better understand cellular responses to ethanol in the developing nervous system, and how these responses might be mitigated by choline, we have undertaken studies of the rat prefrontal cortex in animals treated with vehicle, ethanol, choline, or both ethanol and choline.

Methods: Alcohol (3.0 g/kg/day) was administered by intragastric intubation at postnatal days 2–10; choline (100 mg/kg) by subcutaneous injection at post-natal day 2–20. A third group received concurrent alcohol and choline treatment. Including the sham intubation and saline injected control group, there were 10 animals in each of the 4 groups. The postnatal development of the rat brain mimics the third trimester of human fetal brain development. Genomic DNA from prefrontal cortex of each animal was prepared by digestion with proteinase K, treated with RNase A, extracted with phenol-chloroform-isoamyl and ethanol precipitated. After further purification with Qiagen Minelute PCR columns, DNA was quantitated with Qubit and analyzed for integrity by gel electrophoresis. Fragmentation to an average size of 150 bp, as verified by Bioanalyzer, was performed with a Covaris S2 instrument. Methylated DNA fragments were captured by a methyl binding protein approach (Active Motif Methyl Collector Ultra). Greater than 200 million DNA fragments were sequenced for each treatment group using an ABI 5500 WILDFIRE instrument with 50 bp unidirectional reads. After de-barcoding, sequenced fragments were mapped to the rat reference genome (build RN5) using the Lifescope pipeline (version 2.5.1). Mapped sequence data were normalized using RPKM (reads per kilobase per million) and again across all samples prior to methylome analysis. Methylome features were identified and quantitated using the CLC Bio Genome Workbench (version 6.5b3).

Results: Comparing global DNA methylation patterns by scatter, Q-Q, and volcano plots, we observed overall high intragroup and between group correlations in methylome patterns, with r^2 of >0.98 between treatment groups. However, we also identified hypermethylated and hypomethylated loci in the different treatment groups. Using a modified gene set enrichment analysis (GSEA) to group changes in genic DNA methylation into molecular pathways with statistically significant differences between groups, we detected alcohol-induced and choline-modified DNA methylation changes at retinol binding protein, retinoid, alcohol, and acetaldehyde dehydrogenase genes.

Conclusions: This genomic view of brain methylome in the developing rat exposed to alcohol and choline suggests an important role for retinoid signaling in FAS, and also implicates several other gene networks.

Keywords: FAS, choline, methylation, rat, next-gen sequencing.

Disclosures: K. Schuebel, Nothing to Disclose; K. Blackistone, Nothing to Disclose; I. Mordi, Nothing to Disclose; Q. Yuan, Nothing to Disclose; J. Thomas, Nothing to Disclose; D. Goldman, Nothing to Disclose.

T118. Activity-dependent Phosphorylation of MeCP2 Regulates Interaction with NCoR

Daniel Ebert*, Michael E Greenberg

Harvard Medical School, Brookline, Massachusetts

Background: Rett syndrome is a neurodevelopmental disorder with features of autism that is caused by mutations in *MECP2*. In addition, less severe mutations in *MECP2* can lead to a wider spectrum of neuropsychiatric disorders, including autism and psychotic spectrum disorders. MeCP2 is a nuclear protein that binds DNA at methylated cytosines and represses transcription. In neurons, MeCP2 is expressed at high levels, stochiometrically equivalent to core histones and is bound broadly across the genome. The molecular mechanisms of how loss of MeCP2 leads to Rett syndrome are not well understood. Neuronal activity triggers the phosphorylation of MeCP2 at S421. While S421A knock-in mice have defects in synapse development and behavior, the mutation had no detected effect on transcription. In addition, the proximal molecular impact of phosphorylation at S421 on MeCP2 is not known. Mass spectrometry studies have revealed many additional sites of phosphorylation in MeCP2; however, no other phosphorylation site has reproducibly been shown to be induced by neuronal activity.

Methods: To identify sites of activity-dependent phosphorylation, we used phosphotryptic mapping of MeCP2 derived from ^{32}P-orthophosphate-labeled primary cortical neurons that had been left untreated or membrane-depolarized. We generated phospho-site specific antibodies to each site of phosphorylation. We used these antibodies in Western blotting to determine if various stimuli in neuronal cell culture, or *in vivo*, induce the phosphorylation at each site in MeCP2. We used synthetic peptides in pull-down assays and co-immunoprecipitation assays to determine if phosphorylation of T308 altered MeCP2's ability to bind co-factors. To determine the role of activity-dependent phosphorylation of MeCP2 T308 in regulating gene expression and whether the disruption of this phosphorylation event contributes to Rett syndrome, we generated knock-in mice in which T308 was converted to an alanine (MeCP2 T308A KI).

Results: Using phosphotryptic mapping, we identified activity-dependent phosphorylation sites in MeCP2 at S86, S274, and T308. By Western blotting with the phospho-site specific antibodies, we find that phosphorylation at these sites is differentially induced by neuronal activity, BDNF, and agents that elevate cAMP. We find that phosphorylation of MeCP2 T308 disrupts an interaction with the NCoR co-repressor complex and suppresses MeCP2's ability to provide transcription repression. Consistent with this finding, the mutation of MeCP2 T308 to an alanine reduces the induction of *Npas4* expression by neuronal activity. We find that the common Rett syndrome missense mutations at R306 prevent phosphorylation of MeCP2 T308. MeCP2 T308A KI mice, as compared to wild-type littermates, have decreased brain weight, hindlimb clasp, reduced latency to fall on a rotarod, and higher percentage of seizures after exposure to the GABA antagonist pentylenetetrazol, phenotypes consistent with the acquired microcephaly, motor system abnormalities, and seizure disorder that characterize important aspects of Rett syndrome.

Conclusions: Neuronal activity induces phosphorylation of MeCP2 at multiple sites. These multiple sites of phosphorylation are differentially induced by various stimuli, suggesting that MeCP2 may serve as an epigenetic regulator of gene expression that can integrate different signals from the environment. Phosphorylation of MeCP2 T308 disrupts an interaction with NCoR-HDAC3 co-repressor complex and regulates MeCP2's ability to mediate transcription repression. *Npas4* and *Bdnf* mRNA induction is decreased in response to neuronal activity in neurons harboring the MeCP2 T308A KI mutation. These findings are consistent with a model in which neuronal activity induces the phosphorylation of MeCP2 T308, removing an interaction with the NCoR co-repressor complex and facilitating activity-dependent *Npas4* and *Bdnf* mRNA expression. The common Rett syndrome missense mutations at R306 both disrupt an interaction with NCoR and prevent activity-dependent phosphorylation of MeCP2 at T308. The lower brain weight, motor system abnormalities, and lower seizure threshold found in the MeCP2 T308A KI mice correspond to central features of Rett syndrome. Taken together, these findings suggest that the loss of the regulated interaction between MeCP2 and the NCoR co-repressor complex may underlie key aspects of Rett syndrome.
Keywords: MeCP2 Rett syndrome NCoR-HDAC3 co-repressor complex Activity-dependent neuronal signaling Transcription repression.
Disclosures: D. Ebert, Nothing to Disclose; M. Greenberg, Nothing to Disclose.

T119. Epigenetic and Behavioral Correlates of Adolescent Intermittent Ethanol Exposure at Adulthood

Subhash C Pandey*, Amul J Sakharkar, Lei Tang, Tara Teppen, Huaibo Zhang

University of Illinois at Chicago, Chicago, Illinois

Background: Binge alcohol drinking during adolescence may lead to structural and functional changes in the brain and may be responsible for substance abuse and alcoholism at adulthood. Epigenetic mechanisms, such as histone acetylation and DNA methylation induced changes in gene expression play an important role in brain maturation and synaptic plasticity. We investigated the effects of adolescent intermittent ethanol (AIE) treatment on epigenetically regulated synaptic plasticity associated events in the amygdala and on anxiety-like and alcohol-drinking behaviors at adulthood.
Methods: Adolescent (Sprague-Dawley) rats were exposed with intermittent n-saline or ethanol [2 g/kg, intraperitoneal (IP); 2-days on/2-days off, 4 cycles (8 injections) from postnatal days 28–41]. The behavioral and epigenetic measures were performed at adulthood (postnatal day 92). The dendritic spines were measured by Golgi Cox staining procedure.
Results: It was found that nuclear, but not cytosolic, histone deacetylase (HDAC) activity was increased in the amygdala of AIE adult rats as compared with adolescent intermittent saline (AIS) adult rats. This was due to increase in HDAC2, but not HDAC4, protein levels in the central (CeA) and medial nucleus of the amygdala (MeA), but not basolateral amygdala (BLA) of AIE adult rats compared with AIS adult rats. The global histone H3-K9 acetylation was correspondingly decreased in the CeA and MeA, but BLA of AIE adult rats compared with AIS adult rats. We also found that mRNA levels of activity-regulated cytoskeleton-associated (Arc) protein and various brain-derived neurotrophic factor (BDNF) exons (I & IV) and protein levels of Arc and BDNF were significantly decreased in the CeA and MeA, but not BLA of AIE adult rats compared with AIS adult rats. Interestingly, decreased expression of BDNF and Arc was associated with lower histone H3 acetylation levels in the promoters of these genes in the amygdala, as measured by chromatin immunoprecipitation (ChIP) assay. AIE produced a reduction in dendritic spine density in the CeA and MeA, but not BLA of rats at adulthood. AIE also induced anxiety-like and alcohol-drinking behaviors at adulthood which were attenuated by treatment with the HDAC inhibitor, trichostatin A (TSA). We also measured mRNA levels of CREB-binding protein (CBP) and p300, as they have intrinsic histone acetyltransferase activity, and found that AIE produced reductions in the expression of these genes in the amygdala at adulthood.
Conclusions: These results suggest that adolescent binge ethanol exposure produces abnormal chromatin architecture due to histone modifications in the amygdala, which could play a crucial role in anxiety-like and alcohol-drinking behaviors at adulthood (supported by NADIA grant from NIH-NIAAA to SCP).
Keywords: binge drinking, epigenetic, HDAC, amygdala, anxiety.
Disclosures: S. Pandey, Nothing to Disclose; A. Sakharkar, Nothing to Disclose; L. Tang, Nothing to Disclose; T. Teppen, Nothing to Disclose; H. Zhang, Nothing to Disclose.

T120. Group I Metabotrobic Glutamate Receptor Activation Negatively Regulates Glua2-lacking Ampa Receptors in Cultured Nucleus Accumbens Neurons

Jessica A Loweth*, Jeremy M Reimers, Kuei Y Tseng, Marina E Wolf

University of Chicago, North Chicago, Illinois

Background: After prolonged withdrawal from extended-access cocaine self-administration, Ca^{2+}-permeable AMPA receptors (CP-AMPARs) accumulate in the nucleus accumbens (NAc) and mediate the withdrawal-dependent intensification ('incubation') of cue-induced cocaine craving. We have recently shown that, in slices obtained from 'incubated rats', group I mGluR activation eliminates CP-AMPAR transmission in NAc synapses in an mGluR1-dependent manner and that enhancing mGluR1 activity *in vivo* depresses NAc CP-AMPAR transmission and attenuates incubated cocaine seeking. To further characterize the mechanisms mediating this mGluR-dependent plasticity and directly measure mGluR-mediated changes in AMPAR trafficking, we investigated the effects of group I mGluR activation on AMPAR surface expression in NAc medium spiny neurons co-cultured with prefrontal cortical neurons, which are known to contain a significant population of CP-AMPARs.

Methods: Primary NAc neurons from P1 rats were co-cultured with prefrontal cortex (PFC) neurons from P1 enhanced cyan fluorescent protein (ECFP) expressing mice. The PFC neurons restore excitatory inputs onto the NAc medium spiny neurons, but cell types can be distinguished based on fluorescence. Co-cultures (14–21 days *in vitro*) were treated with DHPG (50 μM) for 10 or 20 min and live-cell staining was used to visualize surface GluA1 and GluA2.
Results: Consistent with our findings in the incubation model, live cell labeling studies showed that acute application of the group I mGluR agonist DHPG (50 μM) led to a significant decrease in GluA1 but not GluA2 surface expression, indicating that group I mGluR activation selectively internalizes GluA2-lacking, CP-AMPARs. Studies are currently underway to determine the group I mGluR subtype (mGluR1 or mGluR5) and signaling mechanisms that produce CP-AMPAR internalization in cultured NAc neurons, and the potential role of concurrent trafficking of GluA2-containing AMPARs.
Conclusions: These studies support our *in vivo* findings that group I mGluR activation in NAc neurons leads to internalization of CP-AMPARs. Additional studies will assess whether group I mGluR transmission exerts tonic control over NAc CP-AMPAR levels by investigating whether long-term (24–48 h) inhibition of group I mGluR transmission increases CP-AMPAR surface expression in cultured NAc medium spiny neurons. Recent evidence from our laboratory suggests that, in the incubation model, a decrease in mGluR1 tone contributes to CP-AMPAR accumulation in NAc synapses and incubation of cue-induced cocaine craving. By further characterizing group I mGluR-mediated regulation of AMPAR trafficking *in vitro*, these studies will provide import insight into the mechanisms mediating enhanced AMPAR transmission and cue-induced cocaine seeking in the incubation model.
Keywords: calcium-permeable AMPA receptor, cocaine, nucleus accumbens, co-culture.
Disclosures: J. Loweth, Nothing to Disclose; J. Reimers, Nothing to Disclose; K. Tseng, Nothing to Disclose; M. Wolf, Nothing to Disclose.

T121. CSF from HD Subjects can Seed Aggregation of Mutant Huntingtin

Steven Potkin*, Zhiqun Tan, Leslie Thompson, Charles Glabe

University of California Irvine, Irvine, California

Background: Huntington's disease (HD) is caused by an expanded CAG trinucleotide repeat, producing a mutant protein (huntingtin) that results in deterioration of the central nervous system, abnormal physical movements, cognitive impairment, psychiatric symptoms, and death. There is a crucial need for early biomarkers that reflect the development of the HD pathological process and are quantitatively associated with the course of illness. Such biomarkers will play a crucial role in developing new and effective treatments.
Methods: 14A2.6 PC-12 cells, which carry the truncated exon 1 of the Huntington gene, are a model system for demonstrating huntingtin aggregation. These cells, when induced with ponasterone A, produce intracellular aggregates of polyglutamine (polyQ) expanded huntingtin exon 1 tagged with enhanced green fluorescent protein (EGFP). Exogenous application of synthetic polyQ oligmers seeds the formation of intracellular aggregates in these inducible PC-12 cells. To determine if seeding could occur in HD subjects, cerebrospinal fluid (CSF) from HD subjects and control subjects were added to the 14A2.6 cell culture to determine the degree of aggregation. The percentage of cells with aggregates was counted using fluorescence microscopy. The cells were then washed and lysed, and the amount of aggregate determined on a filter trap assay following blotting with a specific antibody against EGFP or polyQ expansion. The amount of mHttn was also directly determined by antibody assay.
Results: Synthetic polyQ oligmers, but not monomers, seeded enhancement of the number of cells with intercellular aggregates in inducible PC-12 cells. This seeding takes effect at the nanomolar range. Aβ did not induce seeding. Diluted CSF from subjects with HD, but not controls, enhanced the number of cells with aggregates by greater than two-fold ($p < 0.005$), as well as the amount of aggregates. Following pre-treatment with a polyQ antibody, which depletes expanded polyQ-linked components, the HD CSF had no effects on the formation of intracellular aggregates. Direct measures of CSF huntingtin with an N17 IgG antibody that reacts with the first 17 amino acid domain detected quantifiable immunoactivity in both HD and control CSF. However, antibodies specific for the expanded polyQ repeat demonstrated immunoreactivity only in HD CSF.
Conclusions: We have demonstrated that exogenous seeding with polyQ-linked fragments can enhance aggregation in inducible cellular models of HD. This may reflect cell-to-cell propagation of huntingtinopathy. Seeding with CSF from HD subjects, but not controls, can also increase the cell-to-cell transfer of aggregates as measured by increased number of cells with aggregates and increased amount of aggregates. This suggests that extracellular therapies that block seeding propagation may be effective treatments for HD. Therapies that have an effect on mHttn pathogenesis can be monitored by their ability to block seeding propagation. These data provide evidence that CSF-enhanced-aggregation may be a useful biomarker for diagnosis and monitoring the course of HD, and may aid in future drug development.
Keywords: Huntington's disease, CSF, aggregation, biomarker.
Disclosures: S. Potkin, Nothing to Disclose; Z. Tan, Nothing to Disclose; L. Thompson, Nothing to Disclose; C. Glabe, Nothing to Disclose.

T122. Telomere Length in Schizophrenia as a Function of Age and Illness Duration

Owen M Wolkowitz*, Barton W Palmer, Danielle Glorioso, Wesley Thompson, Elissa S Epel, Jue Lin, Elizabeth Blackburn, Dilip V Jeste

University of California San Francisco School of Medicine, San Francisco, California

Background: Schizophrenia is associated with a substantially increased risk of early mortality even after accounting

for lifestyle factors and suicide, raising the possibility that it may be associated with accelerated biological aging. One index of biological aging is leukocyte telomere shortening, which can happen with repeated mitoses as seen with chronic exposure to inflammation or with oxidative stress. Leukocyte telomere length (LTL) is reportedly shortened in depression and certain anxiety disorders, although not all studies agree, and some suggest a 'dose-response' relationship with illness chronicity. LTL has not been well studied in schizophrenia, and published results differ across studies, with individual studies reporting shortened, lengthened or equivalent LTL. Individual determinants of LTL shortening in schizophrenia, including the possible roles of age and illness chronicity, have been poorly characterized. In this study of demographically well-matched individuals with schizophrenia and healthy comparison subjects, we hypothesized that LTL would be shorter in schizophrenia in proportion to the individual's age (reflecting 'accelerated cellular aging') and the individual's chronicity of illness.

Methods: Thirty-one individuals with schizophrenia (14 men and 17 women; mean age 51.2 yrs + SD 11.1, range: 26–64 years) and 31 matched healthy comparison subjects (14 men and 17 women; age 51.5 + 11.8, range: 23–65 years) were studied after providing informed consent. The duration of schizophrenia was estimated by subtracting the age of onset of illness from the age at time of study participation. The mean illness duration in this sample was 25.7 years + 13.9 (SD), range: 1.1–53.1 years. Severity of psychotic symptoms was rated with the Scales for Assessment of Positive and Negative Symptoms (SAPS and SANS). LTL was determined from buffy coat cells by qPCR as previously described (Cawthon RM, Nucleic Acids Res, 30: e47, 2002). All statistical analyses are reported as two-tailed tests and are covaried for age, gender, ethnicity, and education.

Results: Within the schizophrenia group, there was a highly significant age-associated decrease in LTL ($r = -0.60$, $p = 0.001$), but this relationship was not statistically significant in the healthy comparison group ($r = -0.18$, NS). Comparison of the regression coefficients relating age to LTL revealed a near-significant trend toward greater decreases in LTL with increasing age in the schizophrenia group compared to the healthy comparison group (Fisher r-to-z test: $Z = 1.9$, $p < 0.06$). The average LTL in the schizophrenia group (mean + SD = 5328.6 base pairs + 378.2) was 92 base pairs shorter than that in the healthy comparison subjects (mean + SD = 5420.6 base pairs + 378.2), representing approximately three years of accelerated leukocyte aging in the schizophrenia group, although this difference was not statistically significant ($F = 0.78$, NS). However, lifetime duration of schizophrenia, corrected for age, gender, ethnicity, and education, was strongly inversely correlated with LTL ($r = -0.53$, $p = 0.009$), consistent with a cumulative effect of schizophrenia chronicity on LTL shortening. LTL was not significantly correlated with the current severity of positive or negative psychotic symptoms (SAPS: $r = -0.03$, NS; SANS: $r = 0.15$, NS).

Conclusions: These data provide additional insight into possible accelerated cellular/biological aging in schizophrenia. The data suggest that schizophrenia is not intrinsically associated with shortened LTL, and that LTL shortening does not antedate the onset of schizophrenia or represent a risk factor for developing it. Rather, the data suggest that chronicity of schizophrenia is associated with a progressive acceleration of certain cellular aging processes. This pattern of a 'dose-response' relationship between chronicity of illness and LTL shortening has also been reported in depression, certain anxiety disorders, and psychological stress, suggesting common trans-diagnostic biological underpinnings such as chronic inflammation and oxidative stress, both of which have been reported to be increased in schizophrenia. Future studies will be needed to distinguish between the effects of advanced age and duration of illness in predicting LTL in schizophrenia and to assess the relationship between LTL and treatment history, since the observed correlation could represent effects of psychotropic medication rather than the illness itself. Longitudinal studies will be necessary to assess trajectories of LTL change over the lifespan. The present findings are early results of an ongoing large-scale study of accelerated biological aging in schizophrenia (NIMH Grant: R01 MH094151; Dilip V. Jeste, PI). If replicated, the current results may help explain the surfeit of physical aging disorders among individuals with schizophrenia.

Keywords: schizophrenia, psychosis, telomeres, aging, leukocytes.

Disclosures: O. Wolkowitz, Nothing to Disclose; B. Palmer, Nothing to Disclose; D. Glorioso, Nothing to Disclose; W. Thompson, Nothing to Disclose; E. Epel, Nothing to Disclose; J. Lin, Nothing to Disclose; E. Blackburn, Nothing to Disclose; D. Jeste, Nothing to Disclose.

T123. Cognitive Dysfunction and Higher Levels of Autofluorescence (AF) in Schizophrenia (SZ) Patient-derived Cells and Animal Models

Tsuyoshi Tsujimura*, Chi Ying Lin, Juan A Gallego, Xela Indurkhya, Nao Gamo, Minori Koga, Tess Maseda, Tom Sedlak, Anil Malhotra, Carsten Korth, Koko Ishizuka, Akira Sawa

Dainippon Sumitomo Pharma Co., Ltd., Baltimore, Maryland

Background: Establishment of higher throughput and peripheral biomarkers for schizophrenia (SZ) and psychosis is important. Several groups, including ours, have reported increased oxidative stress associated with SZ. Measuring cellular autofluorescence (AF) has been tried in several physical disorders, such as diabetes, for a possible high throughput biomarker. Here we report a study to address whether AF from blood cells and olfactory neuronal cells is altered in SZ patients compared with matched controls. Furthermore, we have tested whether the levels of cellular AF are correlated with other biochemical characteristics, in particular reactive oxygen species (direct measure of oxidative stress), and clinical manifestations. In parallel, we have examined cellular AF in animal models that display aberrant behaviors relevant to SZ. Finally we explore a means to intervene with aberrant cellular AF in both humans and animals, which may potentially affect behavioral deficits.

Methods: *Recruitment and characterization of human subjects:* Chronic SZ patients and subjects with first episode psychosis are recruited in the Johns Hopkins Schizophrenia Center and Zucker Hillside Hospital, respectively. A battery of neuropsychological tests covering six cognitive domains (psychomotor speed, attention, executive function, fluency, verbal memory, and visual memory) is applied to all the participants. Psychiatric symptoms are evaluated with the Scale for the Assessment of Positive Symptoms (SAPS) and the Scale for the Assessment of Negative Symptoms (SANS). This study has been conducted under approval of the ethical boards of both institutions.
Human cells: Lymphoblasts and olfactory neuronal cells via nasal biopsy are prepared according to the publications from our group (Sawa *et al*, ***Nature Med***, 1999; Kano *et al*, ***Mol Psychiatry***, 2013).
Biochemical characterization of human cells and animal brains: The levels of autofluorescence (AF) are determined by flow cytometry at 488 nm excitation and 530 nm emission. Intracellularreactive oxygen species are detected using the CellROX™ Deep Red Reagent (Invitrogen). The levels of misfolded glyceraldehyde 3-phosphate dehydrogenase (GAPDH) are studied with a misfolded form-specific GAPDH antibody generated in the Korth lab, and normalized by the total levels of GAPDH. *Animal study*: Animal models that display augmented oxidative stress and aberrant behaviors are being utilized, which include excitatory amino-acid transporter 1 (EAAC1) knockout mice that display deficits in glutathione, a major antioxidant, in the brain, and a transgenic model expressing a dominant-negative DISC1 (Johnson *et al*, ***PNAS***, 2013).
Results: The levels of cellular autofluorescence (AF) were significantly elevated in lymphoblasts from SZ patients compared with those from matched controls. Furthermore, the aberrantly elevated AF was also observed in olfactory neuronal cells from SZ compared with control cells. Subchronic treatment of lymphoblasts with antipsychotics did not change the levels of AF. We also found a clear correlation between the levels of cellular AF and reactive oxygen species. Taken together, these results support a notion that high throughput measurement of cellular AF in blood cells is a useful means to access intrinsic and neuronal-relevant abnormalities associated with oxidative stress. Next, we addressed whether the aberrant cellular AF reflected clinical and neuropsychological changes in SZ. We failed to observe any correlation between the levels of AF and scores of SANS/SAPS. However, we observed a specific correlation between the levels of AF and verbal fluency. Given that the cellular AF is a promising marker for oxidative stress and cognitive changes, we further tested molecular mechanisms underlying this cellular change. Among potential sources for cellular AF, Sudan black B is known to specifically inhibit lipofuscin-derived AF. Interestingly, augmented AF in SZ cells were normalized by treatment with Sudan black B. Furthermore, we observed aberrant augmentation of misfolded GAPDH, which is reportedly accumulated in the lipofuscin, in lymphoblasts from SZ patients compared with control cells. These results suggest the lipofuscin/GAPDH pathology in SZ. In animal models that display augmented oxidative stress and aberrant behaviors relevant to SZ, we observed increased levels in AF and misfolded GAPDH, which are consistent with the observations in SZ lymphoblasts and olfactory neuronal cells. As we also obtained preliminarybut promising results that metformin, an anti-diabetic drug, could antagonize elevated AF in SZ cells, we plan to test whether the compound may antagonize not only biological and but also behavioral changes in the animal models.
Conclusions: The measurement of AF in blood cells is high throughput. Thus, these observations may be very useful in mechanistic studies and biomarker explorations, including those for drug screening.
Acknowledgements: MH-084018, MH-094268 Silvo O. Conte center, MH-069853, MH-085226, MH-088753, MH-092443, grants from Stanley, RUSK, S-R foundations, NARSAD and Maryland Stem Cell Research Fund for A.S.
Keywords: autofluorescence, schizophrenia, GAPDH, oxidative stress, lymphoblasts.
Disclosures: T. Tsujimura, **Part 1:** Dainippon Sumitomo Pharma Co., Ltd., **Part 2:** Dainippon Sumitomo Pharma Co., Ltd., **Part 3:** Dainippon Sumitomo Pharma Co., Ltd., **Part 5:** Dainippon Sumitomo Pharma Co., Ltd.; C. Lin, Nothing to Disclose; J. Gallego, Nothing to Disclose; X. Indurkhya, Nothing to Disclose; N. Gamo, Nothing to Disclose; . Koga, Nothing to Disclose; T. Maseda, Nothing to Disclose; T. Sedlak, Nothing to Disclose; A. Malhotra, Nothing to Disclose; C. Korth, Nothing to Disclose; K. Ishizuka, Nothing to Disclose; A. Sawa, **Part 1:** Astellas, Takeda, Tanabe-Mitsubishi, Dainippon-Sumitomo, Johnson and Johnson, Sucampo, Pfizer, Asubio, Eli Lilly, Taisho, Amgen, Afraxis, Sanofi-Aventis, and Astra zeneca, **Part 4:** Astellas, Takeda, Tanabe-Mitsubishi, Dainippon-Sumitomo, Johnson and Johnson, and Sucampo.

T124. Functional Analysis of the Schizophrenia-associated Gene, TCF4

Matthew D Rannals, Andrew Jaffe, Ran Tao, Thomas M Hyde, Joel E Kleinman, Daniel Weinberger, Brady J Maher*

Lieber Institute for Brain Development, Baltimore, Maryland

Background: Schizophrenia is a neurodevelopmental disorder with unknown pathophysiology. The genetic architecture of schizophrenia at the population level appears to be polygenic and current estimates are that hundreds of genetic loci are involved. Genome-wide association studies (GWAS) have identified a number of loci associated with increase risk for SZ and several of these risk variants are located within introns of Transcription Factor 4 (TCF4; E2-2, ITF2). In addition, autosomal dominant mutations in TCF4 result in Pitt Hopkins Syndrome (PTHS), a rare neurodevelopmental disorder characterized by a spectrum of symptoms including hyperventilation, seizures, autistic behaviors, mental retardation, and brain malformations. Currently, the molecular mechanisms and underlying pathophysiology responsible for these two disorders are not understood. Our goal is to determine the function of TCF4 during cortical development and to understand the molecular mechanism of risk that is associated with genetic variants of TCF4. TCF4 is a ubiquitous basic helix-loop-helix (bHLH) protein that binds to E-box DNA sequences

(CANNTG) and regulates transcription. It has 41 exons of which 21 are alternative 5′ exons and potentially code for 18 N-terminally distinct protein isoforms named TCF4A—TCF4R. TCF4 can form homodimers or heterodimers with other bHLH proteins and appears to be regulated by activity through its interaction with the Ca^{2+} binding protein calmodulin.
Methods: To test the function of TCF4 in the developing neocortex we altered its expression by transfecting layer 2/3 pyramidal cells in the rat medial prefrontal cortex by *in utero* electroporation. We knockdown TCF4 expression using two shRNA constructs that target independent sequences within the TCF4 transcript and over-expressed human TCF4 with recombinant TCF4 constructs. Functional analysis was performed using whole-cell electrophysiology and confocal imaging in acute brain slices. To gain insights into the molecular mechanisms responsible for increased risk associated with genetic variants of TCF4, we analyzed RNA sequencing data obtained from the dorsal lateral prefrontal cortex of postmortem brains from schizophrenia patients ($n = 107$) and controls ($n = 107$).
Results: Embryonic knockdown of TCF4 decreased intrinsic excitability and resulted in the ectopic appearance of spike-frequency adaptation ($p < 0.002$; TCF4 shRNA $n = 24$, Con shRNA $n = 32$). To understand the mechanism for these phenotypes we performed an ion channel expression screen to see if TCF4 transcriptionally regulates ion channel expression. We observed enrichment for genes associated with Ca^{2+} signaling and demonstrated that intracellular application of the Ca^{2+} chelator BAPTA ($p < 0.002$; BAPTA $n = 23$; TCF4 shRNA $n = 18$) or lowering extracellular Ca^{2+} concentrations ($p < 0.004$; $n = 9$) can rescue action potential phenotypes associated with knockdown of TCF4. In addition, the L-type Ca^{2+} channel blocker nimodipine was also effective at rescuing these phenotypes ($p < 0.05$; $n = 8$). Over-expression of TCF4B resulted in acceleration of neuronal migration and the abnormal formation of cortical microcolumns. Neuronal migration was measured two days post transfection and greater than 30% of the transfected neurons had reached the intermediate zone or cortical plate compared to less than 10% of the cells transfected with GFP alone ($p < 0.0001$; TCF4B $n = 8$, GFP $n = 8$). The TCF4B-dependent formation of cortical microcolumns was also rescued by co-expressing TCF4B + TCF4 shRNA. Analysis of RNAseq data from postmortem brain identified a specific 5′ exon that uniquely identifies a single TCF4 isoform that shows differential expression between cases ($n = 107$) and controls ($n = 107$), where patients with schizophrenia had lower expression ($p = 1.53 \times 10^{-5}$). We also observed that 3 perfectly correlated and adjacent SNPs were significantly associated with the expression of this unique 5′ exon ($p = 9.52 \times 10^{-3}$) and represent an eQTL. These SNPs are in strong LD with the previously reported TCF4 risk variant (rs17512836). No other TCF4 exons were associated with either illness state or risk associated genotype in our samples.
Conclusions: Our results suggest the dosage of TCF4 is critical to cortical development and neuronal physiology. By analyzing RNA sequencing data in human brain, we have now identified a specific isoform of TCF4 that infers schizophrenia risk, suggesting that this may be the molecular mechanism of the clinical association with schizophrenia. We believe these novel data will allow us to model schizophrenia in our rat model with high fidelity. RNAseq data from prefrontal cortex of rat suggests this specific isoform is present. Future experiments will be designed to specifically knockdown or overexpress this risk associated TCF4 isoform and determine its effects on cortical development and neuronal physiology.
Keywords: schizophrenia transcription factor RNA sequencing neurodevelopment.
Disclosures: M. Rannals, Nothing to Disclose; A. Jaffe, Nothing to Disclose; R. Tao, Nothing to Disclose; T. Hyde, Nothing to Disclose; J. Kleinman, Nothing to Disclose; D. Weinberger, Nothing to Disclose; B. Maher, Nothing to Disclose.

T125. Regulation of Tyrosine Hydroxylase by CLOCK: Potential Mechanisms Underlying the Circadian Control of Dopamine and Reward

Wilbur Williams*, Angela Ozburn, Colleen A McClung

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: Many psychiatric illnesses, including drug addiction, are associated with disturbances in the circadian system, though the mechanisms linking the circadian clock to the mesolimbic reward circuitry remain poorly understood. The circadian gene, *Clock*, is expressed in the ventral tegmental area (VTA) and nucleus accumbens (NAcc) and has been implicated in modulating reward processing. We have previously shown that mice with a mutation in this gene, *ClockD19*, exhibit enhanced sensitivity to rewarding stimuli, including heightened cocaine CPP and self administration. *ClockD19* mice also exhibit upregulated tyrosine hydroxylase (*TH*), the rate-limiting enzyme in dopamine synthesis, in the VTA as well as increased dopamine release in the NAcc. Intriguingly, preventing CLOCK from binding the TH promoter, via mutation to the proximal EBox, upregulates TH transcription. These data suggest a novel, repressive role of the CLOCK protein in dopamine synthesis and provide a potential mechanism by which circadian mutations lead to disruptions in reward processing. We hypothesize that CLOCK inhibits dopamine synthesis through occluding the adjacent CRE site in the TH promoter. We sought to directly test the differential abilities of CLOCK and *ClockD19* to regulate TH and to determine if these differences underlie the sensitivity to cocaine in this model of bipolar mania.
Methods: We utilized PC12 cells transfected with TH-luciferase reporter plasmids to determine if CLOCK directly inhibits TH transcription. Cells were co-transfected with CREB, CREB and CLOCK or CREB and *ClockD19* expression plasmids using lipofectamine LTX (Invitrogen). 24 h later, lysates were analyzed for TH-luciferase activity. To determine if the disinhibition of CREB-mediated dopamine synthesis is behaviorally relevant in *ClockD19* mice, weperformed cocaine conditioned place preference (10 mg/kg i.p.) on WT or *ClockD19* mice following stereotaxic injection of AAV-mCREB or AAV-GFP in the VTA ($n = 10$/group) to inhibit CREB signaling in this nucleus. Finally, we performed Western blots for CRY1 and SIRT1, two proteins known to interact with CLOCK and

lead to a co-repressor complex, on VTA lysates from WT or *ClockD19* mice.
Results: We found that TH-luc activity was significantly reduced when CREB-transfected cells were co-transfected with CLOCK. However, there was no reduction in TH-luc activity when cells were co-transfected with *ClockD19*. This suggests that CLOCK prevents CREB-mediated transcription, while the mutant protein disinhibits CREB-mediated signaling and thus results in an upregulation of dopamine synthesis. AAV-mCREB significantly reduced the heightened cocaine CPP exhibited in *ClockD19* mice, indicating that the increased sensitivity to drugs of abuse can be rescued by reversing VTA-specific CREB signaling in these animals. Finally, both CRY1 and SIRT1 proteins are reduced in the VTAof *ClockD19* mice, which may provide a novel insight into the hyper-dopaminergic state in this model of bipolar mania.
Conclusions: Here, we characterize a novel mechanism by which the CLOCK protein regulates dopamine transcription and connects the circadian system and reward. These data show for the first time that CLOCK inhibits dopamine synthesis, by preventing CREB-mediated transcription of TH. Furthermore, we show that the mutant protein is incapable of inhibiting TH, providing key insight into the mechanisms underlying the hyperdopaminergic state of *ClockD19* mice. Furthermore, we show that *in vivo* inhibition of CREB in the VTA is sufficient to reverse the heightened cocaine CPP in these mice. Finally, CRY1 and SIRT1 protein levels are reduced in the VTA of *ClockD19* mice, providing novel mechanisms underlying their manic phenotype. Taken together, these experiments provide a molecular mechanism by which dopamine synthesis is regulated by CLOCK by which a mutation in the *Clock* gene ultimately leads to elevated dopamine and manic-like behaviors.
Keywords: dopamine circadian bipolar cocaine.
Disclosures: W. Williams, Nothing to Disclose; A. Ozburn, Nothing to Disclose; C. McClung, Nothing to Disclose.

T126. Rats Prone to Obesity Show 'Addiction-like' Deficits in Behavior and Synaptic Plasticity

Robyn M Brown*, Yonatan M Kupchik, Sade Spencer, Constanza Garcia-Keller, Danielle Schwartz, Kelsey Jordan, Thomas C Jhou, Peter W Kalivas

Medical University of South Carolina, Charleston, South Carolina

Background: Difficulty in managing food intake, especially highly palatable food, can result in obesity and the health liabilities associated with being overweight. A cardinal feature of pathological over-eating, is that although the individual can describe the negative consequences of their behavior, they have great difficulty intervening and changing their behavior. Indeed, many overweight individuals express a desire to limit their food consumption, yet struggle to control their intake and repeatedly consume beyond their energy requirements. As such pathological overeating has the capacity to resemble an addictive disorder. The aim of this study was to establish whether rats prone to obesity would show addiction-like impairments in behavior and synaptic plasticity.
Methods: A diet-induced obesity model was used to determine two subpopulations of rats: obesity prone and obesity resistant. These rats were then assessed for operant self-administration of high fat high sugar (HFHS) pellets using fixed and progressive ratios. Two DSM-IV criteria were modelled: (1) The subject has an extremely high motivation to take the substance (palatable food), with activities focused on its procurement and consumption. This criterion was modelled using a progressive ratio schedule: The number of responses required to receive one HFHS pellet was increased progressively within the operant session. The maximal amount of work that the animal will perform beforecessation of responding, referred to as the breakpoint, is considered a reliable index of the motivation for the substance. (2) The subject has difficulty stopping substance use or limiting substance intake. We measured the persistence of HFHS reward-seeking during a period of signaled nonavailability of HFHS pellets. The daily operant session included three 15-min 'food available periods' (S+) that alternated with three 5-min 'food unavailable periods (S−). The two periods were signalled by different contextual cues (house light, auditory cues). 24 h after the last operant session electrophysiological measurements of synaptic strength (AMPA/NMDA ratio) and Long term depression (LTD) were made in the nucleus accumbens core.
Results: Obesity prone rats showed an 'addiction-like phenotype' as demonstrated by both behavior and synaptic plasticity measurements. Obesity prone rats exhibited higher breakpoints on a progressive ratio schedule suggesting enhanced motviation to obtain an HFHS reward. In addition they showed increased responding during periods which signalled reward unavailablity, suggestive of compulsive reward-seeking behavior. Moreover, despite LTD being reliablity induced in obesity resistant rats, we were unable to produce LTD in the nucleus accumbens core of obesity prone rats, consistent with previous findings with drugs of abuse. Lastly, the administration of N-acetylcysteine, a compound that has been trialled in humans for cocaine, nicotine and marijuana addiction, reduced operant responding for HFHS pellets in obesity prone rats down to levels displayed by obesity resistant rats. These findings suggest that in individuals prone to obesity addiction-like impairments in the brain occur which may underlie their ability to limit intake of highly palatable food. This is supported by the observation that N-acetylcysteine, a compound which restores the impairments in glutamate transmission associated with drug addiction, reduces excessive consumption of highly palatable food in rats prone to obesity.
Conclusions: Collectively, these data support the concept of that, akin to drugs of abuse, highly palatable food may in some cases be considered 'addictive' and provides evidence that compounds used to treat drug addiction may also have utility in treatment of the pathological overeating which often underlies obesity.
Keywords: palatable food, obesity, addiction, synaptic plasticity, LTD.
Disclosures: R. Brown, Nothing to Disclose; Y. Kupchik, Nothing to Disclose; S. Spencer, Nothing to Disclose; C.

Garcia-Keller, Nothing to Disclose; D. Schwartz, Nothing to Disclose; K. Jordan, Nothing to Disclose; T. Jhou, Nothing to Disclose; P. Kalivas, Nothing to Disclose.

T127. Analysis of the Pain Transcriptome Using RNA-Seq

Samridhi Goswami, Santosh Mishra, Mark Hoon, Andrew Mannes, Michael Iadarola*

NIH, Bethesda, Maryland

Background: Detailed investigation of the molecular biology of neuronal gene expression is a key element for advancing basic knowledge of neural circuits and translational investigations. The neurons in sensory ganglion present certain advantages for advancing cell- and circuit-specific analyses because of their identified physiological roles and physical shape. The nociceptive subset of ganglionic neurons represent the first step in the pathway to higher nervous system processing of pain. In the present study we determine the complete transcriptional repertoire of dorsal root and trigeminal ganglion neurons (DRG and TG), with emphasis on the subset that expresses the TRPV1 ion channel. This channel transduces painful heat, inflammatory and chemical stimuli and is also active at low pH. Therapeutic chemoablation of TRPV1 neurons shows they are key elements in clinical pain in advanced cancer in either canine or human patients. Based on their pivotal role, determining the molecular signature of TRPV1-expressing DRG neurons is critical for understanding the molecular biology of nociceptive neural transmission and inflammatory clinical pain.
Methods: The present report examines the TRPV1 DRG neuronal transcriptome using either genetic or chemoablative strategies to enrich or delete these neurons. Isolation of the TRPV1-expressing population was done with TRPV1 promoter BAC-transgenic expression of red fluorescent protein and fluorescence activated cell sorting (FACS). This method specifically enriches for DRG neurons that derive from the TRPV1 lineage, which in the adult, encompasses the majority of nociceptive modalities as well as itch. A contrasting transcriptome was obtained by cell deletion: (1) TRPV1 lineage neurons in mouse were deleted by expressing diphtheria toxin in the BAC-TRPV1 promoter mice and (2) intratrigeminal ganglion injection of the TRPV1 agonist resiniferatoxin (RTX). Animal studies were conducted under protocols approved by the institutional animal care and use committee and in accordance with the NIH guidelines. These data also allowed comparison between mouse and rat DRG transcriptomes. Deep sequencing, 170 million paired end reads, was performed on sorted and deleted preparations. Data from rat was obtained from $N=9$ ganglia and RTX lesion was performed on $N=3$ rats and controls all to a depth of 38 million paired end reads. One 'mixed tissue' sample 108 million reads of rat brain areas and peripheral organs and glands was also analyzed. Reads in all cases were 101 bases. The normalized unit of measure is RPKM for reads per kilobase of transcript per million reads.
Results: RNA-Seq has several unique attributes for measurement of cell-specific and state-dependent gene expression alterations. The quantitative results provide objective criteria for stratification and interpretation of the large amount of neuronal gene expression data and both increases and *decreases* in gene expression, as seen with RTX lesions or DTA cell deletion can be clearly assessed. The basal level of expression is a second important factor that quantitatively differentiates transcripts. This parameter is difficult to accurately determine with most other methods for large-scale gene expression, yet is critical in evaluation of potential contributions to physiological or pathophysiological processes. The assignment of precise identity for each transcript is a further benefit resulting from exact sequence determination. Additionally, deep RNA-seq reveals sequence information not yet present, or incompletely represented, in the annotated databases such as extensions of the 5′ and 3′ untranslated regions, which we examine for TRPV1. RNA-Seq provided a panoramic view of ganglionic gene expression. Approximately 12 500 genes are expressed in the DRG using the criterion of >1 RPKM. Another ~ 3500 genes can be added by inclusion of the lower expressed genes. The most highly expressed genes were structural for either axons or myelin and in the range of 2000–3000 RPKM. Sequencing revealed multiple new transcripts in the TRPV1-enriched and depleted populations such as calcium binding proteins from the hippocalcin family. Quantitative relationships among gene families indicate several paracrine communication loops, for example, a purinergic ion channel, adenosine generating enzyme, G-protein receptor cascade that may exert local control of nociceptor excitability following tissue damage and release of cellular ATP. All three elements in this cascade have been targets of analgesic manipulations. Autocrine loops are also suggested by co-expression of ligands and receptors for somatostatin, galanin, and glutamate (kainite receptor) systems. These results allow the beginnings of the 'nociceptome' to be outlined and molecular interrelationships defined.
Conclusions: The present report defines, in a quantitative, cell-specific fashion, the molecular signature of a distinct and clinically important population of pain-sensing neurons. The results provide an overall framework for understanding the transcriptome of TRPV1 nociceptive neurons and yield a rich repertoire of data to further elucidate the pathways and mechanisms involved in acute and neuropathic pain, neurological disorders affecting peripheral nerve, and potential routes to new therapeutic approaches.
Keywords: gene expression transcriptome RNA-Seq pain analgesia.
Disclosures: S. Goswami, Nothing to Disclose; S. Mishra, Nothing to Disclose; M. Hoon, Nothing to Disclose; A. Mannes, Nothing to Disclose; M. Iadarola, Nothing to Disclose.

T128. Actigraphy Measured Sleep Disruption as a Predictor of Survival Among Women with Advanced Breast Cancer

David Spiegel*, Oxana Palesh, Arianna Aldridge-Gerry, Jamie Zeitzer, Cheryl Koopman, Janine Giese-Davis, Booil Jo, Helena Kraemer, Eric Neri, Bita Nouriani

Stanford University School of Medicine, Stanford, California

Background: Poor sleep, prevalent among cancer survivors, is associated with disrupted hormonal circadian rhythms

and poor quality of life. This study aimed to clarify the relationship betweek objective sleep efficiency and overall survival among women with advanced breast cancer using a prospective design.
Methods: We examined sleep quality and duration in 97 women diagnosed with advanced breast cancer (age = 54.6 +9.8 years) via wrist-worn actigraphy for three days and sleep diaries. Sleep quality was operationalized as poor sleep efficiency (ratio of total time asleep to total time in bed).
Results: As hypothesized, poor sleep efficiency was found to predict shorter survival (Hazard Ratio (HR), 0.96, 95% CI, 0.94–0.98, $P<0.001$) at median 6 years follow up. This relationship remained significant (HR, 0.94, 95% CI, 0.91–0.97, $P<0.0001$) even after adjusting for other known prognostic factors (age, estrogen receptor status, cancer treatment, metastatic spread, cortisol levels, and depression). Other sleep parameters such as wake after sleep onset (HR:2.41. CI:1.67–3.5, $P<0.001$), mean number of wake episodes, (HR: 1.07, CI:1.02–1.13, $P<0.01$), and mean wake episode duration (HR: 1.27, CI: 1.12–1.43, $P<0.001$), also showed significant effects on overall survival after adjusting for baseline prognostic factors.
Conclusions: These findings show that sleep dysregulation is a significant independent prognostic factor for more rapid disease progression in women with advanced breast cancer. Further research is needed to determine whether treating sleep disruption with cognitive behavioral and/or pharmacologic therapy could improve survival in women with advanced breast cancer.
Keywords: sleep disruption, sleep efficiency, advanced breast cancer, survival.
Disclosures: D. Spiegel, Nothing to Disclose; O. Palesh, Nothing to Disclose; A. Aldridge-Gerry, Nothing to Disclose; J. Zeitzer, Nothing to Disclose; C. Koopman, Nothing to Disclose; J. Giese-Davis, Nothing to Disclose; B. Jo, Nothing to Disclose; H. Kraemer, Nothing to Disclose; E. Neri, Nothing to Disclose; B. Nouriani, Nothing to Disclose.

T129. Inflammation, Depression and N-3 Fatty Acids: A Case of Personalized Medicine

Mark Hyman Rapaport*, Pamela Schettler, Thaddeus W Pace, Becky Kinkead, Andrew A Nierenberg, David Mischoulon

Emory University School of Medicine, Atlanta, Georgia

Background: The heterogeneity of psychiatric syndromes such as Major Depressive Disorder (MDD) is a significant obstacle to the development of new therapies. We postulated that biomarkers of inflammation may delineate a more homogeneous population of subjects with MDD and that those subjects would be more responsive to N-3 fatty acid monotherapy.
Methods: We measured at baseline 5 biomarkers of inflammation (hs-CRP, IL-6, IL-1RA, leptin, adiponectin) in a cohort of 174 subjects with MDD who participated in a double-blind placebo-controlled trial of monotherapy with either 1 gram/day eicosapentaenoic acid (EPA) or 1 gram/day docosahexaenoic acid (DHA). The Hamilton Depression Rating Scale was the primary outcome measure.
Results: All 5 markers of inflammation were correlated (or inversely correlated foradiponectin) with $p<0.003$ significant levels. Sixty-eight subjects had 1positive (increased level for IL-1RA, IL-6, hs-CRP, leptin and decreasedadiponectin) markers of inflammation; 59 subjects had 2 positive markers ofinflammation; 37 subjects had 3 positive markers of inflammation; 21 subjectshad 4 positive markers of inflammation and 11 subjects had positive of all 5markers of inflammation. The treatmenteffect size difference between EPA and placebo increased significantly as theprevalence of positive inflammatory markers increased: 0 markers: ES + 1.03; 1marker: ES—0.27; 2 markers: ES—0.67; 3–5 markers: ES— .67; 4–5 markers: ES –1.11.
Conclusions: In this proof of concept study, we used biomarkers of inflammation to define an increasingly homogeneous group of subjects. Subjects with MDD who had 2 or more positive markers of inflammation demonstrated a moderate to large ES difference between EPA and placebo monotherapy. This suggests the importance of measuring multiple biomarkers of inflammation simultaneously in order to better characterize one's study population.
Keywords: inflammation, depression, N-3 fatty acids, personalized medicine.
Disclosures: M. Rapaport, Nothing to Disclose; P. Schettler, **Part 1:** Statistical Consulting to: Cedars-Sinai Medical Ctr., Dept of Psychiatry, Los Angeles, CA (2011–2012); Methylation Sciences, Barbados West Indies (2011); Brain Cells, Inc., San Diego, CA (2012); Massachusetts General Hospital, Depression Clinical & Research Program, Boston, MA (2011, 2012), **Part 2:** Cedars-Sinai Medical Center, Department of Psychiatry, Los Angeles, CA (2011 and 2012), **Part 3:** Cedars- Sinai Medical Center, Department of Psychiatry, Los Angeles, CA (2011 and 2012); T. Pace, Nothing to Disclose; B. Kinkead, Nothing to Disclose; A. Nierenberg, **Part 1:** Dr Nierenberg has served as a consultant to: Appliance Computing Inc., (Mindsite), Brain Cells, Inc., Brandeis University, Bristol Myers Squibb, Clintara, Dianippon Sumitomo (Now Sonovion), Eli Lilly and Co., EpiQ, Forest, Novartis, PamLabs, PGx Health, Shire, Schering-Plough, Sunovion, Takeda Pharmaceuticals, Teva and Targacept. He has consulted thruogh MGH Clinical Trials Network and Institute (CTNI): Astra-Zeneca, Brain Cells, Inc., Dianippon Sumitomo/Sepracor, Johnson and Johnson, Labopharm, Merck, Methylation Science, Novartis, PGX Health, Shire, Schering-Plough, Targacept and Takeda/Lundbeck Pharmaceuticals. Dr Nierenberg received honoraria or travel expenses including CME activities from APSARD, Belvoir Publishing, Boston Center for the Arts, University of Texas SW, Dallas, Hillside Hospital, American Drug Utilization Review, American Society for Clinical Psychopharmacology, Bayamon Region Psychiatric Society, San Juan PR, Baystate Medical Center, Canadian Psychiatric Association, Columbia University, Douglas Hospital/McGill University, IMEDEX, International Society for Bipolar Disorders, Israel Society for Biological Psychiatry, Johns Hopkins University, MJ Consulting, New York State, Massachusetts Association of College Counselors, Medscape, MBL Publishing, Physicians Postgraduate Press, Ryan Licht Sang Foundation, Slack Publishing, SUNY Buffalo, University of FL, University of Miami, University of Wisconsin, University of Pisa, and SciMed. Dr Nierenberg

is a presenter for the Massachusetts General Hospital Psychiatry Academy (MGHPA). The education programs conducted by the MGHPA were supported through independent medical education (IME) grants from the following pharmaceutical companies in 2008: Astra Zeneca, Eli Lilly and Janssen Pharmaceuticals; in 2009, Astra Zeneca, Eli Lilly, and Bristol-Myers Squibb. No speaker bureaus or boards in 2003. Dr Nierenberg owns stock options in Appliance Computing, Inc and Brain Cells, Inc. Additional income is possible from Infomedic.com depending on overall revenues of the company but no revenue has been received to date. Through MGH, Dr Nierenberg is named for copyrights to: the Clinical Positive Affect Scale and the MGH Structured Clinical Interview for the Montgomery Asberg Depression Scale exclusively licensed to the MGH Clinical Trials Network and Institute (CTNI). **Part 4:** Dr Nierenberg has received grant/research support through MGH form AHRQ, Cephalon, Forest, Mylan, NIMH, PamLabs, Pfizer Pharmaceuticals, Takeda and Shire.; D. Mischoulon, **Part 4:** Research support from the FisherWallace, Ganeden, Nordic Naturals, Methylation Sciences, Inc. (MSI). Honoraria for consulting, speaking and writing from Pamlab, Nordic Naturals, and the Massachusetts General Hospital Psychiatry Academy. Royalties from Lippincott Williams & Wilkins for published book 'Natural Medications for Psychiatric Disorders: Considering the Alternatives.'.

T130. The Opiate Antagonist, Naltrexone, in the Treatment of Trichotillomania: Results of a Double-blind, Placebo-controlled Study

Jon E Grant*, Brian Odlaug, Suck Won Kim

University of Chicago, Chicago, Illinois

Background: Trichotillomania is characterized by repetitive pulling resulting in hair loss. With an estimated prevalence of 1–4%, trichotillomania is a fairly common condition in which individuals repeatedly pull their hair. Despite the promise from current treatments such as habit reversal therapy and medication, treatments are not effective for all individuals with trichotillomania, and so additional options are needed. The opioid receptor antagonist, naltrexone, has previously been examined in the treatment of grooming behaviors in animals and in human trichotillomania. Opioid antagonists reduce self-licking or self-chewing in 63% to 91% of dogs with acral lick dermatitis. In a small ($n = 17$), 6-week, double-blind study, Christenson and colleagues examined the possibly efficacy of naltrexone (50 mg/day) for adults with trichotillomania. Subjects treated with naltrexone exhibited statistically significant improvement compared to placebo on one of three measures of hair pulling severity. More recently, an open-label study of naltrexone (50 mg–100 mg/day) in 14 children with trichotillomania reported that 11 (78.6%) exhibited significant improvement. The efficacy of opioid antagonists in the treatment of repetitive behaviors has been proposed to involve opioidergic modulation of mesolimbic dopamine circuitry, leading to diminished urges to engage in the behavior.

Methods: Men and women aged 18–75 with a primary diagnosis of trichotillomania were recruited by newspaper advertisements and referrals. Only subjects who reported urges to pull (at least 50% of the time and a score of $\geqslant 1$ on each of the first three items of the Massachusetts General Hospital Hair Pulling Scale) were included. 51 individuals (44 [86.3%] women; mean age $= 32.7 \pm 9.8$ years) were randomized to naltrexone or placebo. Demographics and clinical features of trichotillomania were assessed with a semi-structured interview. Subjects reported severity of trichotillomania symptoms using the self-rated Massachusetts General Hospital Hair Pulling Scale (MGH-HPS), the primary outcome measure for the study. Cognition was assessed using the Intra-dimensional/Extra-dimensional (IDED) shift task and the Stop Signal Task (SST). Differences in response between placebo and naltrexone were adjusted for baseline disparities using the baseline score as a covariate. Primary and secondary measures were examined using ANOVA modeling analyses.

Results: There were no statistically significant imbalances regarding demographics or baseline trichotillomania symptoms between treatment groups. There were no significant differences observed for those assigned to naltrexone on the primary efficacy variable (MGH-HPS score) compared to placebo by study endpoint. Secondary measures also failed to reflect any significant differences between treatment groups. By study endpoint, 9 (36%) of those assigned to naltrexone were 'much' or 'very much' improved compared to 9 (34.6%) of those on placebo (Yates chi-square $= 0.04$; df $= 1$; $p = 0.920$). Cognitive flexibility significantly improved in the naltrexone group compared to the placebo group ($t = 2.697$; $p = 0.028$). Within group analysis also demonstrated that the naltrexone group significantly improved on cognitive flexibility from baseline to endpoint ($t = 2.329$; $p = 0.026$). We also found that reduction in urges to pull from baseline to endpoint (using the first three items of the MGH-HPS) in the naltrexone group was numerically greater in those with a family history of an AUD or SUD, but not statistically significant ($t = 2.007$; $p = 0.057$; effect size $= 0.837$).

Conclusions: This randomized, double-blind, clinical trial indicates that naltrexone is generally not more effective than placebo for trichotillomania based on our primary and secondary outcome measures. The study hypothesis was not supported by the data, but post-hoc analyses of the data yielded two potentially important findings. First, although it did not reduce urges in all trichotillomania subjects, there is at least a preliminary suggestion that naltrexone may reduce urges to pull in individuals with trichotillomania who also have a family history of substance addiction. The second important finding from this study was that neurocognitive testing indicated significant improvements in cognitive flexibility after treatment with naltrexone. Cognitive flexibility may represent a promising target for treatment in some individuals with trichotillomania. Individuals with problems in cognitive flexibility may have difficulties disengaging attention from a task or resolving interference from previous stimuli or tasks. Cognitive inflexibility might therefore prevent individuals from shifting from one thought to another and thus lock them in to a specific behavior. Whether a subgroup of subjects with trichotillomania who have cognitive flexibility problems would benefit

preferentially from naltrexone awaits further research in larger samples.
Keywords: trichotillomania, treatment, opioid, naltrexone, cognition.
Disclosures: J. Grant, **Part 1:** Research grants from Transcept, Forest, Roche, and Psyadon Pharmaceuticals, **Part 4:** Research grants from Transcept, Forest, Roche, and Psyadon Pharmaceuticals ; B. Odlaug, **Part 1:** Consulting for Lundbeck Pharmaceuticals.; S. Kim, **Part 1:** Research grant from Roche Pharmaceuticals., **Part 4:** Research grant from Roche Pharmaceuticals.

T131. Perinatal Choline Supplementation is Associated with Earlier Maturation of P50 Sensory Gating and May Improve Preschool Attentional Function

Randal Ross*, Sharon Hunter, Lizbeth McCarthy, Amanda Hutchison, Brandie Wagner, Sherry Leonard, Karen Stevens, Robert Freedman

University of Colorado Denver, Aurora, Colorado

Background: Attentional dysfunction is a core symptom of multiple psychiatric disorders, including schizophrenia, bipolar disorder, ADHD, and autism. Sensory gating is often considered an early preconscious component of attention and, because it can be measured in infancy, is a potential infant biomarker of later attentional performance. P50 sensory gating impairments are associated, in both human and mouse models, with low expression of alpha7 nicotinic cholinergic receptors. In mouse models, perinatal exposure to increased dietary choline, an alpha7 nicotinic receptor agonist, leads to improved sensory gating in adulthood. In humans, maternal prenatal serum choline levels have been correlated with preschool attentional performance. This report seeks to determine if, as in the animal models, perinatal dietary choline supplementation has a similar effect on human infants and to present preliminary data on the relationship between perinatal dietary choline supplementation and preschool attention.
Methods: Pregnant women received placebo or 6300 mg of phosphatidylcholine (the equivalent of approximately 900 mg of choline) per day in a divided dose from approximately 15 weeks gestation through delivery. Infants then received either placebo or 700 mg of phosphatidylcholine (the equivalent of approximately 100 mg of choline) per day until 52 weeks post last menstrual period (approximately 12 weeks of age). P50 sensory gating was assessed at 44 and 52 weeks gestational age. Suppression of the P50 response to a second sound of at least 50% compared to the first sound was considered robust sensory gating; suppression less than 50% was considered diminished sensory gating. To explore stimulation of the alpha7 nicotinic cholinergic receptor as a potential mechanism for any choline supplementation effect, infants were genotyped for CHRNA7 rs3087454, a marker associated with abnormal P50 sensory gating. Forty (23 males, 17 females) of the infants also had a parent complete a Child Behavior Checklist (CBCL) when the children were at a mean + S.D. of 40.8 + 1.0 months. The CBCL Attention subscale score was used as the outcome for the 40 month report.
Results: No adverse effects of supplementation on pregnant mothers or their infants were identified. At one month of age, choline treated infants were more likely to have robust sensory gating than placebo treated infants ($\chi^2 = 6.23$; $p = 0.013$). CHRNA7 genotype associated with risk for schizophrenia diminished P50 sensory gating in the placebo-treated but not the choline-treated infants. There was no effect of treatment group on P50 sensory gating at the 3 month time point. For males, 62.5% of preschoolers who had received perinatal choline supplementation had an Attention subscale score below the mean as compared to 27.3% of preschoolers who had received placebo [Fisher's Exact Test (one-sided) = 0.070]. For females, the percentage of females below the mean score was similar for choline and placebo treated groups [62.5 *vs* 66.6%; Fisher's Exact Test (1-sided) = 0.627].
Conclusions: Perinatal dietary choline supplementation leads to earlier maturation of P50 sensory gating. An interaction between choline supplementation and a CHRNA7 genotype suggests choline acts by alpha7 nicotinic cholinergic receptor activation. While sample size at the follow-up age of 40 months of age follow-up was small, there is a suggestion that perinatal choline supplementation may be associated with improved parent-reported attention at 40 months of ages, particularly for males. Clinical Trials Registration for the perinatal choline trial: NCT00332124. This work was supported by the Anschutz Family Foundation, the Institute for Children's Mental Disorders, and National Institutes of Health grants P50MH086383 and R01MH056539.
Keywords: choline, prevention, attention, pregnancy, infant.
Disclosures: R. Ross, Nothing to Disclose; S. Hunter, Nothing to Disclose; L. McCarthy, Nothing to Disclose; A. Hutchison, Nothing to Disclose; B. Wagner, Nothing to Disclose; S. Leonard, Nothing to Disclose; K. Stevens, Nothing to Disclose; R. Freedman, Nothing to Disclose.

T132. An International Study of the GRID-HAMD: Has It Fulfilled Its Promise?

Janet BW Williams*, Matej Ondrus, Melanie Kitzinger, Jennie Persson, Marlene Popescu, Risto Valjakka

MedAvante, Hamilton, New Jersey

Background: The Hamilton Depression Rating Scale (HAM-D) has been in widespread use since its debut in the 1960s. Because so many critiques have been levied about the HAMD (Bagby *et al*, 2004), an international group representing academia, clinical practice, the pharmaceutical industry and government developed the GRID-HAMD in an attempt to improve the HAM-D and its most widely used structured interview guide (the SIGH-D) (Williams, 1988; Williams *et al*, 2008). The GRID-HAMD provides a novel grid scoring structure that separates frequency and intensity for most items to allow clinicians to rate these as independent axes. The newly formulated instrument also provides a structured interview guide and scoring conventions directly in the instrument on the same page as every item. Finally, although the overall intent was to approximate the scoring profile of the original HAM-D scale, the GRID-HAMD presents revised anchor points for several items that

were inconsistently rated or that were especially problematic for raters. The GRID-HAMD has now been available for five years and has been the major outcome measure for several large clinical trials. This poster presents the results of a survey of a global cohort of 74 highly trained and calibrated clinical interviewers ('central raters') who have collectively administered the GRID-HAMD 4850 times in global clinical trials.

Methods: The survey was distributed to 74 central raters worldwide via Survey Monkey in July 2013. All raters work for a pharmaceutical-services company that provides remote ratings of subjects in CNS clinical trials by videoconferencing or telephone. The survey included basic demographic questions, and 20 statements about the GRID-HAMD that respondents were asked to rate on a 7 point scale that ranged from strongly disagree (1) through neutral (4) to strongly agree (7). Questions covered usability and ease of use as well as the major innovations incorporated into the GRID-HAMD, including the new page layout (item, interview questions and conventions on same page), the revised item wordings, and the grid format separating ratings for severity and intensity. Raters were also asked to indicate which items they found easiest, and which most difficult to administer. The final section of the questionnaire listed four statements asking raters to compare the GRID-HAMD with the SIGH-D, with a response from 1 = GRID-HAMD through 4 = neutral to 7 = SIGH-D.

Results: Fifty-seven questionnaires were completed (77%). Mean age of respondents was 40; 37 (65%) were psychiatrists or doctorate-level psychologists. The remaining respondents were other mental health professionals. Half (51%) of the respondents live in Europe, 42% in the US, and the rest in Russia (5%) and South Africa (2%). All reported at least three years' experience assessing depression, and 81% reported more than seven years. All had used the GRID-HAMD, and nearly all (96.5%) had administered it 10 or more times. Most raters agreed that the wording of the questions in the GRID-HAMD made it easy to administer (77%), the conventions were clear (82%) and helpful (86%), and the guidelines for rating symptom intensity were clear (79%). Fewer rated it 'easy to decide on a frequency level' (61%). A large majority (89%) of raters thought that 'having the scoring conventions integrated into the interview guide has made scoring easier.' 75% agreed that 'assessing symptom intensity and frequency separately makes it easier to score the items.' And 71% responded affirmatively that 'experience with the GRID scoring system has helped me balance symptom intensity and frequency when scoring other scales.' The section comparing the GRID-HAMD to the SIGH-D was completed only by raters with experience using both scales ($N = 44$). More than half (54%) of the raters preferred the graphical layout of the GRID to that of the SIGH-D. A slightly higher percentage preferred the SIGH-D for its 'ease of use' (50 *vs* 45%) and 'efficiency' (39 *vs* 36%). Finally, slightly more raters expressed 'overall preference for the SIGH-D' (45 *vs* 43%).

Conclusions: These central raters rated the clarity and ease of use of the GRID-HAMD positively. Surprisingly, however, they did not indicate an overall preference for the GRID-HAMD over the SIGH-D. Several areas of improvement were indicated for both scales, and the 'most difficult' items were highlighted. In ongoing data analyses, demographic and other predictors of these views are being explored and will be presented.

References:

Bagby RM, Ryder AG, Schuller DR, Marshall MB (2005). The Hamilton Depression Rating Scale: Has the gold standard become a lead weight? *Am J Psychiatry* **161**: 2163–2177.

Williams JBW (1988). A structured interview guide for the Hamilton Depression Rating Scale. *Archives of General Psychiatry* **45**: 742–747.

Williams JBW, Kobak KA, Bech P, Engelhardt N, Evans K, Lipsitz J, Olin J, Pearson J, Kalali A (2008). The GRID-HAMD: Standardization of the Hamilton Depression Rating Scale. *International Clinical Psychopharmacology* **23**: 120–129.

Keywords: depression rating scale, Hamilton depression scale, assessment, severity, central raters.

Disclosures: J. Williams, **Part 1:** Full-time employee of MedAvante, Inc., **Part 2:** Full-time employee of MedAvante, Inc., **Part 5:** MedAvante, Inc.; M. Ondrus, **Part 1:** Employed by MedAvante, Inc., **Part 2:** MedAvante, Inc., Private practice; M. Kitzinger, **Part 1:** Employed by MedAvante, Inc., **Part 5:** MedAvante, Inc.; J. Persson, **Part 1:** Employed by MedAvante, Inc., **Part 2:** MedAvante, Inc., National Health Service, England; M. Popescu, **Part 1:** Full-time employee of MedAvante, **Part 2:** MedAvante, **Part 5:** MedAvante; R. Valjakka, **Part 1:** Work part-time for Medavante, Inc., **Part 2:** MedAvante, Inc., Varsinais-Suomi hospital district, psychotherapy patients.

T133. Methadone and Suboxone for Subutex Injectors: Primary Outcomes of Pilot RCT

George E Woody*, David Otiashvili, Gvantsa Piralishvili, Zura Sikharulidze, George Kamkamidze, Sabrina Poole

University of Pennsylvania, Philadelphia, Pennsylvania

Background:

- Non-medical use of buprenorphine has been reported in a number of countries
- Widespread injecting use of Subutex® has been documented in the Republic of Georgia
- Data on treatment of buprenorphine injection abuse are lacking.

Methods: Treatment setting The study was done at the Addiction Research Center, Alternative Georgia; an independent non-profit research institution located in Tbilisi and affiliated the Medical Centre Uranti. Uranti is the second largest addiction program in the country and routinely provides in-patient detoxification, traditional psychosocial-based outpatient treatment, and medication-assisted treatment. Participants Patients were recruited through word of mouth, fliers and advertisements given to the staff of addiction clinics, harm reduction programs, and other facilities frequented by injection drug users. All screening, assessment and follow-up evaluations were done at the Uranti clinic.

Results: 112 potential subjects were screened between January 25 and September 27, 2011, of which 80 (4 females) were randomly assigned to methadone or Suboxone®. Subjects were white; average age was 34; mean years of opioid injection use was 5.77 (SD4.6); and heroin, Subutex®, other opioids (opium, desomorphine) and home-produced amphetamine type stimulants were the main drugs reported to have been injected. Injecting more than one drug was reported by 68.4% of methadone patients and 72.5% of Suboxone® patients. None were HIV positive however 73.4% were positive for hepatitis C. There were no significant differences in socio-demographic and clinical characteristics between two groups.
Conclusions: Daily observed methadone or buprenorphine-naloxone were effective in terms of reducing illicit buprenorphine and other opioid use, and reducing HIV risk behavior in non-medical buprenorphine and other opioid users in the Republic of Georgia. The results suggest that increasing availability and accessibility of opiate agonist treatment with either methadone or buprenorphine is an effective public health approach for treating non-medical use of buprenorphine and other opioids in the Republic of Georgia.
Keywords: methadone; suboxone; subutex injectors.
Disclosures: G. Woody, Nothing to Disclose; D. Otiashvili, Nothing to Disclose; G. Piralishvili, **Part 1:** Suboxone was provided free of charge for this study by Reckitt Benckiser; Z. Sikharulidze, Nothing to Disclose; G. Kamkamidze, Nothing to Disclose; S. Poole, Nothing to Disclose.

T134. Treatment-related Improvement in Neuropsychological Functioning in Depressed Patients at High Risk for Suicidal Behavior: Paroxetine *vs* Bupropion

Marianne Gorlyn, John Keilp, Ainsley Burke, Maria Oquendo, J John Mann, Michael Grunebaum*

Columbia University, New York, New York

Background: Suicidal behavior is associated with cognitive deficits that exceed those associated with depression. It is unknown whether antidepressant medication treatment is effective in reducing cognitive difficulties in patients explicitly at risk for suicidal behavior, or whether specific medications have differential effects on cognition in this population. Changes in cognitive performance may be associated with reduced risk of suicidal behavior.
Methods: Within a randomized, double-blind clinical trial comparing paroxetine and bupropion in depressed patients with elevated suicide risk ($N=74$), we administered a comprehensive neuropsychological battery at baseline and after eight weeks of treatment. Patients presented with current suicidal ideation and/or prior suicide attempt. Test scores were adjusted for age and education using established norms.
Results: Overall, patients demonstrated post-treatment reductions on ratings of depression and suicidal ideation as well as improved cognitive functioning. There was no clear advantage for either medication in terms of clinical or neuropsychological gains. Improved memory performance was associated with the reduction in suicidal ideation independent of the effects of reduced depression severity.
Conclusions: Antidepressant medication is effective in reducing clinical symptoms and cognitive impairment in patients with elevated suicide risk. Neuropsychological test performance changes exceeded those associated with practice effects. Changes in verbal memory were independently associated with lower ideation scores, and reduction in suicidal ideation was best predicted by the combination of improvements in depression severity and verbal memory. Targeted treatment of cognitive dysfunction may serve as a method of reducing suicidal behavior risk.
Keywords: cognitive function, depression, suicidal ideation, clinical trial, antidepressant.
Disclosures: M. Gorlyn, Nothing to Disclose; J. Keilp, Nothing to Disclose; A. Burke, **Part 1:** Receives royalties from use of the Columbia Suicide Severity Rating Scale (C-SSRS).; M. Oquendo, **Part 1:** Dr Oquendo receives royalties for use of the Columbia Suicide Severity Rating Scale and received financial compensation from Pfizer for the safety evaluation of a clinical facility, unrelated to this study. She has received unrestricted educational grants and/or lecture fees from Astra-Zeneca, Bristol Myers Squibb, Eli Lilly, Janssen, Otsuko, Pfizer, Sanofi-Aventis, and Shire. Her family owns stock in Bristol Myers Squibb.; J. Mann, **Part 1:** Past unrelated grants from Novartis and GSK. Royalities from Research Foundation for Mental Health for commercial use of the C-SSRS.,; M. Grunebaum, Nothing to Disclose.

T135. How Far are Duplicate Subjects Willing to Go? Changing Indications and Identifiers in Order to Participate in Studies at Distant Sites

Lilit Gevorgyan, Zoe Shiovitz, Marlene Zarrow, Thomas Shiovitz*

California Neuroscience Research, Sherman Oaks, California

Background: Subject registries attempt to reduce the number of duplicate and professional subjects enrolling in clinical trials by identifying them prior to randomization.The nature of these subjects remains poorly understood, perhaps because of HIPAA and privacy concerns. While there are some case descriptions of how these subjects behave, data on specifically what duplicate subjects do to avoid detection is currently lacking.
Methods: Subjects presenting at participating CNS clinical trial sites between October 31, 2011 and July 31, 2013, signed an IRB-approved authorization at prescreen to allow certain partial identifiers to be entered into CTSdatabase, a privately available subject registry. The data set was comprised of subjects who matched enough key identifiers to be considered a *Virtually Certain* match (ie $<10^{-7}$ likely to have matched by chance) *and* matched at a second, unique site. The distances between matching sites were compared, as were the frequencies with which there was a change in presenting indications/diagnoses, initials, dates of birth and/or last 4 digits of Social Security Number (SSN) between sites. Distances between sites were calculated with Google Maps, using the shortest option (by car) available.

Results: 3932 subjects were entered at 22 participating Southern California sites. 352 same site matches (ie those that occurred when a subject returned to the same site at a later date) were excluded from the data set. 211 matching pairs representing 422 (11.8%) potential duplicate subjects matched enough key identifiers to be considered Virtually Certain matches. 51 (24%) of these matched subjects presented at the second site with a different diagnosis or indication; 57 (27%) presented with some change in their initials, 38 (8%) with a different (or missing) SSN and 9 (4%) with a different day, month or year of birth.
Of matched subjects, 74 (18%) attempted to screen at a second site within 30 days of pre-screening at the first site. 121 (29%) attempted to screen at a second site within 60 days and 268 (64%) went to another site within 180 days. Within 6 months of presenting at the first site, 74 (42.5%) matching subjects were willing to travel to another site over 25 miles from the original site and 14 (8%) were willing to travel to sites that were over 50 miles apart in order to attempt to participate in another study. Two subjects traveled to sites that were over 100 miles apart.
Conclusions: Placebo response is rising in CNS clinical trials and disturbing rates of non-compliance with study medication have been reported. Duplicate and professional subjects may be both more likely to respond to placebo and to not take study medication.
A culture of dishonesty, a declining economy, internet support for duplicate study participation and easy web-based *shopping* for studies have worsened the duplicate subject problem. Subject registries attempt to address this problem by tracking potential subjects who are either prescreening or have signed study consent but have not yet been randomized. CTSdatabase uses partial subject identifiers entered into an online registry to detect potential duplicate subjects. This registry can identify duplicate subjects who seek to avoid detection by changing sites, indications or personal identifiers. Some identifiers that cannot be easily altered, like height and gender, are also used in this registry. Other subject registries use de-identified personal information or fingerprints. In the future, use of fingerprinting, retinal scanning or other more specific (but more intrusive) methods of tracking subjects may become more acceptable. Investigators and sponsors alike should be aware of problems that duplicate and professional subjects pose if they are allowed to enter clinical trials. A likely contributor to failed studies, these subjects may be willing to travel near and far, change their diagnoses and alter their personal identifiers in order to avoid detection.
Keywords: duplicate subjects, duplicate enrollment, professional patients, changing diagnoses, changing identifiers.
Disclosures: L. Gevorgyan, Nothing to Disclose; Z. Shiovitz, Nothing to Disclose; M. Zarrow, Nothing to Disclose; T. Shiovitz, **Part 1:** President and Principal Investigator, California Neuroscience Research Medical Group, Inc., a research site., President and equity shareholder, CTSdatabase, LLC, **Part 2:** President and Principal Investigator, California Neuroscience Research Medical Group, Inc., a research site, 90% of income;, Thomas M. Shiovitz, M.D., Inc., Private Practice of Psychiatry, 10% of income;, **Part 3:** See above. My primary source of personal income is as Investigator and President of California Neuroscience Research, a clinical trial site., **Part 4:** Research payments for performing clinical trials:, Astra-Zeneca, Avanir, Bristol-Myers Squib, Forest, Eli Lilly, Novartis, Otsuka, Pfizer, Shire, Takeda.

T136. Corticostriatothalamic Circuit Dysfunction in Major Depressive Disorder

Olusola Ajilore*, Melissa Lamar, Jamie Cohen, Anand Kumar

University of Illinois, Chicago, Illinois

Background: Corticostriatothalamic (CST) circuits have been implicated in the pathophysiology of major depression. Recent translational efforts have delinated specific cognitive functions associated specific segments of the CST circuit. The purpose of the present study was to examine cognitive functions associated with segments of the CST circuit in patients with major depression and investigate their neuroanatomical correlates.
Methods: We recruited 112 subjects from the community (54 who met DSM-IV criteria for major depressive disorder and 58 healthy comparison subjects). All subjects underwent a neurocognitive protocol. Tasks probing the CST circuit included the Continuous Performance Task (CPT = . visuospatial/sustained attention), the Self-Ordered Pointing Task (SOPT = spatial working memory), the Stroop (set-shifting and reversal cognitive processes) and Object Alternation (OA = decision-making and impulsivity). MRI scans were obtained on a subset of participants ($n = 83$; 40 depressed subjects) using a 3.0 T Philips Achieva scanner. Volumetric and diffusion tensor imaging data were analyzed using Freesurfer and DTI Studio.
Results: While there was no significant difference on the SOPT, depressed subjects performed significantly worse on the CPT ($p = 0.022$) with specific deficits in hit RT, RT variability, and perseverations. Stroop performance was also significantly impaired in the depressed group driven by worse interference scores ($p = 0.02$). Depressed subjects also performed significant worse on all aspects of the OA ($p = 0.018$) with more trials to completion, longer RTs, and fewer correct responses per trial. Across the entire sample, CPT performance were associated with left rostral anterior cingulate volumes, Stroop performance with right superior frontal volumes, and OA with caudate volumes. Analysis of DTI predictors of performance revealed that orbitofrontal to caudate and rostral middle frontal to caudate fractional anisotropy (FA) was associated with CPT performance in the entire sample.
Conclusions: This study indicates significant impairment in cognitive function linked to CST circuits associated with major depression and CST circuit involvment in specific congitive functions regardless of group. Differential contributions of cortical and subcortical regions and their connectivity may serve as a neural substrate for the cognitive impairment seen in major depression.
Keywords: executive function, corticostriatal, major depression, neuroimaging, cognition.

Disclosures: O. Ajilore, Nothing to Disclose; M. Lamar, Nothing to Disclose; J. Cohen, Nothing to Disclose; A. Kumar, Nothing to Disclose.

T137. Aripiprazole Lauroxil (ALKS 9070), a Novel Once-monthly Prodrug of Aripiprazole, Achieves Therapeutically Relevant Levels and Is Well-tolerated in Adult Patients with Schizophrenia Following Deltoid Administration

Ryan Turncliff*, Marjie Hard, David Brown, Mark Lerman, Adam Lowy, Morteza Marandi, Yangchun Du, Robert Risinger, Elliot W Ehrich

Alkermes, Inc., Waltham, Massachusetts

Background: Aripiprazole lauroxil (ALKS 9070, ALKS 9072) is a linker lipid ester prodrug of aripiprazole (ARP) for extended-release intramuscular (IM) injection. A water-insoluble lauroyl prodrug approach was selected to chemically 'mask' ARP following injection, minimizing the potential for injection site reactions and optimizing the ARP release profile for once-monthly dosing. The purpose of this study was to determine the safety, tolerability and relative bioavailability of aripiprazole lauroxil administered as a single intramuscular (IM) injection in the deltoid muscle compared to the gluteal muscle in adult subjects with chronic stable schizophrenia or schizoaffective disorder.
Methods: This was a multicenter, randomized, open label, single-dose study conducted at four study centers in the US. Subjects (46) with chronic, stable schizophrenia participated in the clinical study: mean age was 42.5 years old; subjects were predominantly male, black or African American, and not Hispanic or Latino; mean BMI was 28.69 kg/m^2. Most subjects (84.8%) were classified by the CGI-S as mildly ill. Most subjects were CYP 2D6 extensive metabolizers (63%) or intermediate metabolizers (approximately 33%); 2 subjects (4%) were poor metabolizers. Two subjects received an open-label aripiprazole lauroxil 150 mg (ARP eq.) IM injection in the deltoid muscle. After the safety data were reviewed, 44 subjects were randomized in a 1 : 1 ratio to receive an open label aripiprazole lauroxil 300 mg (ARP eq.) IM injection in either the deltoid or gluteal muscle. Forty-three (93.5%) subjects completed all study assessments. PK samples collected on Day 1 predose, and at 1, 4, 8, and 12 h postdose and on days 2–7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 28, 31, 38, 45, 52, 59, 70, 80 and 89. PK samples were assayed for levels of aripiprazole lauroxil, ARP, and dehydroaripiprazole (dARP) by LC-MS/MS (LLOQ = 1 ng/ml). PK parameters were derived using WinNonLin version 5.3. Relative bioavailability comparing deltoid to gluteal IM administration of aripiprazole lauroxil was summarized based on aripiprazole exposure ($Frel = AUC_{deltoid}/AUC_{gluteal}$). ANOVA was performed on log-transformed PK parameters. The relationship between PK parameters (C_{max}, AUC_{0-last}, $AUC_{0-\infty}$) and BMI was explored.
Results: Plasma profiles of ARP and dARP following a single 150 or 300 mg IM injection of aripiprazole lauroxil demonstrated the slow dissolution properties of the prodrug with no evidence of early aripiprazole release. During the PK sampling period, deltoid administration was associated with a higher mean exposure (AUC_{0-last}) of ARP (23%) and dARP (24%), compared to gluteal administration; ARP exposure was similar in subjects with sufficient data to calculate $AUC_{0-\infty}$ [GMR 0.84 (0.57, 1.24); $n = 7$–10]; thus the range of exposures overlapped. There was no apparent relationship between exposure and BMI. Single IM doses of aripiprazole lauroxil 150 or 300 mg injection in the deltoid or gluteal muscle were well tolerated. There were no apparent drug effects on vital signs, ECG recordings, ESRS scores, or C-SSRS scores. The most commonly reported treatment emergent AEs overall were injection site pain in 20 subjects (43.5%), headache in 6 subjects (13.0%); all of these events were mild. Mean changes from baseline for the PANSS total score and each of the 3 subscales were similar between the two.
Conclusions: Deltoid muscle provides a more accessible injection site and could facilitate patient acceptance of an injectable medication. Deltoid and gluteal administrations of aripiprazole lauroxil result in similar safety and tolerability. The PK data suggest that deltoid and gluteal administrations of aripiprazole lauroxil result in similar efficacy.
Keywords: aripiprazole, schizophrenia, depot, pharmacokinetics, deltoid.
Disclosures: R. Turncliff, **Part 5:** Alkermes Inc.; M. Hard, **Part 5:** Alkermes Inc.; D. Brown, **Part 1:** Speakers bearaus for Jansen, Otsuka. Clinical Trials performed for Alkermes, Otsuka, Bristol Meyers, Abbott, Jansen, Eli Lilly, CeNe Rx.; M. Lerman, **Part 1:** Compensation recieved as a clinical investigator for Alkermes Inc.; A. Lowy, **Part 1:** Compensated as a clinical investigator for Alkermes Inc.; M. Marandi, **Part 1:** Compensation recieved as a clinical investigator for Alkermes Inc.; Y. Du, **Part 5:** Alkermes Inc.; R. Risinger, **Part 5:** Alkermes Inc.; E. Ehrich, **Part 5:** Alkermes Inc.

T138. Clinical Assessment of Lurasidone Benefit and Risk in the Treatment of Bipolar I Depression Using Number Needed to Treat, Number Needed to Harm, and Likelihood to be Helped or Harmed

Leslie Citrome*, Terence A Ketter, Josephine Cucchiaro, Antony Loebel

New York Medical College, Suffern, New York

Background: Prior to recent approval of lurasidone for the treatment of bipolar depression there were only two United States Food and Drug Administration approved treatments for bipolar depression (quetiapine and olanzapine-fluoxetine combination, OFC), and these each were similarly as likely to provide therapeutic benefit as adverse effects. We assessed efficacy, safety, and tolerability of lurasidone for major depressive episodes associated with bipolar I disorder using number needed to treat (NNT, for benefits), number needed to harm (NNH, for harms), and likelihood of being helped or harmed (LHH, ratio of NNH to NNT, for trade-offs between benefits *vs* harms).
Methods: Data were from two 6-week multicenter, randomized, double-blind, placebo-controlled, flexibly-dosed acute bipolar I depression studies, one using lurasidone

monotherapy at 20–60 mg/d or 80–120 mg/d, and another using lurasidone 20–120 mg/d adjunctive to lithium or valproate. For lurasidone and placebo, we assessed rates of response ($\geqslant$50% reduction from baseline on Montgomery Asberg Depression Rating Scale (MADRS) total score); remission (final MADRS total score $\leqslant$12); discontinuation due to an adverse event (AE); weight gain $\geqslant$7% from baseline; incidence of spontaneously reported AEs; and incidence of total cholesterol $\geqslant$240 mg/dl, low-density lipoprotein cholesterol $\geqslant$160 mg/dl, fasting triglycerides $\geqslant$200 mg/dl and glucose $\geqslant$126 mg/dl post baseline; as well as NNT for response and remission, NNH for safety/tolerability outcomes, and LHH.

Results: NNT *vs* placebo for response was 5 for lurasidone monotherapy (both dose ranges) and 7 for adjunctive therapy. NNT *vs* placebo for remission for lurasidone monotherapy was 6 for 20–60 mg/d and 7 for 80–120 mg/d and 7 for adjunctive lurasidone. NNH *vs* placebo for discontinuation due to an AE for lurasidone monotherapy was 642 for 20–60 mg/d and −181 for 80–120 mg/d, and for adjunctive lurasidone was −54. Lurasidone was not associated with any clinically meaningful mean weight or mean metabolic disadvantages compared to placebo; NNH *vs* placebo for weight gain $\geqslant$7% was 29 for 20–60 mg/d and 5550 for 80–120 mg/d and 42 for adjunctive lurasidone. The three most frequently encountered AEs with the largest difference in incidence for lurasidone *vs* placebo were nausea, akathisia, and somnolence, with NNH values for lurasidone *vs* placebo ranging from 11 (nausea with lurasidone monotherapy 80–120 mg/d) to 130 (somnolence with lurasidone monotherapy 20–60 mg/d). LHH was substantially and consistently greater than 1 (indicating help being more likely than harm) when contrasting response or remission *vs* AEs or weight gain.

Conclusions: NNT, NNH, and LHH can help quantify relative benefits (efficacy) and harms (side effects), thus placing lurasidone findings in research studies into clinical perspective. Lurasidone compared to earlier treatments approved for bipolar depression (quetiapine, OFC) yielded comparable benefits (all had single-digit NNT *vs* placebo for response or remission), and less risk of harm (double-digit or greater NNHs with lurasidone compared to single-digit NNHs for sedation with quetiapine and for $\geqslant$7% weight gain with OFC), and thus a substantially more favorable LHH ($>$ or $\geqslant$1) with lurasidone monotherapy and adjunctive therapy, compared to quetiapine and OFC (LHH$<$ or $\sim$1).

Keywords: bipolar I depression, efficacy, lurasidone, number needed to harm, number needed to treat, safety, tolerability.

Disclosures: L. Citrome, **Part 1:** Alexza, Alkermes, AstraZeneca, Avanir, Bristol-Myers Squibb, Eli Lilly, Envivo, Forest, Genentech, Janssen, Lundbeck, Merck, Mylan, Novartis, Noven, Otsuka, Pfizer, Reckitt Benckiser, Shire, Sunovion and Valeant, **Part 2:** Alkermes, AstraZeneca, Eli Lilly, Genentech, Janssen, Merck, Mylan, Novartis, Otsuka, Pfizer, Sunovion, **Part 3:** AstraZeneca, Eli Lilly, Janssen, Merck, Novartis, Otsuka, Sunovion ; T. Ketter, **Part 1:** Dr Ketter has received grant/research support from Agency for Healthcare Research and Quality, AstraZeneca Pharmaceuticals LP, Teva Pharmaceuticals/Cephalon Inc., Eli Lilly and Company, National Institute of Mental Health, Pfizer Inc., Sepracor Inc./Sunovion Pharmaceuticals; has served as a consultant for Allergan Pharmaceuticals, Avanir Pharmaceuticals, Teva Pharmaceuticals/Cephalon Inc., Forest Pharmaceuticals, Janssen Pharmaceutica Products, LP, Sunovion Pharmaceuticals; has received CME Lecture Honoraria (NOT Speaker's Bureau fees) from AstraZeneca Pharmaceuticals LP and Otsuka Phramaceuticals; his spouse (Nzeera Ketter, MD) is an employee of and owns stock in Janssen Pharmaceutica Products, LP/Johnson & Johnson, **Part 2:** For Terence Ketter only Otsuka Pharmaceuticals for 2012; for Nzeera Ketter only Janssen Pharmaceutica Products, LP/Johnson & Johnson for each year., **Part 3:** For Terence Ketter none; for Nzeera Ketter only Janssen Pharmaceutica Products, LP/Johnson & Johnson for each year., **Part 4:** All grants from pharmaceutical, biotechnology companies, companies providing clinical assessment, scientific or medical products whether they come directly from the company or indirectly through a foundation, university, or any other organization –, C10953/3072 (SPO # 48966) (Ketter Site-PI) Teva Pharmaceuticals/Cephalon Inc. 'A Double-blind, Placebo-controlled, Parallel-group, Fixed-dosage Study to Evaluate the Efficacy and Safety of Armodafinil Adjunctive Therapy in Adults With Major Depression Associated With Bipolar I Disorder'—3/4/11—12/31/12—$140 131, WS819640 (SPO # 49927) (Ketter PI) Pfizer Pharmaceuticals. 'Effectiveness of Ziprasidone in a Clinical Setting' 12/01/10—11/01/11—$77 044, D1050256 (SPO # 45413) (Ketter Site PI). Quintiles, Inc. (Prime Sponsor: Sepracor Inc./Sunovion Pharmaceuticals) 'A 24-Week, Flexible-Dose, Open-Label Extension Study of Lurasidone for the Treatment of Bipolar I Depression'—8/25/10—8/24/12—$120 624, D1050235 (SPO # 45621) (Ketter Site PI). Quintiles, Inc. (Prime Sponsor: Sepracor Inc./Sunovion Pharmaceuticals) 'A Randomized, 6-Week, Double-Blind, Placebo-Controlled, Flexible-Dose, Parallel-Group Study of Lurasidone Adjunctive to Lithium or Divalproex for the Treatment of Bipolar I Depression'—8/25/10—8/24/12—$166 596, IRUSQUET0463 (SPO # 41037) (Ketter PI). AstraZeneca. 'The Long Term Effectiveness of Quetiapine plus LTG Therapy in Bipolar Patients'—2/27/07—6/30/12—$44 255, F1D-US-X279 (SPO # 30246) (Ketter PI). Eli Lilly and Company. 'Double-Blind Placebo-Controlled Olanzapine Monotherapy in the Treatment of Acute Syndromal And Subsyndromal Exacerbations of Bipolar Disorders'—6/28/05—9/30/11—$150 000, IRUSQUET0333 (SPO # 30119) (Ketter Site PI). AstraZeneca. 'A Double-blind, Placebo-controlled Trial of Seroquel for the Treatment of Dysphoric Hypomania in Bipolar II Patients'—1/1/04—06/30/11—$355 098, D1050296 (SPO # 107051) (Ketter Site PI). Sunovion Pharmaceuticals, Inc. 'A randomized, double-blind, placebo-controlled, flexible-dose, parallel-group title: study of lurasidone adjunctive to lithium or divalproex for the prevention of recurrence in subjects with bipolar I disorder'—12/20/12—12/20/13—$477 085, D1050308 (SPO # 110379) (Ketter Site PI). Sunovion Pharmaceuticals, Inc. 'A Multicenter, Open Label, Flexible-Dose Extension Study of Lurasidone Adjunctive to Lithium or Divalproex In Subjects With Bipolar I Disorder'—6/12/13—6/12/14—$95 221, **Part 5:** Janssen Pharmaceutica Products, LP/Johnson & Johnson (Nzeera Ketter, MD, Spouse); J. Cucchiaro, **Part 1:** Full-time employee of Sunovion Pharmaceuticals, **Part 2:** Full-time employee of Sunovion Pharmaceuticals, **Part 3:** Full-time employee of

Sunovion Pharmaceuticals, **Part 5:** Full-time employee of Sunovion Pharmaceuticals; A. Loebel, **Part 1:** Full-time employee of Sunovion Pharmaceuticals, **Part 2:** Full-time employee of Sunovion Pharmaceuticals, **Part 3:** Full-time employee of Sunovion Pharmaceuticals, **Part 5:** Full-time employee of Sunovion Pharmaceuticals.

T139. Intranasal Ketamine in Treatment-resistant Depression

Kyle A Lapidus*, Cara F Levitch, Laili Soleimani, Andrew M Perez, Jess W Brallier, Michael K Parides, Dan V Iosifescu, Dennis S Charney, James W Murrough

Mount Sinai Medical Center, New York, New York

Background: Current treatments for depression are only partially effective and exhibit delays in onset of therapeutic efficacy. Several studies have reported a rapid onset of antidepressant action for intravenous (IV) ketamine—and NMDA receptor antagonist—in patients with treatment resistant depression (TRD). Despite potential efficacy, the requirement for IV administration imposes potential limitations to therapeutic delivery. In contrast, ketamine delivery via an intranasal (IN) route may provide a more feasible treatment approach. Herein we report the first placebo-controlled study of IN ketamine in TRD.
Methods: Twenty subjects with TRD in a current major depressive episode were randomized to receive IN ketamine hydrochloride (50 mg) or 0.9% saline solution, in a crossover design with one of two treatment orders: either ketamine-placebo (KET-PBO) or placebo-ketamine (PBO-KET); KET or PBO were administered 1–2 weeks apart. 18 subjects received both treatments under randomized, double blind conditions. Before administration, subjects were admitted to a clinical research unit and study drug was administed by an anesthesiologist. Continuous vital signs monitoring was employed during and for 4 h following treatment. The primary efficacy outcome measure was change from baseline in the Montgomery-Asberg Depression Rating Scale (MADRS) score at 24 h following KET, compared to PBO. Response and remission rates at 24 h represented secondary outcomes. Clinical response was defined as MADRS decrease $\geqslant$50% from baseline and remission was defined as a MADRS score of $\leqslant$9. To assess hemodynamic side effects, vital signs were monitored and clinically significant changes were defined as systolic or diastolic blood pressure (BP) $>$180/100 or $>$20% increase above baseline level or tachycardia with heart rate $>$110 beats/min. Management by medication interventions and treatment discontinuation was to be provided for significant hemodynamic changes. Other secondary outcomes included general adverse events, acute psychotomimetic effects, and dissociative effects, measured with the Systematic Assessment for Treatment Emergent Effects (SAFTEE), Brief Psychiatric Rating Scale (BPRS), and Clinician-Administered Dissociative States Scale (CADSS) respectively.
Results: Subjects evidenced significant improvement in depressive symptoms within 24 h after ketamine compared to placebo ($t=4.4$, $p<0.001$). Following KET, 8 of 19 subjects responded (42%), compared to 2 of 19 (11%) following PBO. Among study completers (those who received both treatment conditions), 8 (44%) met the response criterion. Of these 8, 1 (6%) also responded to placebo, while no completer met response criteria only for placebo ($p=0.0325$ based on McNemar's test). Intranasal ketamine was well tolerated with few side effects. Specifically, following ketamine, the mean increase in BPRS was 0.3, and in CADSS was 1.37. No patient exhibited clinically significant changes in hemodynamics.
Conclusions: In this study, we demonstrated a rapid antidepressant effect of IN ketamine in patients with TRD. IN KET was well tolerated with almost no psychotmimetic or dissociative side effects and with no significant hemodynamic effects including blood pressure and heart rate changes. No interventions by anesthesiology were needed. Future studies are indicated to identify strategies for maintaining antidepressant response in patients who respond to intranasal ketamine.
Keywords: ketamine, glutamate, major depression, antidepressant.
Disclosures: K. Lapidus, **Part 1:** Dr Lapidus has received support for this project from the Brain and Behavior Research Foundation's Young Investigator Award and Apire Janssen Psychiatric Resident Research Scholars Award and serves as scientific advisor for Halo Neuro, Inc. He also participated in an interview on the future of antidepressants with LCN Consulting, Inc., **Part 4:** Dr Lapidus has received support for this project from the Brain and Behavior Research Foundation's Young Investigator Award and Apire Janssen Psychiatric Resident Research Scholars Award.; C. Levitch, Nothing to Disclose; L. Soleimani, Nothing to Disclose; A. Perez, Nothing to Disclose; J. Brallier, Nothing to Disclose; M. Parides, Nothing to Disclose; D. Iosifescu, **Part 1:** In the previous 36 months, Dr Iosifescu has received research funding through Mount Sinai School of Medicine from AstraZeneca, Brainsway, Euthymics, Neosync, and Roche; he has received consulting fees from CNS Response, Otsuka, Servier and Sunovion., **Part 4:** In the previous 36 months, Dr Iosifescu has received research funding through Mount Sinai School of Medicine from AstraZeneca, Brainsway, Euthymics, Neosync, and Roche; he has received consulting fees from CNS Response, Otsuka, Servier and Sunovion.; D. Charney, **Part 1:** Dr Charney has been named as inventors on a pending use-patent of ketamine for the treatment of depression. If ketamine were shown to be effective in the treatment of depression and received approval from the Food and Drug, Administration for this indication, Dr Charney and Mount Sinai School of Medicine could benefit financially.; J. Murrough, **Part 1:** Dr Murrough has received research support from Janssen Pharmaceuticals and Avanir Pharmaceuticals., **Part 4:** Dr Murrough has received research support from Janssen Pharmaceuticals and Avanir Pharmaceuticals.

T140. Do Structured, Taped and Reviewed Rating Interviews Improve Outcomes in Antidepressant Trials?

Arif Khan*, James Faucett, Walter A Brown

Northwest Clinical Research Center, Bellevue, Washington

Background: Although the earliest antidepressants such as amitriptyline and imipramine were shown to reduce depressive symptoms in placebo controlled clinical trials using a simple measurement of a clinician's overall

impression, in the past 60 years specific measurement tools have been developed to evaluate the efficacy of new antidepressants. The most recent of these developments have been efforts to increase reliability of depression ratings through use of structured interviews, taped interviews and by having outside reviewers gauge the quality of interviews throughout a trial. These methods have become commonplace in antidepressant clinical trials based on the assumption that variability of traditional unstructured interviews have contributed to the high failure rate of antidepressant clinical trials. The evidence suggesting that these techniques improve antidepressant-placebo differences comes from one retrospective study by Dr Cogger published in 2007. In order to replicate the finding that methods such as taping and external review of the depression interviews enhance signal detection we examined the data from two recent antidepressant trials using such a model, three recent trials using traditional methods of interviewing depressed patients, and data from previously published studies that were included as a historical control.

Methods: Randomization codes for 5 clinical trials conducted at Northwest Clinical Research Center (NWCRC) from 2009–2011 were obtained from the pharmaceutical company sponsors with each trial using the Montgomery-Asberg Depression Rating Scale (MADRS) to assess outcome. The 2 trials using structured and taped interviews enrolled 63 patients and the 3 trials using traditional interview methods enrolled 87 patients. The previously published data used as a historical control is from 8 trials conducted at NWCRC from 1996 to 2000 that enrolled 208 patients.

Results: Overall, patient characteristics were similar between the three groups of trials (historical control trials using traditional interviews, recent trials using traditional methods, recent trials with structured and taped interviews). The trials using structured and taped interviews resulted in an antidepressant-placebo difference of 1.67, $t(\mathrm{df}=61)=0.60$, $p=0.55$, $d=0.16$. Recent trials using traditional methods resulted in an antidepressant-placebo difference of 3.6, $t(\mathrm{df}=85)=2.42$, $p=0.037$, $d=0.54$. The historical control data from trials using traditional interview methods were similar in outcome to the recently conducted trials using traditional interviewing methods with an antidepressant-placebo difference of 6.6, $t(\mathrm{df}=159)=3.78$, $p<0.001$, $d=0.49$.

Conclusions: The expected increased signal detection from taped and structured interviews considered to increase reliability in recently conducted antidepressant clinical trials did not occur. In contrast, the data from recently conducted antidepressant clinical trials using the traditional method of patient evaluation and data from antidepressant clinical trials that were used as a historical control showed strong signal detection. The difference in outcome in trials with traditional interview methods as compared to those with structured and taped interview methods was due to a two-fold increase in placebo response in the trials using structured interviews. These data warrant further evaluation of the value and usefulness of using structured *vs* unstructured interviews in antidepressant clinical trials.

Keywords: antidepressant clinical trials; rater behavior; traditional psychiatric interview; structured interview techniques; Montgomery-Asberg depression rating scale.

Disclosures: A. Khan, **Part 1:** Arif Khan, M.D., principal investigator of over 340 clinical trials sponsored by over 65 pharmaceutical companies and 30 CROs, has done no compensated consulting or speaking on their behalf, nor does he own stock in any of these or other pharmaceutical companies. Dr Khan is not compensated for his role as author of medical manuscripts. In 2009 Dr Khan founded Columbia Northwest Pharmaceuticals LLC, and is Medical Director of the company. Columbia Northwest Pharmaceuticals owns intellectual property rights for potential therapies for Central Nervous System disorders and other medical conditions. J. Faucett, **Part 3:** James Faucett is an employee of the Northwest Clinical Research Center.; W. Brown, Nothing to Disclose.

T141. Patient-reported Outcome of Antipsychotic Treatment—Relationships to Psychopathology, Compliance and Remission

Dieter Naber*

University Medical Centre Hamburg Eppendorf, Hamburg, Germany

Background: Only recently success criteria became more ambitious and include a more thorough consideration of negative symptoms and cognitive dysfunction. The most important change within the last decade is the long overdue consideration of the patient's perspective, neglected for a long time. One reason was the prejudice that schizophrenic patients are not able to self-rate their well-being or quality of life, another the belief that such data are not necessary because the psychiatrist's perspective, 'objective' psychopathology, includes these domains. However, recent data indicate that in addition to the positive influence of a good therapeutic relationship, the subjective experience of antipsychotic treatment is a major predictor of compliance. In addition to motor symptoms that often accompany treatment with antipsychotics, marked adverse effects on drive and emotion may also be experienced. Particularly under treatment with high dosage typical antipsychotics (and high D2-receptor blockade), patients often report a reduced quality of life with restricted emotionality, straight thinking and spontaneity, a syndrome similar to negative symptoms of schizophrenia, coined 'akinetic depression' or 'neuroleptic-induced-deficit syndrome'.

Methods: Among other scales, a self-report instrument has been constructed to evaluate 'subjective well-being under neuroleptics' (SWN).[1] This scale was used in numerous open and controlled trials, indicating that most schizophrenia patients (80–95%), if no longer acutely psychotic or suffering from severe cognitive deficits, are able to reliably assess their subjective well-being.

Results: Psychopathology (PANSS total score) and SWN are not strongly related ($r=-0.30$ to -0.50). Regarding the different psychopathological syndromes, SWN correlates, particularly during long-term treatment, mostly with the degree of depressive and negative symptoms ($r=-0.30$ to -0.50), barely with positive symptoms ($r=-0.10$ to -0.20).[1] In a twelve months trial, antipsychotic compliance was highly correlated with SWN, but not with psychopathology or its improvement.[2] Dopamine D2 receptor blockade, in the temporal lobe as well as in the striatum, is highly correlated to reduced SWN ($r=0.66$–0.76).[3] Recent trials reveal the prognostic relevance of early improvement of

SWN: In a 12-week trial, 95% of Patients with early subjective response (within 4 weeks) showed further subjective and psychopathological improvement in the following 8 weeks, but only 9% without early subjective response showed later improvement.[4] In a 3-year trial, again psychopathological response as well as symptomatic and functional remission were not only related to young age and treatment with atypical antipsychotics, but mostly to early (within the first 3 months) subjective improvement.[5] Moreover, in a five year trial of first episode patients, marked improvement of SWN within the first 6 weeks of antipsychotic treatment was found to be related to enduring remission, while early improvement of PANSS did not predict outcome.[6].

Conclusions: Clinical studies, particularly if they reflect 'real-world conditions', indicate the usefulness of a systematic evaluation of the patients' perspective. Patient-reported-outcome, also in schizophrenia, is at least a valuable addition to expert rated assessment of psychopathology, social functioning and other success criteria. SWN is found as 'a unique measure capturing potential treatment effects not captured by the other measurements scales.'[7].

References:

1) Naber D *et al* (2001). Improvement of schizophrenic patients' subjective well-being under atypical antipsychotic drugs. *Schizophr Res* **30**;50:79–88.
2) Karow A *et al* (2007). Association of subjective well-being, symptoms, and side effects with compliance after 12 months of treatment in schizophrenia. *J Clin Psychiatry* **68**: 75–80.
3) Mizrahi R *et al* (2009). The relationship between subjective well-being and dopamine D2 receptors in patients treated with a dopamine partial agonist and full antagonist antipsychotics. *Int J Neuropsychopharmacol* **12**: 715–721.
4) Lambert M *et al* (2007). Remission of severely impaired subjective well-being in 727 patients with schizophrenia treated with amisulpride. *Acta Psychiatr Scand* **115**: 106–113.
5) Lambert M *et al* (2006). Prediction of remission as a combination of symptomatic and functional remission and adequate subjective well-being in 2960 patients with schizophrenia. *J Clin Psychiatry* **67**: 1690–1697.
6) De Haan L *et al* (2008). Improvement of subjective well-being and enduring symptomatic remission, a 5-year follow-up of first episode schizophrenia. *Pharmacopsychiatry* **41**: 125–128.
7) Chen L *et al* (2010). Relationships among multiple outcome measures in the study of schizophrenia. *Schizophr Res* **117**: 400–400.

Keywords: Schizophrenia, patient-reported-outcome, quality of life, subjective well-being.

Disclosures: D. Naber, Nothing to Disclose.

T142. The Efficacy of Vortioxetine *vs* Placebo in the Treatment of Adults with Major Depressive Disorder: Patient Level Data from 10 Short-term Studies and a Meta-analysis

Michael E Thase*, Atul Mahableshwarkar, Marianne Dragheim

Perelman School of Medicine of the University of Pennsylvania, Philadelphia, Pennsylvania

Background: Vortioxetine (Lu AA21004) is an investigational antidepressant currently being evaluated for the treatment of major depressive disorder (MDD). The mechanism of action of vortioxetine is thought to be related to its multimodal activity, which is a combination of two pharmacological modes of action: direct modulation of receptor activity and inhibition of the serotonin (5-HT) transporter. *In vitro* studies indicate that vortioxetine is a 5-HT3, 5-HT7, and 5-HT1D receptor antagonist, 5-HT1B receptor partial agonist, 5-HT1A receptor agonist and inhibitor of the 5-HT transporter. The aim of the present analyses was to compare the efficacy of therapeutic doses of vortioxetine (5–20 mg/day) *vs* placebo using data from all the completed randomised placebo-controlled short-term clinical trials of adults with major depressive disorder (MDD) included in the FDA submission.

Methods: Analyses were based on patient-level data from 10 randomised, double-blind short-term placebo-controlled studies in MDD in which vortioxetine (5–20 mg/day) was compared to placebo (NCT00672958, NCT00672620, NCT00735709, NCT01153009, NCT01163266, NCT01179516, NCT00839423, NCT00635219, NCT01140906, NCT00811252). The meta-analyses included the 9 short-term studies in adults, both positive and negative, while the elderly study (NCT00811252) was described separately, as this only included patients $\geqslant$65 years of age. For inclusion, patients had to meet the DSM-IV criteria for a major depressive episode (MDE) and be at least 18 years old. Patients had a score of at least 22 (1 study), 26 or 30 on the Montgomery-Åsberg Depression Rating Scale (MADRS). The principal outcome measure for this meta-analysis was the estimated treatment difference in change from baseline to endpoint of study (week 6 or 8) in the MADRS total score, using MMRM or ANCOVA (LOCF) based on the full-analysis set (FAS), comprising all treated patients with at least 1 post-baseline MADRS assessment. Secondary outcome measures were the broad effect on depressive symptoms (MADRS single items and HAM-A), the response to treatment (at least 50% reduction in baseline MADRS total score), remission rate (MADRS $\leqslant$10), and Clinical Global Impressions (CGI-I/S) scores.

Results: In the 9 adult studies, 1215 patients were treated with placebo and 2416 with vortioxetine (5 mg/day: $n=847$, 10 mg/day: $n=687$, 15 mg/day: $n=436$, 20 mg/day: $n=446$). Patient characteristics were similar, with a mean age of 44 years, a ratio of men to women of approximately 1 : 2, and a median of 2 previous MDEs. In the individual studies (including more than one dose) there was a dose response across the therapeutic dose range of 5–20 mg, which was supported by the meta-analysis. The mean difference from placebo for vortioxetine in change from baseline to week 6/8 in MADRS total score was -2.6 points (5 mg, $p=0.008$), -3.5 (10 mg, $p<0.001$), -2.6 (15 mg, $p=0.105$) and -4.5 points (20 mg, $p<0.001$) (FAS, MMRM). When the meta-analysis was repeated using ANCOVA (LOCF), similar results were obtained. Vortioxetine showed a broad clinical effect across the MADRS single items and on the HAM-A. The clinical relevance of the observed effect was further shown by significant differences from placebo in the MADRS response and remission rates (FAS, LOCF) as well as CGI-S and CGI-I scores for all vortioxetine doses (5–20 mg). In the elderly study, vortioxetine (5 mg/day) showed a significantly ($p<0.001$) greater improvement *vs* placebo (-4.7 MADRS

points; FAS, MMRM) as well as on the other secondary efficacy endpoints.
Conclusions: In this meta-analysis of patient-level data from over 2000 vortioxetine-treated patients from 9 randomised placebo-controlled short-term adult MDD studies, vortioxetine (5–20 mg/day) is efficacious with increasing efficacy *vs* placebo with increasing dose. Robustness and a broad clinical effect are shown by the secondary outcomes as well as efficacy in elderly patients.
Keywords: MDD, novel MOA, vortioxetine, efficacy, meta-analysis.
Disclosures: M. Thase, **Part 1:** Dr Thase has provided scientific consultation to Alkermes, Astra-Zeneca, Bristol-Myers Squibb Company, Dey Pharma, L.P., Eli Lilly & Company, Forest Pharmaceuticals, Inc., Gerson Lehman Group, GlaxoSmithKline, Guidepoint Global, H. Lundbeck A/S, MedAvante, Inc., Merck and Co. Inc., Neuronetics, Inc., Novartis, Otsuka, Ortho-McNeil Pharmaceuticals, PamLab, L.L.C., Pfizer (formerly Wyeth-Ayerst Laboratories), Schering-Plough (formerly Organon, Inc.), Shire US Inc., Sunovion Pharmaceuticals, Inc., Takeda (Lundbeck), and Transcept Pharmaceuticals. He has equity holdings in MedAvante, Inc. and receives royalty income from American Psychiatric Foundation, Inc., Guilford Publications, Herald House, Oxford University Press, and W.W. Norton & Company. His wife is employed as the Group Scientific Director for (Embryon—formerly Advogent; which does business with BMS and Pfizer/Wyeth)., **Part 4:** Dr Thase receives grant funding from the Agency for Healthcare Research and Quality, Eli Lilly & Company, GlaxoSmithKline (ended 7/10), National Institute of Mental Health, Otsuka Pharmaceuticals, and Sepracor, Inc.; A. Mahableshwarkar, **Part 1:** Takeda Developement Center Americas, Inc., GlaxoSmithKline (GSK), and Johnson & Johnson (J&J), **Part 2:** Takeda Developement Center Americas, Inc., GlaxoSmithKline (GSK), and Johnson & Johnson (J&J), **Part 3:** Takeda Developement Center Americas, Inc., GlaxoSmithKline (GSK), and Johnson & Johnson (J&J), **Part 5:** Takeda Development Center America, Inc.; M. Dragheim, **Part 1:** H. Lundbeck A/S, **Part 2:** H. Lundbeck A/S, **Part 3:** H. Lundbeck A/S, **Part 5:** H. Lundbeck A/S.

T143. Varenicline Effects on Smoking, Cognition, and Psychiatric Symptoms in Schizophrenia

Robert C Smith*, Revital Amiaz, SiTian Mei,
Lawrence Maayan, Hua Jin, Sylvia Boules, Henry Sershen, Chunbo Li, Juanjuan Ren, Liu Yanhong,
Harshita Ravishankar, Abel Lajtha, Alessandro Guidotti, Mark Weiser, John M Davis

Nathan Kline Institute for Psychiatric Research, Hewlett, New York

Background: Schizophrenics have a high rate of smoking and cognitive deficits which may be related to a decreased number or responsiveness of nicotinic receptors in their brains. Varenicline is a partial nicotinic agonist which is effective as an antismoking drug in normals, although concerns have been raised about potential psychiatric side-effects. Nicotinic agonists or partial agonists have been proposed as potential treatments for cognitive deficits in schizophrenia. We conducted a double-blind placebo controlled study to evaluate effects of varenicline on measures of smoking, cognition, psychiatric symptoms, and side-effects in schizophrenic patients who were cigarette smokers.
Methods: 87 patients with diagnosis of schizophrenia or schizoaffective disorder at 4 sties (2 US, 1 Israel, and 1 China) participated in a double-blind placebo-controlled study in which they received varenicline (2 mg/day) or matched placebo for 8 weeks, with some sites extending selected measures to 12 weeks. All subjects received brief weekly structured behavioral counseling sessions on smoking cessation. Smoking was evaluated with objective measures of breathalyzer CO, and serum nicotine and cotinine, and self-report measures of cigarettes smoked and a smoking urges scale. Cognition was measured by MATRICS cognitive battery. Psychiatric symptoms were evaluated with PANSS, SANS, and Calgary Depression scales. Side effects were evaluated with a side-effect check list and additional probing questions for depression and suicidal ideation, Statistical analysis used SAS mixed model ANCOVA (baseline covariate) and SPSS GLM ANCOVA supplemented by non-parametric tests on observed cases and LOCF ANCOVA.
Results: Varenicline significantly decreased objective measures of smoking, and responses on a smoking urges scale, more than placebo. However, only 22–24% of varenicline subjects had measures indicating they had quit smoking by 8 weeks, and these 'quit' percentages did not differ between varenicline and placebo. Varenicline did not improve either overall MATRICS Composite scores or summary scores on Cognitive Domains. Placebo patients improved significantly more than varenicline patients on Reasoning and Problem Solving domain. Varenicline patients tended to improve more on Trial Making Test Part A. There were no significant differences between varenicline and placebo on total scores on PANSS, SANS, or Calgary Depression Scale. There was no increase in any measure of psychiatric symptoms with varenicline, and varenicline patients showed trends for a decrease in symptoms scores on several symptom measures (although the decreases were not significantly greater than placebo for most measures). Varenicline patients showed a significantly greater decrease in PANSS Depression Factor sub-score than placebo patients, and had a greater decrease in SANS Avolition sub-score. Varenicline patients did not show greater side-effects than placebo treated patients at any time point.
Conclusions: Varenicline was a safe and effective drug for decreasing cigarette smoking in schizophrenic patients. It was not a cognitive enhancer on the MATRICS battery measure. It did not increase psychiatric symptoms and may decrease some components of depression or negative symptoms in schizophrenic patients.
Keywords: varenicline, schizophrenia, smoking, nicotine, cognition.
Disclosures: R. Smith, **Part 4:** Suplly of active and palcebo varencline by Pfizer for a study primarily fudned b y Stanely Reserch Orgaization; R. Amiaz, **Part 4:** co-investigator on several pharmaceutical company grants; S. Mei, Nothing to Disclose; L. Maayan, Nothing to Disclose; H. Jin, **Part 4:** Invesitigator or co-investigagtor on grants from severql pharmceutical companies.; S. Boules, Nothing to Disclose;

H. Sershen, Nothing to Disclose; C. Li, **Part 4:** Independent investigator grant from Pifzier.; J. Ren, Nothing to Disclose; L. Yanhong, Nothing to Disclose; H. Ravishankar, Nothing to Disclose; A. Lajtha, Nothing to Disclose; A. Guidotti, Nothing to Disclose; M. Weiser, **Part 4:** Grants from several paceutical companies for reserch on drug studies., Associate Director for clinical studies, Stanley Reserch Foudation.; J. Davis, Nothing to Disclose.

T144. Citalopram Decreases Acute CSF Aβ Production in Young Healthy Subjects

Yvette Sheline*, Tim West, Kevin Yarasheski, Robert A Swarm, John Cirrito, Jin-Moo Lee, Mateusz S Jasielec, Christine Frederiksen, Robert Chott, John C Morris, Mark A Mintun

University of Pennsylvania, Philadelphia, Pennsylvania

Background: Asymptomatic participants in prospective primary prevention trials for Alzheimer's disease (AD) may be exposed to an experimental compound for multiple years. The compound thus should have a proven safety record, as is true for SSRIs. Based on preliminary transgenic mouse and retrospective human studies (Cirrito *et al*, 2011) we examined the hypothesis that the SSRI citalopram prospectively lowers Amyloid beta (Aβ) levels in humans, thus demonstrating key target engagement for AD prevention studies.
Methods: A randomized double-blind placebo controlled study was performed to determine the central nervous system effect of 60 mg of the SSRI antidepressant citalopram on Aβ protein production and levels. Healthy male and female volunteers age 18–50 years received either placebo or 60 mg of citalopram prior to the start of the central nervous system stable isotope labeling kinetics study of Aβ. A lumbar catheter was placed and hourly sampling of blood and CSF was conducted during and after administration of a stable-isotope labeled amino acid ($^{13}C_6$-leucine) as previously described. Metabolism of Aβ was measured using stable isotope labeling kinetics (SILK-Aβ®) assay (Bateman, 2009) with the addition of stable isotope spike absolute quantitation (SISAQ™) to allow for quantitation of Aβ concentrations (C2N Diagnostics). General linear mixed models with fixed effects of treatment group, time and a treatment group by time interaction, with time treated as a nominal variable, were utilized to analyze the patterns of change in newly generated Abeta and Aβ concentrations over time. Subsequent model derived approximate *t*-tests were used to assess differences in the mean levels of the outcome variables between treatment groups, at specific time points.
Results: Placebo ($n=9$) and citalopram ($n=15$) treated volunteers did not differ on any demographic variables. The citalopram-treated individuals had significantly different trajectories of newly generated Abeta over time (time * group interaction $p=0.0003$, treatment group $p=0.004$) compared with placebo. Further, the citalopram-treated group had significantly different total Aβ concentration trajectories over time (time * treatment group $p=0.0004$, treatment group $p=0.001$). Additional testing revealed that citalopram exposed individuals had lower total Aβ concentration over both the 9 h production phase and the total 36 h measurement ($p<0.05$). At hour 19, the mean citalopram-treated Abeta concentration was 33.33 ng/ml ± 11.23 *vs* the mean placebo-treated Abeta concentration of 52.35 ng/ml ± 10.65. Effect size = 1.75.
Conclusions: We show for the first time in humans that prospective administration of an SSRI drug acutely lowers CSF Aβ production, resulting in lower CSF Aβ concentrations. Decreasing Aβ concentrations is important because early in AD pathogenesis, soluble Aβ monomers aggregate at increasing concentrations into soluble Aβ oligomers and insoluble Aβ plaques, both of which can alter synaptic transmission and kill neurons under certain conditions. Maintaining low Aβ levels with any agent should limit the rate at which these toxic species of Aβ are formed. The ability of citalopram to decrease Aβ concentrations suggests that it may be a candidate agent for preventive trials for AD.
Keywords: amyloid beta, CSF, SSRI, prophylaxis.
Disclosures: Y. Sheline, **Part 4:** I have received research funds from Neosynch, Inc.; T. West, **Part 5:** C2N Diagnostics; K. Yarasheski, Nothing to Disclose; R. Swarm, Nothing to Disclose; J. Cirrito, Nothing to Disclose; J. Lee, Nothing to Disclose; M. Jasielec, Nothing to Disclose; C. Frederiksen, Nothing to Disclose; R. Chott, Nothing to Disclose; J. Morris, **Part 1:** Pfizer; Janssen Alzheimer Immunotherapy Program; Eisai, **Part 2:** none, **Part 3:** none, **Part 4:** none, **Part 5:** none; M. Mintun, **Part 5:** I am employed by Avid Radiopharmaceuticals, Inc, a wholly owned subsidiary of Eli Lilly Inc.

T145. The Differential Effect of Early and Efficient Interventions for Acute PTSD Declines with Time

Arieh Y Shalev*, Yael Ankri, Moran Gilad, Yossi Israeli-Shalev, Meng Qian, Isaac Galatzer—Levy, Sara Freedman

New York University, New York, New York

Background: Early, trauma focused, cognitive behavioral therapy (CBT) reduces the prevalence of PTSD in the year that follows a traumatic event. Little is known about the long-term effect of early CBT relative to no- or inefficient interventions. This study evaluated the three year outcomes of providing early interventions.
Methods: Longitudinal survey of adult civilian survivors of traumatic events ($n=756$) with an embedded randomized controlled study of early interventions ($n=231$). Hadassah Hospital emergency department (ED) unselectively receives trauma survivors from Jerusalem and vicinity. Consecutively admitted trauma survivors were screened, assessed, and, upon consent, randomized to receive early treatment for Acute PTSD. The interventions started 29.8 ± 5.7 days after the traumatic event and included twelve weekly sessions of prolonged exposure (PE; $n=63$), or cognitive therapy (CT; $n=40$), or twelve weeks of (blinded) SSRI (escitalopram 20 mg daily; $n=23$) or Placebo ($n=23$). Eighty-two eligible survivors declined an offer of treatment (a DT group). Treatment eligible survivors, including those who declined, were re-evaluated three years after the traumatic event. Subjects lost to follow up ($n=111$) were missing completely at random in the entire sample and within each intervention group. The main outcome

measures were PTSD and PTSD symptoms as measured by the *Clinician Administered PTSD Scale* (*CAPS*). Trained clinicians who administered the CAPS were blind to treatment allocation and participation.

Results: Upon treatment termination, five months after the traumatic event, the study groups differed significantly ($F(4, 187) = 4.74$, $p. < 0.001$), PE and CT doing better than the other groups (Shalev *et al*, 2012). At three years, however, the groups had similar levels of PTSD symptoms (respectively for PE, CT, SSRI, Placebo and DT: 31.51 ± 32.7, 32.08 ± 27.47, 34.31 ± 29.39, 32.13 ± 29 and 30.39 ± 31.04, $F < 1$) and similar prevalence of PTSD (Chi-square (df-4) = 3.53, $p = 0.62$). A linear mixed effect model analyses using PTSD symptoms as within subjects variable showed non-significant Group by Time interaction for the pre-treatment to three-year comparison, with piecewise linear spline modeling showing significant Group by Time interaction for the one and five months comparison.

Conclusions: Despite its beneficial early effect, the longer-term effect of initially efficient trauma-focused early CBT does not differ from that of receiving inefficient interventions or declining treatment. Accelerating recovery is a highly significant achievement. However, the shared non-recovery remains the major clinical and theoretical challenge.

Keywords: post-traumatic stress disorder; prevention; cognitive behavioral therapy, SSRI, barriers to care, longitudinal study.

Disclosures: A. Shalev, Nothing to Disclose; Y. Ankri, Nothing to Disclose; M. Gilad, Nothing to Disclose; Y. Israeli-Shalev, Nothing to Disclose; M. Qian, Nothing to Disclose; I. Galatzer—Levy, Nothing to Disclose; S. Freedman, Nothing to Disclose.

T146. Reduced p11 in Blood Cells Predicts Antidepressant Response to Citalopram

Per Svenningsson*, Louise Berg, Daniel Matthews, Alan Malinger, Marisa Toups, Madhukar Trivedi, Carlos A Zarate, Paul Greengard

Karolinska Institutet, Stockholm, Sweden

Background: The diagnosis and evaluation of treatment response of many psychiatric disease states is hampered by the lack of useful biomarkers. For the identification and assessment of biomarkers, it is important to find molecules which are measurable in peripheral compartments and have documented functions relevant to disease states. Preclinical studies of the multifunctional protein p11 (also S100A10) are shedding light on molecular and cellular mechanisms underlying depression. p11 exerts its function by modulating serotonergic signaling and regulating gene transcription. P11 levels are reduced in frontal cortex, nucleus accumbens and hippocampus from depressed individuals, but can be up-regulated in these regions by chronic administration of various antidepressant treatments, including selective serotonin reuptake inhibitors (SSRIs). p11 is widely distributed in the body. Here, we studied the levels of p11 in distinct subsets of leukocytes in response to citalopram treatment in patients with major depressive disorder (MDD).

Methods: After signing informed consent, 27 patients with MDD in a current major depressive episode were recruited, screened and diagnosedusing DSM-IV criteria and SCID. The depressed patients were prescribed with a daily dosage of citalopram and clinically assessed with MADRS for 8 weeks. Blood samples were drawn and peripheral blood mononuclear cells (PBMC) prepared at baseline, and following 2 and 8 weeks of treatment. For comparison, onsite healthy controls, with no history of mental or neurological disorder, were recruited. The protocol was approved by the National Institutes of Mental Health (http://clinicaltrials.gov/ct2/show/NCT00697268). In a separate cohort, 21 patients with MDD were recruited, assessed with IDS-C30 and treated with SSRIs (incl 9 with citalopram) for 12 weeks. Blood samples were drawn and PBMC prepared at baseline, and following 1 and 12 weeks of SSRI treatment. In both cohorts, PBMC was permeabilized and incubated with anti-human p11unconjugated mouse IgG monoclonal antibody. Following incubation, cells were washed and bound antibody detected by PE conjugated anti-mouse antibody. Surface staining (CD3-PerCP, CD14-PerCP, CD19-FITC, CD56-APC, NK1.1-APC) was performed after blocking of unboundanti-mouse antibody. After labeling, cells underwent flow cytometric analysis to detect p11 levels in gated populations of leukocytes. Linear regression analysis and Pearson's correlation test were used for statistical analysis.

Results: PBMC was prepared from the patients at baseline, and following 2 and 8 weeks of citalopram treatment. Flow cytometry analysis showed that p11 is expressed in essentially all monocytes and NK cells, in most T lymphocytes and myeloidic dendritic cells, and less in plasmacytoid dendritic cells and B lymphocytes. No differences in p11 levels were found between controls and depressed individuals at baseline. Moreover, baseline levels of p11 could not predict antidepressant response. However, after 2 and 8 weeks of citalopram treatment, linear regression analyses showed negative correlations between changes of p11 in NK cells and improvements on MADRS (Pearson r 0.45 and 0.66, respectively; $p < 0.05$). There was also a negative correlation between p11 after 2 weeks of citalopram treatment and improvement on MADRS after 8 weeks (Pearson r 0.61; $p < 0.05$). In a subgroup of patients from a separate cohort, a negative correlation between p11 in NK cells after 1 week of citalopram and the improvement on IDS30 after 12 weeks was evident (Pearson r 0.87; $p < 0.05$). In monocytes, there was a negative correlation between p11 after 2 weeks of citalopram and improvement on MADRS after 8 weeks (Pearson r 0.58; $p < 0.05$). Likewise, citalopram-treated patients from a separate cohort showed a correlation between p11 in monocytes after 1 week of citalopram and improvement on IDS30 after 12 weeks (Pearson r 0.81; $p < 0.05$).

Conclusions: We provide evidence that the widely expressed protein p11 has the characteristics necessary to serve as a biomarker for treatment effects of psychiatric disorders. p11 is not only expressed in certain brain regions known to be afflicted in depression, but also in leukocytes. Surprisingly, the antidepressant response to citalopram correlates to decreases of p11 in both NK cells and monocytes. Interestingly, there is a correlation between an early reduction in p11 levels and later antidepressant response suggesting that p11 may predict antidepressant response.

Keywords: S100A10, SSRI, depression, biomarkers.
Disclosures: P. Svenningsson, Nothing to Disclose; L. Berg, Nothing to Disclose; D. Matthews, **Part 1:** Current employee of Lundbeck, **Part 3:** Current employee of Lundbeck, **Part 5:** Lundbeck ; A. Malinger, Nothing to Disclose; M. Toups, Nothing to Disclose; M. Trivedi, Nothing to Disclose; C. Zarate, **Part 1:** Dr Zarate is listed as a coinventor on a patent application for the use of ketamine and its metabolites in major depression. Dr Zarate has assigned his rights in the patent to the US government but will share a percentage of any royalties that may be received by the government.; P. Greengard, Nothing to Disclose.

T147. Effects of Acute and Sustained Administration of the Antidepressant Vilazodone on Monoaminergic Systems: *In Vivo* Electrophysiological Studies

Pierre Blier*, Agnes Crnic, Mostafa El Mansari

University of Ottawa, Ottawa, Ontario, Canada

Background: Vilazodone has an IC50 of 0.2 nM at the human 5-hydroxytryptamine1A (h5-HT1A) receptor and 0.5 nM for the serotonin reuptake transporter (Heinrich *et al*, 2004; Dawson and Watson, 2009). *In vivo* microdialysis revealed that a single treatment with vilazodone, dose-dependently increases extracellular 5-HT levels in rat median prefrontal cortex and ventral hippocampus (Page *et al*, 2002), but not in norepinephrine (NE) or dopamine (DA) levels in the dorsal lateral frontal cortex of freely moving rat (Hughes *et al*, 2005). Also, behavioral studies showed that vilazodone inhibited ultrasonic vocalizations in rats, a behavioral model for anxiolytic effects produced by activation of presynaptic 5-HT1A receptors. Moreover, vilazodone resulted in significant decrease of immobility and increase of swimming in the forced swim test in rats (Page *et al*, 2002).
Methods: Adult male Sprague-Dawley rats (Charles River, Saint-Constant, QC, Canada) weighing 250–350 g at the time of the experiments were used. For acute experiments, vilazodone, dissolved in 40% hydroxylpropyl-beta-cyclodextrin, was administered intravenously at dose of 0.2 mg/kg. Following vilazodone administration, the 5-HT1A receptor antagonist WAY100635 was administered at a dose of 0.1 mg/kg (i.v.) to reverse the initial drug effects. For 2-day and 14-day regimens, vilazodone (5 mg/kg/day) was administered, once daily, via intraperitoneal (i.p) injections at a dose of 5 mg/kg. Control rats received an equivalent injection of the vehicle. Single-unit, extracellular recordings of monoaminergic neurons were obtained utilizing single-barreled glass electrodes filled with 2 M NaCl solution.
Results: Acute administration of vilazodone produced a dose-dependent decrease in DR 5-HT neuronal firing that was reversed by the injection of the 5-HT1A receptor antagonist WAY100635. To examine whether vilazodone could inhibit 5-HT neuronal firing by a direct action on 5-HT1A receptors or indirectly because of its ability to block 5-HT reuptake, rats were pretreated with the 5-HT synthesis inhibitor PCPA. No significant difference in inhibition of neuronal firing of 5-HT neurons by vilazodone was seen in PCPA-treated rats compared to controls. Twenty four hours after the 2-day administration of vilazodone, there was a robust decrease of 51% in 5-HT neuronal firing that was no longer present following the 14-day regimen. Two-day administration of vilazodone decreased the mean firing rate of DA neurons by 38% in comparison to control animals; this attenuation of firing was still present after a 14-day regimen (23%, $p<0.05$). There were no significant changes in the number of DA neurons spontaneously active per electrode trajectory through the VTA in either the 2- or 14- day treated rats. Burst firing parameters of DA neurons were significantly attenuated after 2- and 14-day regimens (bursts/min per minute: 70 and 43%; spikes/burst: 30 and 33%, respectively). Most importantly, the percentage of spikes occurring in bursts was decreased in a sustained fashion after 2 and 14 daily injections of vilazodone (62 and 54%, respectively). Two daily injections of vilazodone significantly decreased the mean firing rate of LC NE neurons (30%), which was still dampened by 25% following the 14-day regimen. The mean number of spontaneously firing NE neurons per electrode trajectory as well as their burst firing pattern were not affected by vilazodone regimen.
Conclusions: Vilazodone was still effective in suppressing the firing rate of 5-HT neurons after PCPA, which indicates that vilazodone can still directly activate the 5-HT1A autoreceptor in the presence of a much reduced levels of 5-HT. As was demonstrated for SSRIs, the decrease in firing activity of 5-HT neurons observed after 2-day administration of vilazodone was normalized to the control level after 14-day administration. This suggests that the 5-HT1A autoreceptor had desensitized. Similarly, vilazodone inhibited the firing rate and pattern of VTA DA neurons in a sustained manner, as previously reported with SSRIs. In contrast to SSRIs, vilazodone inhibited the mean firing rate of NE neurons but not their burst firing activity; this may have resulted from the excitatory effect of 5-HT1A receptor activation on NE neurons exerted directly by the potent 5-HT1A receptor agonism of vilazodone. The determination of the increase in 5-HT transmission in a projection structure (ie the hippocampus) produced by sustained vilazodone administration, in comparison to a SSRI, will allow to determine if its 5-HT1A agonistic property provides a greater degree of enhancement.
References:
Heinrich T, Böttcher H, Schiemann K, Hölzemann G, Schwarz M, Bartoszyk GD, van Amsterdam C, Greiner HE, Seyfried CA. Dual 5-HT1A agonists and 5-HT re-uptake inhibitors by combination of indole-butyl-amine and chromenonyl-piperazine structural elements in a single molecular entity. Bioorg Med Chem 2004, 12:4843–4852.
Dawson LA, Watson JM. Vilazodone: a 5-HT1A receptor agonist/serotonin transporter inhibitor for the treatment of affective disorders. CNS Neurosci Ther 2009, 15: 107–117.
Page ME, Cryan JF, Sullivan A, Dalvi A, Saucy B, Manning DR, Lucki I. Behavioral and neurochemical effects of 5-(4-[4-(5-Cyano-3-indolyl)-butyl)-butyl]-1-piperazinyl)-benzofuran-2-carboxamide (EMD 68843): a combined selective inhibitor of serotonin reuptake and 5-hydroxytryptamine(1A) receptor partial agonist. J Pharmacol Exp Ther 2002, 302: 1220–1227.
Keywords: serotonin, norepinephrine, dopamine, antidepressant, electrophysiology.
Disclosures: P. Blier, **Part 1:** Financial involvements with: Eli Lilly and Co, Forest, Janssen, Lundbeck, Astra-Zeneca,

Pfizer, Takeda, Bristol Myers, Otsuka, Merck, Servier. **Part 4:** Bristol Myers Squibb, Servier, Lundbeck, Pfizer; A. Crnic, Nothing to Disclose; M. El Mansari, Nothing to Disclose.

T148. The Efficacy of Levomilnacipran ER in Patients with Prominent Fatigue Symptoms: *Post Hoc* Pooled Analyses of Double-blind Placebo-Controlled Trials

Carl Gommoll*, Adam Ruth, Changzheng Chen, William M Greenberg, Maurizio Fava

Forest Research Institute, Jersey City, New Jersey

Background: Fatigue, tiredness, and lack of energy are very prevalent symptoms in patients with major depressive disorder (MDD). High levels of fatigue-related depression symptoms at baseline have also been associated with poorer treatment outcomes. Fatigue-related symptoms are strongly correlated with diminished functioning, depression recurrence, and progression to a chronic course of illness. Fatigue associated with MDD appears to be less responsive to antidepressant treatment than other depressive symptoms, and among patients who fully respond to treatment, fatigue is one of the most commonly reported residual symptoms. Noradrenergic neurotransmission may be specifically related to alertness and energy; limited evidence has suggested that antidepressants that include a strong noradrenergic component may be beneficial in treating patients with MDD and prominent fatigue symptoms. Levomilnacipran extended-release (ER) is a potent and selective serotonin and norepinephrine reuptake inhibitor recently approved by the FDA for the treatment of MDD in adults. Levomilnacipran has approximately 2-fold greater potency for reuptake inhibition of norepinephrine relative to serotonin. *Post hoc* analyses of data from 5 short-term randomized, placebo-controlled, clinical trials evaluated the efficacy of levomilnacipran ER in patients with MDD and prominent fatigue-related symptoms.

Methods: Data from 2 fixed- and 3 flexible-dose randomized, double-blind, placebo-controlled trials of 8 or 10 weeks' duration evaluating levomilnacipran ER 40–120 mg/day were pooled and analyzed. Patients were 18–80 years of age and met DSM-IV-TR criteria for MDD. The primary efficacy measure in all studies was change from baseline to endpoint in Montgomery-Asberg Depression Rating Scale (MADRS) total score. *Post hoc* analyses evaluated the efficacy of levomilnacipran ER compared with placebo in patients with high levels of fatigue. Baseline score on MADRS Item 7 (Lassitude) was used to stratify patients into 2 subgroups: patients with high fatigue levels (score $\geqslant 4$) and without high fatigue levels (score < 4). Least squares mean (LSM) change from baseline for levomilnacipran ER and placebo in patients with and without high fatigue levels was evaluated on various efficacy measures including MADRS total score, and percent of patients meeting response ($\geqslant$50% MADRS improvement at Week 8), early response ($\geqslant$20% MADRS improvement at Week 2), and early and sustained response ($\geqslant$20% MADRS improvement at Week 1 or 2 and $\geqslant$50% MADRS improvement at last 2 visits). Additional efficacy outcomes included time to response ($\geqslant$50% MADRS improvement) that was maintained at all consecutive visits. The between-group difference in LSM change in MADRS scores was analyzed using a mixed-model repeated measures (MMRM) approach. Response rates were analyzed using a logistic regression model (LOCF) with corresponding odds ratios (OR) and numbers needed to treat (NNT). Time to response was evaluated using a Cox proportional hazards regression model.

Results: Most patients had prominent fatigue symptoms at baseline; 73% of placebo- and 74% of levomilnacipran ER-treated patients metthe criteria for high baseline fatigue (MADRS Item 7 score $\geqslant 4$). The least squares mean difference (LSMD) in MADRS total score change from baseline for levomilnacipran ER *vs* placebo was statistically significant in patients with high (LSMD $= -3.1$, $P < 0.0001$) and without high (LSMD $= -2.8$, $P = 0.0018$) baseline fatigue. In both groups, differences *vs* placebo on the MADRS exceeded the 2-point threshold that has been suggested to represent clinically meaningful improvement. Response rates were significantly greater for levomilnacipran ER *vs* placebo in subgroups of patients with high fatigue (43 *vs* 33%; OR $= 1.6$; $P < 0.0001$) and without high fatigue (50 *vs* 39%; OR $= 1.7$; $P = 0.0019$); the NNT was 9 in each subgroup, suggesting clinically relevant treatment effects. Time to response that was maintained until the end of treatment ($\geqslant$50% MADRS improvement at all consecutive visits) was significantly faster for levomilnacipran ER compared with placebo in patients with high (hazard ratio [HR] $= 1.53$; $P < 0.0001$) and without high (HR $= 1.65$; $P < 0.0001$) baseline fatigue. Using early response criteria ($\geqslant$20% improvement at Week 2), early response was seen for levomilnacipran ER *vs* placebo in patients with high (49 *vs* 44%; OR $= 1.3$; $P = 0.0123$) and without high (49 *vs* 41%; OR $= 1.4$; $P = 0.0428$) baseline fatigue; significant advantages for levomilnacipran ER relative to placebo were also seen for early and sustained response in patients with (30 *vs* 23%; OR $= 1.5$: $P = 0.0015$) and without (31 *vs* 23%; OR $= 1.6$; $P = 0.0221$) high baseline fatigue.

Conclusions: In these exploratory *post hoc* pooled analyses, levomilnacipran ER compared with placebo showed statistically significant and clinically meaningful improvement in adult MDD patients with prominent fatigue symptoms at baseline; results were comparable to those for patients without high levels of baseline fatigue. The results suggest that levomilnacipran ER may be an efficacious treatment option for patients with prominent fatigue symptoms. Given the high prevalence of depression-related fatigue symptoms and their association with worse clinical outcomes in patients with MDD, further research in this area is warranted.

Keywords: levomilnacipran, major depressive disorder, fatigue, SNRI.

Disclosures: C. Gommoll, **Part 5:** Employee of Forest Research Institute, subsidiary of Forest Laboratories, Inc.; A. Ruth, **Part 5:** Employee of Prescott Medical Communications Group, a contractor for Forest Research Institute.; C. Chen, **Part 5:** Forest Research Institute, a subsidiary of Forest Laboratories, Inc.; W. Greenberg, **Part 5:** Forest Research Institute, a subsidiary of Forest Laboratories, Inc.; M. Fava, **Part 1:** Research Support:, Abbot Laboratories; Alkermes, lnc.;Aspect Medical Systems; AstraZeneca; BioResearch;, BrainCells Inc.; Bristol-Myers Squibb; CeNeRx BioPharma; Cephalon; Clintara, LLC;, Covance; Covidien;

Eli Lilly and Company; EIMindA, Ltd.; EnVivo Pharmaceuticals, Inc.;, Euthymics Bioscience, Inc.; Forest Pharmaceuticals, Inc.; Ganeden Biotech, Inc.;, GlaxoSmithKline; Harvard Clinical Research Institute; Icon Clinical Research; i3, lnnovus/lngenix; Janssen R&D, LLC; 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); SanofiAventis, US LLC; Shire; Solvay Pharmaceuticals, Inc.; Synthelabo; Wyeth-Ayerst, Laboratories., Advisory/Consulting:, Abbott Laboratories; Affectis Pharmaceuticals AG; Alkermes, Inc.; Amarin Pharma Inc.;, Aspect Medical Systems; AstraZeneca; Auspex Pharmaceuticals; Bayer AG; Best, Practice Project Management, Inc.; BioMarin Pharmaceuticals, Inc.; Biovail Corporation;, BrainCells Inc; Bristol-Myers Squibb; CeNeRx BioPharma; Cephalon, Inc.; Cerecer; CNS, Response, Inc.; Compellis Pharmaceuticals; Cypress Pharmaceutical, Inc.;, DiagnoSearch Life Sciences (P) Ltd.; Dinippon Sumitomo Pharma Co. Inc.; Dov, Pharmaceuticals, Inc.; Edgemont Pharmaceuticals, Inc.; Eisai Inc.; Eli Lilly and Company;, EnVivo Pharmaceuticals, Inc.; ePharmaSolutions; EPIX Pharmaceuticals, Inc.; Euthymics, Bioscience, Inc.; Fabre-Kramer Pharmaceuticals, Inc.; Forest Pharmaceuticals, Inc.;, GenOmind, LLC; GlaxoSmithKline; Grunenthal GmbH; i3 lnnovus/lngenis; Janssen, Pharmaceutica; Jazz Pharmaceuticals, Inc.; Johnson & Johnson Pharmaceutical, Research & Development, LLC; Knoll Pharmaceuticals Corp.; Labopharm Inc.; Lorex, Pharmaceuticals; Lundbeck Inc.; MedAvante, Inc.; Merck & Co., Inc.; MSI Methylation, Sciences, Inc.; Naurex, Inc.; Neuralstem, Inc.; Neuronetics, Inc.; NextWave, Pharmaceuticals; Novartis AG; NuPathe;Nutrition 21; Orexigen Therapeutics, Inc.;, Organon Pharmaceuticals; Otsuka Pharmaceuticals; Pamlab, LLC.; Pfizer Inc.;, PharmaStar; Pharmavite® LLC.; PharmoRx Therapeutics; Precision Human, Biolaboratory; Prexa Pharmaceuticals, Inc.; Puretech Ventures; PsychoGenics; Psylin, Neurosciences, Inc.; Rexahn Pharmaceuticals, Inc.; Ridge Diagnostics, Inc.; Roche;, Sanofi-Aventis US LLC.; Sepracor Inc.; Servier Laboratories; Schering-Plough, Corporation; Solvay Pharmaceuticals, Inc.; Somaxon Pharmaceuticals, Inc.; Somerset, Pharmaceuticals, Inc.; Sunovion Pharmaceuticals; Supernus Pharmaceuticals, Inc.;, Synthelabo; Takeda Pharmaceutical Company Limited; Tai Medical, Inc.; Tetragenex, Pharmaceuticals, Inc.; Teva; TransForm Pharmaceuticals, Inc.; Transcept, Pharmaceuticals, Inc.; Vanda Pharmaceuticals, Inc., Speaking/Publishing:, Adamed, Co; Advanced Meeting Partners; American Psychiatric Association; American, Society of Clinical Psychopharmacology; AstraZeneca; Belvoir Media Group; Boehringer, lngelheim GmbH; Bristol-Myers Squibb; Cephalon, Inc.; CME Institute/Physicians, Postgraduate Press, Inc.; Eli Lilly and Company; Forest Pharmaceuticals, Inc.;, GlaxoSmithKline; lmedex, LLC; MGH Psychiatry Academy/Primedia; MGH Psychiatry, Academy/Reed Elsevier; Novartis AG; Organon Pharmaceuticals; Pfizer Inc.;, PharmaStar; United BioSource,Corp.; Wyeth-Ayerst Laboratories, Equity Holdings: Compellis; PsyBrain, Inc., Royalty/patent, other income:, Patent for Sequential Parallel Comparison Design (SPCD), which are licensed by MGH, to RCT Logic, LLC; and patent application for a combination of azapirones and, bupropion in Major Depressive Disorder (MOD)., Copyright for the MGH Cognitive & Physical Functioning Questionnaire (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.

T149. Efficacy and Safety of Intravenous Esketamine in Patients with Treatment-resistant Depression: A Double-blind, Double-randomization, Placebo-controlled Phase 2a Study

Jaskaran Singh*, Margaret Fedgchin, Ella Daly, Liwen Xi, Caroline Melman, Geert De Bruecker, Andre Tadic, Pascal Sienaert, Frank Wiegand, Husseini K Manji, Wayne Drevets, Luc Van Nueten

Janssen R&D US, Titusville, New Jersey

Background: Although there are many effective pharmacotherapies available for the treatment of major depressive disorder (MDD), a substantial proportion of patients with MDD do not respond to currently available biogenic amine-based antidepressants. Recent studies have demonstrated that ketamine, a nonselective N-methyl-D-aspartate (NMDA) receptor antagonist, has a rapid onset of effect and robust antidepressant properties. Esketamine, the *S* enantiomer of ketamine, has 3–4 times higher potency at the NMDA receptor than racemic ketamine, allowing antidepressant efficacy at lower doses, with potentially fewer side effects. The efficacy, safety and dose response of intravenous (IV) esketamine was evaluated in patients with treatment resistant depression (TRD).

Methods: This double-blind (DB), double-randomization, placebo-controlled, multicenter, phase 2a study conducted in 30 adult patients with TRD, consisted of a screening phase [up to 2 weeks], DB treatment phase [1 week] and follow up phase [4 weeks that included optional open-label treatment [OL]). Men and women (18–64 years old) who met the Diagnostic and Statistical Manual of Mental Disorders—Fourth Edition (DSM-IV-TR) diagnostic criteria for recurrent MDD, without psychotic features and an IDS-C_{30} total score $\geqslant 34$ at screening and day -1 were randomized (1 : 1 : 1) to receive IV esketamine: 0.4 mg/kg or 0.2 mg/kg, or placebo over 40 min on day 1. Following the primary endpoint measurements (change from baseline [day 1, predose] to day 2 in the Montgomery Åsberg Depression Rating Scale [MADRS] total score), patients were classified into responders (those with reduction in MADRS total score of $>50\%$ *vs* day 1 baseline on day 2, 3, or 4) and non-responders. Responders received the same treatment on day 4 while the non-responders who had received placebo were re-randomized (1 : 1) on day 4 to IV esketamine 0.2 mg/kg or 0.4 mg/kg. Non-responders who

had received 0.2 mg/kg had the dose increased to 0.4 mg/kg on day 4 and non-responses who received 0.4 mg/kg continued with the same treatment on day 4. Key safety assessments included assessment of heart rate, blood pressure, blood oxygen, dissociative symptoms & psychosis like symptoms.

Results: Overall, 96.7% of the enrolled patients (29/30) completed the study. The mean age of patients was 43 years (range: 20–62 years), majority were white (97%) and 60% were women. One patient withdrew after incorrectly receiving double the dose (0.8 mg/kg) and experiencing the adverse event of dissociation. All patients received 2 infusions (days 1 and 4) during the DB phase. The least square (LS) mean (SE) change from day 1 baseline to day 2 in the MADRS total score was similar for both dose groups: −16.8 (3.00) (0.2 mg/kg dose group) and −16.9 (2.61) (0.4 mg/kg dose group) and showed significant improvement (one-sided $p = 0.001$ in both dose groups) *vs* placebo – 3.8 [2.97]). 2 of 3 non responders to 0.2 mg/kg dose group responded to 0.4 mg/kg dose group. There were no deaths or serious treatment-emergent adverse events (TEAEs). Most common TEAEs were headache, nausea and dissociation. TEAEs such as dissociation did not persist longer than 2 h from the start of the esketamine infusion. Patients in the 0.2 mg/kg dose group had fewer adverse events.

Conclusions: The current study showed that IV esketamine at both doses (0.2 mg/kg and 0.4 mg/kg) had a rapid onset (within hours) and robust antidepressant effects in patients with TRD. Lower dose may allow for better tolerability while maintaining the robust efficacy.

Keywords: antidepressants, esketamine, ketamine, major depressive disorder, treatment-resistant depression.

Disclosures: J. Singh, Nothing to Disclose; M. Fedgchin, Nothing to Disclose; E. Daly, Nothing to Disclose; L. Xi, Nothing to Disclose; C. Melman, Nothing to Disclose; G. De Bruecker, Nothing to Disclose; A. Tadic, Nothing to Disclose; P. Sienaert, Nothing to Disclose; F. Wiegand, Nothing to Disclose; H. Manji, Nothing to Disclose; W. Drevets, Nothing to Disclose; L. Van Nueten, Nothing to Disclose.

T150. The Triple Reuptake Inhibitor Antidepressant Effects (TRIADE) Trial: Amitifadine for the Treatment of Major Depressive Disorder

Marlene P Freeman*, Anthony McKinney, Mark Bradshaw, Pierre Tran, Timothy Hsu, Maurizio Fava

Harvard Medical School, Boston, Massachusetts

Background: Major depressive disorder (MDD) is a prevalent and disabling disorder, and a majority of patients do not experience remission from MDD with an initial treatment with an available first-line antidepressant such as a selective serotonin reuptake inhibitor (SSRI). Available treatments are limited by efficacy and side effect profiles. Novel treatments for MDD are needed, in terms of efficacy in treatment resistant depression (TRD), as well as tolerability. Sexual side effects are common side effects of available antidepressants with serotonergic activity. Amitifadine is a novel inhibitor of serotonin, norepinephrine, and dopamine reuptake with a ratio of 1 : 2 : 8 for the three transporters, respectively, with promising preliminary data for the dose of 100 mg in the treatment of MDD. A previous proof-of-concept trial demonstrated a significant difference of amitifadine 100 mg compared to placebo in a six week, randomized controlled trial for MDD (Tran *et al*, 2012).

Objective: The objective of the study was the assessment of amitifadine 50 mg and 100 mg in the treatment of MDD not adequately responsive to treatment with an antidepressant, with a focus on dose-related efficacy and the associated side effect profile. The hypotheses regarding tolerability were that amitifadine would have a low burden of sexual side effects relative to SSRIs.

Methods: This was a multicenter, randomized, double blind, placebo- and paroxetine- controlled trial of amitifadine for MDD non-responsive to an adequate trial of an antidepressant trial in the current depressive episode. A sequential parallel comparison design was utilized. All patients were initially randomized to receive either amitifadine 50 or 100 mg, paroxetine 40 mg, or placebo. The study was 12 weeks in duration, with two 6-week phases. The primary outcome measure was change on the Montgomery Asberg Depression Rating Scale (MADRS). Secondary outcomes included the Hamilton Rating Scale for Depression (HAM-D), Clinical Global Impression- Improvement (CGI-I), Sexual Functioning Inventory (SFI), Clinical Global Impression (Improvement and severity versions), and the MGH Cognitive and Physical Functioning Questionnaire (MGH-CPFQ). Male and female patients, between the ages of 18–65 (inclusive), were eligible who had a diagnosis of MDD, based on the SCID interview, and validated by remote raters, a history of non-response to an adequate trial of an antidepressant during the current episode of depression (an adequate dose for at least 8 weeks), and a HAM-D-17 score of >18 at screening.

Results: A total of 342 patients were enrolled in the study. Efficacy: In the primary analysis, there was no significant difference in MADRS change from baseline for amitifadine 50 and 100 mg compared to placebo. There was a significant difference between paroxetine and placebo, verifying the internal validity of the trial. There were no significant differences on the CGI-Improvement between amitifadine 50 or 100 mg and placebo; there was a significant difference on the CGI-I between paroxetine and placebo at both 6 and 12 weeks. Despite lack of efficacy on the primary outcome, exploratory analyses did demonstrate potential signals for a dose related antidepressant effect of amitifadine. In exploratory analyses, paroxetine demonstrated significantly better improvement between placebo and amitifadine 50 mg, although the difference between paroxetine and amitifadine 100 mg was non-significant, although the trial was not powered as a non-inferiority trial. For patients with confirmed adherence based on PK levels, the improvement in terms of change on the MADRS from baseline with amitifadine 100 mg was similar to that seen with paroxetine. Neither dose was associated with significant changes in heart rate or blood pressure. Sexual side effects: Those receiving amitifadine 100 mg had significantly less overall worsening of sexual function than those receiving paroxetine (SFI scores, total). On most individual items of the SFI, amitifadine 100 mg was significantly less impairing than paroxetine, such as anorgasmia, arousal, and overall satisfaction.

Conclusions: Amitifadine is a triple reuptake inhibitor with a unique profile of monoamine affinity for reuptake inhibition. An earlier study demonstrated the efficacy of amitifadine 100 mg compared with placebo for MDD. This study was focused on those with MDD who were not responsive to a verified adequate trial of an FDA-approved antidepressant. Results of this proof-of-concept study demonstrated relative ineffectiveness of amitifadine dosed at 50 and 100 mg per day for MDD, although amitifadine was well tolerated, with a low burden of sexual side effects, particularly at the 100 mg dose. At the higher dose of 100 mg, amitifadine may adequately engage the dopamine transporter to mitigate sexual side effects. Neither dose led to changes in heart rate or blood pressure, as surrogate markers of clinically excessive norepinephrine transporter engagement. Higher doses of amitifadine may be assessed for efficacy in MDD non-responsive to a first trial of an FDA-approved antidepressant and are likely to exhibit good tolerability based on this study and previous trials.

Keywords: major depressive disorder, triple reuptake, amitifadine, sexual side effects.

Disclosures: M. Freeman, **Part 1:** Research support: Lilly, Forest, Glaxo SmithKline; Consulting: PamLab; Advisory Boards: Lundbeck, Takeda, Otsuka; Medical Editing: DSM Nutritionals, **Part 2:** None, **Part 3:** None, **Part 4:** Research grants: Lilly, Forest, GSK; A. McKinney, **Part 1:** Founder and Stockholder in Euthymics, patent holder of amitifadine; more than 5% of personal income, **Part 2:** Yes, as per above, **Part 5:** Euthymics Bioscience; M. Bradshaw, **Part 1:** Consultant to Euthymics Bioscience, **Part 2:** Consultant to Euthymics Bioscience; P. Tran, **Part 1:** Employee of Euthymics Bioscience Inc (2011-April 2013), **Part 2:** Employee of Euthymics Bioscience Inc (2011-April 2013), **Part 5:** Employee of Euthymics Bioscience Inc (2011-April 2013), not current; T. Hsu, **Part 1:** Full time employee of Euthymics, Neurovance, Cephalon, Teva, **Part 2:** Full time employee of Euthymics, Neurovance, Cephalon, Teva, **Part 3:** Full time employee of Euthymics, Neurovance, Cephalon, Teva, **Part 5:** Euthymics ; M. Fava, **Part 1:** Dr Fava only provides lifetime disclosure, will send in separately by email., **Part 2:** Dr Fava only provides lifetime disclosure, will send in separately by email., **Part 4:** Dr Fava only provides lifetime disclosure, will send in separately by email.

T151. Reliability of Behavioral Phenotyping Predictors of Treatment Response in the EMBARC Study

Diego A Pizzagalli Daniel Dillon, Pia Pechtel, Phillip Adams, Thomas Carmody, Crystal Cooper, Patricia J Deldin, Maurizio Fava, Benji T Kurian, Patrick J McGrath*, Melvin McInnis, David W Morris, Ramin V Parsey, Madhukar Trivedi, Myrna M Weissman, Gerard Bruder

Harvard Medical School, Belmont, Massachusetts

Background: Despite the availability of a variety of antidepressant treatments, up to 50% of patients fail to respond to treatment. The likelihood of remission is even lower: only one in three patients achieved remission in the STAR*D study. Unfortunately, attempts to identify clinical or sociodemographic variables predicting antidepressant response have met with limited success. Consequently, treatment in clinical practice often follows a trial-and-error approach. Identification of reliable predictors of antidepressant response would constitute major progress. One of the overarching goals of the EMBARC study (Establishing Moderators/Mediators For A Biosignature Of Antidepressant Response in Clinical Care) is to identify mediators and moderators of treatment response in a large sample of MDD patients ($n = 400$). A variety of neurocognitive tests—including measures of psychomotor slowing, cognitive control, working memory, and reward responsiveness—have shown promise in discriminating antidepressant responders and nonresponders. Their reproducibility and test-retest reliability remains, however, largely unknown. The goal of the current analyses was to evaluate the test-retest reliability of these behavioral phenotyping measures in healthy adults at four research centers in the EMBARC study.

Methods: A neurocognitive battery that included a word fluency task (executive function and cognitive slowing), four-choice reaction time test (psychomotor slowing), AnotB test (speed of reasoning and working memory), Flanker task (executive function and cognitive control), and a probabilistic reward task (reward responsiveness) was administered to 40 healthy adults (10 at each of four EMBARC centers) with a test-retest interval of about 1 week.

Results: *Word Fluency*. The mean number of valid words reported was 44.3 (SD = 10.3) at baseline and 45.6 (SD = 10.1) at week 1, which matches normative data for the verbal fluency test. There was no significant difference across sessions or sites. The overall test-retest reliability was high ($r = 0.81$). *Choice Reaction Time*. There was no significant difference across sites in the 4-choice reaction time (RT). Mean RT was faster in the second session (449 ms, SD = 93) than the baseline session (488 ms, SD = 109, $p < 0.01$). Overall test-retest reliability was excellent ($r = 0.95$). *AnotB Test*. There was no significant difference across sites, but mean reaction time was faster in the second session (2734 ms, SD = 1215) than the baseline session (3228 ms, SD = 2121, $p < 0.001$). Overall test-retest reliability was excellent ($r = 0.90$). *Flanker effects*. Participants were significantly slower and less accurate for incongruent relative to congruent trials (ps < 0.001). There were no differences across sites or sessions. The overall test-retest reliability was high (RT: $r = 0.84$; accuracy: $r = 0.75$). *Probabilistic Reward Task*. Participants showed a preference for the more frequently rewarded stimulus ($p < 0.01$). There were no differences across sites or sessions. Contrary to our hypotheses and unlike prior studies, the test-retest reliability for response bias was poor ($r = 0.27$).

Conclusions: These findings demonstrate that neurocognitive measures previously found to predict antidepressant response can be measured with high reliability in a multisite study, with some of the variability due to practice effects as expected. The only exception was the probabilistic reward task, which showed poor test-retest reliability. Interestingly, however, preliminary analyses indicate that the test-retest reliability for the probabilistic reward task was significant for MDD patients ($n = 50$). These findings provide a foundation for investigating the predictive validity of these behavioral phenotyping markers with respect to treatment outcome in major depression.

Keywords: depression, biomarkers, neurocognitive tasks, executive function, antidepressants.

Disclosures: D. Pizzagalli, Nothing to Disclose; D. Dillon, Nothing to Disclose; P. Pechtel, Nothing to Disclose; P. Adams, Nothing to Disclose; T. Carmody, Nothing to Disclose; C. Cooper, Nothing to Disclose; P. Deldin, Nothing to Disclose; M. Fava, **Part 1:** Advisory/Consulting: Abbott Laboratories; Affectis Pharmaceuticals AG; Alkermes, Inc.; Amarin Pharma Inc.; Aspect Medical Systems; AstraZeneca; Auspex Pharmaceuticals; Bayer AG; Best Practice Project Management, Inc.; BioMarin Pharmaceuticals, Inc.; Biovail Corporation; BrainCells Inc; Bristol-Myers Squibb; CeNeRx BioPharma; Cephalon, Inc.; Cerecor; CNS Response, Inc.; Compellis Pharmaceuticals; Cypress Pharmaceutical, Inc.; DiagnoSearch Life Sciences (P) Ltd.; Dinippon Sumitomo Pharma Co. Inc.; Dov Pharmaceuticals, Inc.; Edgemont Pharmaceuticals, Inc.; Eisai Inc.; Eli Lilly and Company; EnVivo Pharmaceuticals, Inc.; ePharmaSolutions; EPIX Pharmaceuticals, Inc.; Euthymics Bioscience, Inc.; Fabre-Kramer Pharmaceuticals, Inc.; Forest Pharmaceuticals, Inc.; GenOmind, LLC; GlaxoSmithKline; Grunenthal GmbH; i3 Innovus/Ingenis; Janssen Pharmaceutica; Jazz Pharmaceuticals, Inc.; Johnson & Johnson Pharmaceutical Research & Development, LLC; Knoll Pharmaceuticals Corp.; Labopharm Inc.; Lorex Pharmaceuticals; Lundbeck Inc.; MedAvante, Inc.; Merck & Co., Inc.; MSI Methylation Sciences, Inc.; Naurex, Inc.; Neuralstem, Inc.; Neuronetics, Inc.; NextWave Pharmaceuticals; Novartis AG; NuPathe;Nutrition 21; Orexigen Therapeutics, Inc.; Organon Pharmaceuticals; Otsuka Pharmaceuticals; Pamlab, LLC.; Pfizer Inc.; PharmaStar; Pharmavite® LLC.; PharmoRx Therapeutics; Precision Human Biolaboratory; Prexa Pharmaceuticals, Inc.; Puretech Ventures; PsychoGenics; Psylin Neurosciences, Inc.; Rexahn Pharmaceuticals, Inc.; Ridge Diagnostics, Inc.; Roche; Sanofi-Aventis US LLC.; Sepracor Inc.; Servier Laboratories; Schering-Plough Corporation; Solvay Pharmaceuticals, Inc.; Somaxon Pharmaceuticals, Inc.; Somerset Pharmaceuticals, Inc.; Sunovion Pharmaceuticals; Supernus Pharmaceuticals, Inc.; Synthelabo; Takeda Pharmaceutical Company Limited; Tal Medical, Inc.; Tetragenex Pharmaceuticals, Inc.; Teva; TransForm Pharmaceuticals, Inc.; Transcept Pharmaceuticals, Inc.; Vanda Pharmaceuticals, Inc., Speaking/Publishing: Adamed, Co; Advanced Meeting Partners; American Psychiatric Association; American Society of Clinical Psychopharmacology; AstraZeneca; Belvoir Media Group; Boehringer Ingelheim GmbH; Bristol-Myers Squibb; Cephalon, Inc.; CME Institute/Physicians Postgraduate Press, Inc.; Eli Lilly and Company; Forest Pharmaceuticals, Inc.; GlaxoSmithKline; Imedex, LLC; MGH Psychiatry Academy/Primedia; MGH Psychiatry Academy/Reed Elsevier; Novartis AG; Organon Pharmaceuticals; Pfizer Inc.; PharmaStar; United BioSource, Corp.; Wyeth-Ayerst Laboratories, Equity Holdings: Compellis; PsyBrain, Inc., Royalty/patent, other income: Patent for Sequential Parallel Comparison Design (SPCD), which are licensed by MGH to RCT Logic, LLC; and patent application for a combination of Scopolamine and Ketamine in Major Depressive Disorder (MDD). Copyright for the MGH Cognitive & Physical Functioning Questionnaire (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, **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, ; B. Kurian, **Part 1:** Pfizer, Inc.; Johnson & Johnson; Evotec; Rexahn; Naurex; Forest Pharmaceuticals, **Part 4:** Pfizer, Inc.; Johnson & Johnson; Evotec; Rexahn; Naurex; Forest Pharmaceuticals; P. McGrath, Nothing to Disclose; M. McInnis, Nothing to Disclose; D. Morris, Nothing to Disclose; R. Parsey, Nothing to Disclose; M. Trivedi, Nothing to Disclose; M. Weissman, Nothing to Disclose; G. Bruder, Nothing to Disclose.

T152. Can Oxytocin Enhance Learning During Social Cognitive Skills Training in Schizophrenia?

Michael C Davis*, Michael F Green, Junghee Lee, William Horan, Jonathan K Wynn, Stephen R Marder

VA VISN 22 MIRECC; UCLA Semel Institute for Neuroscience and Human Behavior, Los Angeles, California

Background: Impairments across multiple domains of social cognition are common in schizophrenia and they predict poor functional outcome. Psychosocial remediation programs developed to target these deficits have been successful at improving processes involving low-level cue detection (eg, facial affect identification) but show limited benefits for high-level inferential processes (eg, empathy). The current study evaluated a novel pharmacological approach for enhancing social cognitive training using the neuropeptide oxytocin (OT). Oxytocin is an important regulator of social behaviors that appears to increase the salience of social information. Furthermore, we recently found that acute administration of OT improved high-level social cognition in schizophrenia. We evaluated whether administering OT immediately prior to training sessions could enhance learning from social cognitive skills training exercises.

Methods: Twenty-seven male outpatients (mean age = 40) who met DSM-IV-TR criteria for schizophrenia and were taking antipsychotic medications participated in a 6-week

(12-session) course of Social Cognitive Skills Training (SCST). Participants within each group were randomized (double-blind) to receive intranasal OT (40 IU) or placebo 30 min prior to each session. Hence, each session included both patients taking OT and placebo. Baseline (1-week before treatment), post-treatment (1-week after treatment), and one-month follow-up assessments included measures of low-level (1. facial affect recognition, 2. social perception, 3. detection of lies) and high-level (4. detection of sarcasm, 5. empathy, 6. management of emotions) social cognition; we evaluated scores on the individual social cognitive tests, as well as low-level, high-level, and total composite scores. Additional measures at each assessment examined clinical symptoms (Brief Psychiatric Rating Scale (BPRS) and Clinical Assessment Interview for Negative Symptoms (CAINS)), neurocognition (MATRICS Consensus Cognitive Battery (MCCB)), and event-related potentials during a face processing paradigm (N170 and N250). Participants only received OT immediately prior to each training session; they did not receive OT between sessions or on the day of assessments. Generalized linear mixed models were used to evaluate time effects (across the three assessments) and time X treatment group (OT, placebo) effects.

Results: 13 patients were randomized to receive OT and 14 to placebo, and there were no significant demographic differences between the groups. Subjects were unable to accurately tell which treatment they had received (OT *vs* placebo) during the study. On the social cognitive tests, subjects receiving OT demonstrated significantly greater improvements on empathic accuracy than those receiving placebo ($p = 0.03$, $d = 0.92$ post-treatment and $p = 0.03$, $d = 0.98$ at 1 month follow-up). There were no OT-related effects for the other individual tests but there was a main effect of time on facial affect recognition ($p < 0.0001$ post-treatment and $p < 0.0001$ at 1 month follow-up). On the social cognition composite scores, there was a trend for larger improvements on high-level social cognition in the OT *vs* placebo group ($p = 0.07$, $d = 0.50$ post-treatment). There were also significant main effects of time for both the total ($p = 0.03$ post-treatment and $p < 0.01$ at 1 month follow-up) and low-level composite scores ($p < 0.01$ post-treatment and $p < 0.0001$ at 1 month follow-up), indicating general improvements across groups. On the other measures, there were no significant changes in clinical symptoms, though a main effect of time for the MCCB neurocognitive composite score indicated improvement across both groups ($p < 0.01$ at 1-month follow-up). In addition, there was a significant increase in left-hemisphere N170 amplitude for emotion identification in participants who received OT ($p < 0.05$ at 1 month follow-up), but not in those receiving placebo. Finally, there were no differences in side effects between the treatment groups and were no serious adverse events related to study participation.

Conclusions: This study demonstrates the feasibility and possible therapeutic benefits of administering OT prior to psychosocial remediation training sessions that target social cognition. The effects of OT were most pronounced for empathic accuracy, a high-level social cognitive process, which are generally more difficult to modify with current social cognition remediation programs. The benefits of OT can be attributed to enhancement of learning from training sessions rather than acute effects on testing ability since all assessments were scheduled at least 1 week apart from treatment administration. These initial results support further investigation of this novel use of intranasal OT in schizophrenia.

Keywords: schizophrenia, social cognition, oxytocin, psychotherapy, clinical trial.

Disclosures: M. Davis, Nothing to Disclose; M. Green, **Part 1:** Consultant—Abbott Laboratories (AbbVie), Biogen, and Roche, Scientific Advisory Board: Mnemosyne, Research Support: Amgen, **Part 4:** Research Support: Amgen; J. Lee, Nothing to Disclose; W. Horan, Nothing to Disclose; J. Wynn, Nothing to Disclose; S. Marder, **Part 1:** Consultation and Advisory Boards: Astellas, Abbott, Roche, Targacept, Otsuka, Pfizer, Shire, Lundbeck, Genentech, Boehringer-Ingelheim, Research Support: Amgen, Novartis, Sunovion, Psychogenics, Stockholder: Med Avante, **Part 2:** Stockholder: Med Avante, **Part 4:** Research Support: Amgen, Novartis, Sunovion, Psychogenics.

T153. Examining for Potential Duplicate Patients in Clinical Trials: CATIE Analysis

Jonathan Rabinowitz*, Yaacov Z Rabinowitz

Bar Ilan University, Raanana, Israel

Background: Including patients who participate concurrently in more than one clinical trial, or who have recently participated in another trial, could severely bias trial results. There is mounting anecdotal evidence from people conducting clinical trials, but scarcely no published data from analyses of clinical trial data, that suggests that there are people who are concomitantly multiply enrolled in clinical trials. Some have suggested that this is being accelerated by the emergence of websites to help people find trials, promote trial recruitment, flaunt the economic benefit and may be used to easily locate clinical trials.

Methods: Using the DupCheck algorithm, patients in the CATIE (Clinical Antipsychotic Trials of Intervention Effectiveness) study ($n = 1460$) matching on all of the following 8 criteria were identified: (1) age (date of birth not provided) within one year to account for possible second enrollment after birthday, (2) height, (3) BMI category (5 categories), (4) sex, (5) race (Caucasian yes/no), (6) Hispanic (yes/no), (7) Marital status (5 categories), (8) Level of education (8 categories). Simulations were done to examine the likelihood that the above criteria would identify true duplicates.

Results: 49 matches on the 8 criteria were found representing 87 study patients (29 had one match, seven had two matches and two had three). The results of the simulations of 1000 trials with the sample size of CATIE, based on the distribution of CATIE subjects on the matching variables, suggests that the chance of incorrect positive identification of duplicates using this algorithm was less than 7%.

Conclusions: Results suggest that the CATIE study appears to have included duplicate patients. Using age artificially narrows the net as people may have re-entered at different ages (date of randomization was not provided in data set so age could not be adjusted); also having date of birth would have further reduced the chance of false positives. We only examined matches *within* the CATIE study, leaving open the question how many patients were concomitantly

enrolled in other studies. We are currently establishing a cross sponsor registry of doubly encrypted patient demographic data to examine, prospectively and historically, the occurrence of duplicate patients using *de-identified* data. This will allow screening out duplicate patients before enrolment and also re-analyzing completed trials after removing duplicate patients.

Keywords: professional patients, duplicate patients.

Disclosures: J. Rabinowitz, **Part 1:** Received research support, and/or consultancy fees and/or travel support from Janssen (J&J), Eli Lilly, Pfizer, BiolineRx, F. Hoffmann-La Roche, Amgen, Avraham Pharmaceuticals and Newron Pharmaceuticals, **Part 2:** J&J, BiolineRx, Avraham Pharma, F. Hoffman-La Roche, Eil Lilly, **Part 3:** Abraham Pharama, BiolineRx, **Part 4:** Eli Lilly; Y. Rabinowitz, Nothing to Disclose.

T154. A Double-blind Placebo-controlled Study of Long-chain Omega-3 Fatty Acid Supplementation for Depression in Youth at Ultra-high Risk for Bipolar Disorder

Melissa DelBello*, Jeffrey Welge, Jeffrey Strawn, Luis R Patino Duran, Lauren Stahl, Thomas Blom, Stephen Strakowski, Robert McNamara

University of Cincinanti, Cincinnati, Ohio

Background: Family studies demonstrate that offspring of bipolar parents have an elevated risk of developing mood disorders compared with the general population. When these offspring develop major depressive disorder, their risk of developing mania (and by definition bipolar I disorder) is increased further (ie, they are at ultra-high risk). Moreover, antidepressant medications that are commonly used to treat depressive symptoms may increase risk of developing manic symptoms. Therefore, studies of potential treatments for mood symptoms in ultra-high risk youth are urgently needed to establish early intervention, and ultimately prevention, strategies. The aim of our 12-week double-blind placebo-controlled study was to examine the efficacy and tolerability of long-chain omega-3 (LC*n*-3) fatty acids for the treatment of depressive symptoms in ultra-high risk youth and to investigate predictors and mediators of treatment response.

Methods: Sixty youth (ages 9–20 years) with a current DSM-IV-TR diagnosis of Major Depressive Disorder or Depressive Disorder NOS, a Childhood Depression Rating Scale-Revised Version (CDRS-R) of score $\geqslant 28$, and at least one biological parent with bipolar I disorder, were recruited to participate in this 12-week double-blind placebo-controlled study. Fifty-six subjects were randomized to LC*n*-3 fatty acid supplements (2100 mg/d) or placebo (olive oil). Symptom and tolerability ratings were performed weekly and red blood cell (RBC) LC*n*-3 fatty acid levels were obtained at baseline and endpoint from each participant.

Results: The mean age of participants was 13.7 + 4.0 years, and a majority of participants were girls (82%) and White (67%). At baseline subjects displayed moderate symptoms of depression (mean CDRS-R: 47 + 8), and there was a trend for an inverse correlation between baseline CDRS scores and RBC LCn-3 fatty acid levels ($R^2 = 0.06$, $p = 0.085$). Among participants with post-baseline LC*n*-3 measurements, RBC LC*n*-3 fatty acid (EPA + DHA) levels increased and arachidonic acid (AA) to EPA + DHA (AA/EPA + DHA) ratios decreased, significantly from baseline to endpoint in the group treated with LC*n*-3 fatty acids ($n = 20$) but not in the placebo group ($n = 20$). There were statistically significant baseline to endpoint reductions in CDRS-R scores in both treatment groups (LC*n*-3 fatty acids: −21.1 + 8.9 and placebo: −18.9 + 8.4), although there was no significant group difference in improvement ($p = 0.42$). The mean + SD percent reduction in CDRS-R score was 66 + 27% in the LC*n*-3 fatty acid group and 53 + 27% in the placebo group ($p = 0.11$). The baseline AA/EPA + DHA ratio was inversely correlated with baseline to endpoint change in CDRS-R score (ie, smaller baseline ratios were associated with greater CDRS-R improvement, $p = 0.031$), and larger baseline to endpoint decreases in the AA/DHA + EPA ratio were associated with smaller reductions in CDRS-R scores. In an ANCOVA model, this effect appeared to be similar in both treatment groups ($p = 0.044$ for main effect of change in AA/EPA + DHA, and no significant interaction with treatment assignment, $p = 0.69$). After adjusting for the baseline to endpoint change in the AA/EPA + DHA ratio, treatment with LC*n*-3 fatty acids was associated with greater reductions in CDRS-R scores than placebo ($p = 0.017$). The most commonly reported adverse events were headache (Placebo: 83%, LC*n*-3: 77%) and drowsiness (Placebo: 72%, LC*n*-3: 77%). Reported gastrointestinal adverse events were nausea (Placebo: 52%, LC*n*-3: 54%), vomiting (Placebo: 38%, LC*n*-3: 19%), diarrhea (Placebo: 17%, LC*n*-3: 27%), and heartburn (Placebo: 17%, LC*n*-3: 31%).

Conclusions: This study provides preliminary evidence that LC*n*-3 fatty acid supplementation is well-tolerated and improves depressive symptoms in youth at high risk for developing mania. Additionally, we found that higher baseline LC*n*-3 fatty acid levels predicted greater improvement in depressive symptoms. Although the results suggest that supplementation may produce treatment benefits, in contrast to our expectation, larger decreases in AA to DHA + EPA ratios were associated with less improvement in depressive symptoms. These findings indicate that the amount, duration, and/or rate of change in LC*n*-3 fatty acid levels may impact the therapeutic benefits of omega-3 fatty acid supplementation. Additional dose finding studies to identify a plausible biological mechanism for our findings and to determine whether omega-3 fatty acid supplementation is more effective and better tolerated than conventional antidepressant medications for the treatment of ultra-high risk youth are necessary.

Keywords: Omega-3 fatty acid, depression, at-risk, bipolar disorder, youth.

Disclosures: M. DelBello, Nothing to Disclose; J. Welge, Nothing to Disclose; J. Strawn, Nothing to Disclose; L. Patino Duran, Nothing to Disclose; L. Stahl, Nothing to Disclose; T. Blom, Nothing to Disclose; S. Strakowski, Nothing to Disclose; R. McNamara, **Part 1:** Dr McNamara was recently a paid member of the Inflammation Research Foundation's Scientific Advisory Board, which receives significant funding from Zone Labs, the manufacturer of the Omega-3 diet supplement being investigated., **Part 2:** Dr McNamara was recently a paid member of the Inflammation Research Foundation's Scientific Advisory Board, which

receives significant funding from Zone Labs, the manufacturer of the Omega-3 diet supplement being investigated., **Part 3:** Dr McNamara was recently a paid member of the Inflammation Research Foundation's Scientific Advisory Board, which receives significant funding from Zone Labs, the manufacturer of the Omega-3 diet supplement being investigated., **Part 4:** Dr McNamara was recently a paid member of the Inflammation Research Foundation's Scientific Advisory Board, which receives significant funding from Zone Labs, the manufacturer of the Omega-3 diet supplement being investigated., **Part 5:** n/a.

T155. Reliability of Electrophysiological Predictors of Treatment Response in the EMBARC Study

Diego A Pizzagalli*, Craig E Tenke, Jürgen Kayser, Pia Pechtel, Daniel Dillon, Crystal Cooper, Patricia J Deldin, Maurizio Fava, Benji T Kurian, Patrick J McGrath, Ramin V Parsey, Eva Petkova, Madhukar Trivedi, Myrna M Weissman, Sarah Weyandt, Gerard Bruder

Harvard Medical School, Belmont, Massachusetts

Background: Three electrophysiological measurements have been prospectively associated with antidepressant treatment responses: the amplitude of loudness dependent auditory evoked potentials (LDAEP), EEG power in the alpha band, and theta current density localized to the rostral anterior cingulate cortex (rACC). However, the reliability of these measures has not been established. The current analyses evaluated the test-retest reliability of these EEG measures in healthy adults enrolled in the multi-site Establishing Moderators and Biosignatures of Antidepressant Response for Clinical Care (EMBARC) project.
Methods: Resting EEG (four min each eyes open and closed) and auditory event-related potentials (1000 Hz tones, five intensities from 60 to 100 dB) were collected from 40 healthy adults with a test-retest interval of about one week; 10 participants were assessed at each of the four EMBARC sites. EEG alpha and LDAEP measures of N1 dipole were quantified using principal components analysis of current source density (CSD) estimates. Low-resolution electromagnetic tomography (LORETA) was used for EEG source localization, and resting theta current density was extracted from a predefined rACC region-of-interest.
Results: For the LDAEP, N1 dipole amplitudes increased monotonically along with tone intensity ($p<0.001$), with no differences across sessions or centers. The overall reliability of N1 was high ($r=0.87$; range: 0.70–0.98 across centers). For EEG alpha, there was a significant difference in posterior CSD alpha across centers ($p<0.001$), with one center (MGH) showing lower alpha than the other three centers, and a center by session interaction ($p<0.05$), with one center (Columbia) showing greater alpha in the second session. There was, however, no overall session effect and test-retest reliability was high ($r=0.89$; range: 0.72–0.99 across centers. LORETA measures of theta activity localized to rACC required spatial smoothing to minimize differences across sites. Nevertheless, there was a significant center effect ($p<0.001$), with one center (MGH) having higher current density than the others. There was, however, no significant difference between the two sessions with respect to rACC current density for each level of spatial smoothing, and test-retest reliability ranged from $r=0.70$ to $r=0.91$ for different levels of spatial smoothing.
Conclusions: These findings demonstrate that CSD measures of N1 dipole loudness modulation, EEG alpha, and source-localized rACC theta activity can be obtained with good to excellent reliability in a multi-site study. These findings lay the foundation for investigating the predictive validity of these EEG markers with respect to treatment outcome in major depression.
Keywords: depression, biomarkers, neurocognitive tasks, executive function, antidepressants.
Disclosures: D. Pizzagalli, Nothing to Disclose; C. Tenke, Nothing to Disclose; J. Kayser, Nothing to Disclose; P. Pechtel, Nothing to Disclose; D. Dillon, Nothing to Disclose; C. Cooper, Nothing to Disclose; P. Deldin, Nothing to Disclose; M. Fava, **Part 1:** Advisory/Consulting: Abbott Laboratories; Affectis Pharmaceuticals AG; Alkermes, Inc.; Amarin Pharma Inc.; Aspect Medical Systems; AstraZeneca; Auspex Pharmaceuticals; Bayer AG; Best Practice Project Management, Inc.; BioMarin Pharmaceuticals, Inc.; Biovail Corporation; BrainCells Inc; Bristol-Myers Squibb; CeNeRx BioPharma; Cephalon, Inc.; Cerecor; CNS Response, Inc.; Compellis Pharmaceuticals; Cypress Pharmaceutical, Inc.; DiagnoSearch Life Sciences (P) Ltd.; Dinippon Sumitomo Pharma Co. Inc.; Dov Pharmaceuticals, Inc.; Edgemont Pharmaceuticals, Inc.; Eisai Inc.; Eli Lilly and Company; EnVivo Pharmaceuticals, Inc.; ePharmaSolutions; EPIX Pharmaceuticals, Inc.; Euthymics Bioscience, Inc.; Fabre-Kramer Pharmaceuticals, Inc.; Forest Pharmaceuticals, Inc.; GenOmind, LLC; GlaxoSmithKline; Grunenthal GmbH; i3 Innovus/Ingenis; Janssen Pharmaceutica; Jazz Pharmaceuticals, Inc.; Johnson & Johnson Pharmaceutical Research & Development, LLC; Knoll Pharmaceuticals Corp.; Labopharm Inc.; Lorex Pharmaceuticals; Lundbeck Inc.; MedAvante, Inc.; Merck & Co., Inc.; MSI Methylation Sciences, Inc.; Naurex, Inc.; Neuralstem, Inc.; Neuronetics, Inc.; NextWave Pharmaceuticals; Novartis AG; NuPathe;Nutrition 21; Orexigen Therapeutics, Inc.; Organon Pharmaceuticals; Otsuka Pharmaceuticals; Pamlab, LLC.; Pfizer Inc.; PharmaStar; Pharmavite® LLC.; PharmoRx Therapeutics; Precision Human Biolaboratory; Prexa Pharmaceuticals, Inc.; Puretech Ventures; PsychoGenics; Psylin Neurosciences, Inc.; Rexahn Pharmaceuticals, Inc.; Ridge Diagnostics, Inc.; Roche; Sanofi-Aventis US LLC.; Sepracor Inc.; Servier Laboratories; Schering-Plough Corporation; Solvay Pharmaceuticals, Inc.; Somaxon Pharmaceuticals, Inc.; Somerset Pharmaceuticals, Inc.; Sunovion Pharmaceuticals; Supernus Pharmaceuticals, Inc.; Synthelabo; Takeda Pharmaceutical Company Limited; Tal Medical, Inc.; Tetragenex Pharmaceuticals, Inc.; Teva; TransForm Pharmaceuticals, Inc.; Transcept Pharmaceuticals, Inc.; Vanda Pharmaceuticals, Inc., Speaking/Publishing: Adamed, Co; Advanced Meeting Partners; American Psychiatric Association; American Society of Clinical Psychopharmacology; AstraZeneca; Belvoir Media Group; Boehringer Ingelheim GmbH; Bristol-Myers Squibb; Cephalon, Inc.; CME Institute/Physicians Postgraduate Press, Inc.; Eli Lilly and Company; Forest Pharmaceuticals, Inc.; GlaxoSmithKline; Imedex, LLC; MGH Psychiatry Academy/Primedia; MGH Psychiatry Academy/Reed Elsevier; Novartis AG; Organon Pharmaceuticals; Pfizer Inc.; PharmaStar; United BioSource,Corp.; Wyeth-

Ayerst Laboratories, Equity Holdings: Compellis; PsyBrain, Inc., Royalty/patent, other income: Patent for Sequential Parallel Comparison Design (SPCD), which are licensed by MGH to RCT Logic, LLC; and patent application for a combination of Scopolamine and Ketamine in Major Depressive Disorder (MDD). Copyright for the MGH Cognitive & Physical Functioning Questionnaire (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, **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; B. Kurian, **Part 1:** Pfizer, Inc.; Johnson & Johnson; Evotec; Rexahn; Naurex; Forest Pharmaceuticals, **Part 4:** Pfizer, Inc.; Johnson & Johnson; Evotec; Rexahn; Naurex; Forest Pharmaceuticals; P. McGrath, Nothing to Disclose; R. Parsey, Nothing to Disclose; E. Petkova, Nothing to Disclose; M. Trivedi, Nothing to Disclose; M. Weissman, Nothing to Disclose; S. Weyandt, Nothing to Disclose; G. Bruder, Nothing to Disclose.

T156. Modulation of N-methyl-D-aspartate (NMDAR)-type Glutamate Receptors in Psychiatric Disorders

Joshua T Kantrowitz*, Michael Epstein, Odeta Beggel, Nayla Scaramello, Gail Silipo, Elisa Dias, Stephanie Rohrig, Batsheva Halberstam, Marlene Carlson, Daniel C Javitt

Columbia University, New York, New York

Background: Over the past 20 years, attention has turned increasingly to dysfunction of the N-methyl-D-aspartate (NMDAR)-type glutamate receptors as a fundamental deficit underlying pathophysiology in major psychiatric disorders such as schizophrenia and affective illnesses. In schizophrenia, a major focus has been on development of compounds to enhance NMDAR function. Proof-of-principle trials have been conducted with glycine-site agonists including glycine and D-serine, and with high affinity glycine transport inhibitors. Although significant improvement has been observed on negative and total symptoms in some, but not all, studies, effects of these compounds on cognition remain relatively understudied. Furthermore, relatively low doses of D-serine have been used because of concerns regarding nephrotoxicity. Two studies have been done to assess potential effects of D-serine on cognitive function. The first investigated effects of high dose (60 mg/kg/d) D-serine X 6 weeks on neurocognition as assessed both with neurophysiological and neurocognitive measures. A second piloted effects of acute D-serine treatment in the enhancement of cognitive plasticity during an auditory learning task. In affective disorders, NMDAR antagonists represent a potential treatment modality for treatment resistant major depression and bipolar depressive disorders. Acute treatment with intravenous ketamine induces near-immediate relief for treatment resistant depressive symptoms that last for up to 2 weeks. However, practical approaches to prolong this acute benefit are still being developed. D-Cycloserine (DCS) is an antibiotic hat cross reacts with the glycine site of the NMDAR, and, at high dose, acts as a net NMDAR antagonist. Antidepressant effects of DCS were first described in the 1950's and have recently been confirmed in a double blind RCT of treatment resistant MDD. The present study investigates utility of acute ketamine challenge, added to ongoing treatment, to induce improvement followed by high-dose DCS to maintain improvement, as a potential near-term, practical approach for MDD treatment. A concern with use of NMDAR antagonists is a risk of treatment emergent psychosis. In this study, DCS is added to ongoing treatment with antipsychotics (Seroquel, fluoxetine/olanzapine, lurasidone) also approved for treatment of bipolar depression. **Methods:** We will present data from three NMDAR modulator studies, as follows: 1. *ERP biomarker*: In this study, neurophysiological and neurocognitive data were collected during a double blind, crossover study of high dose (60 mg/kg/d) D-serine *vs* placebo added to existing antipsychotic medication ($n = 16$). These neurophysiological data were combined with previously unpublished measures obtained as part of a previously reported open label dose finding study (Kantrowitz 2010) ($n = 19$). Primary outcome measures for neurophysiology included amplitude of the mismatch negativity (MMN) and visual P1 potentials, analyzed as described previously (Friedman 2012). The primary neurocognitive outcome measure was composite score on the MATRICS neuropsychological battery. Clinical symptoms were assessed using the PANSS. 2. *D-serine + cognitive remediation*: In this study, MMN was measured before and after subjects completed an auditory training program (coinciding with peak d-serine levels) and post intervention ERP (NCT01474395). Cognitive remediation consisted of an auditory frequency discrimination task shown to promote learning in healthy controls (Ahissar 2006). Outcome measures included tone matching accuracy and MMN. 3. *DCS in bipolar depression*: DCS has been reported to be effective in treatment of refractory MDD. This study evaluates effectiveness of DCS as maintenance treatment for bipolar depression after acute ketamine administration. DCS (1000 mg) was added to standard treatment for 8 weeks in an open label tolerability investigation.

Results: *ERP biomarker:* 33 subjects with a mean age of 42.7 ± 9.9 and a mean chlorpromazine equivalent dose of 749 ± 545 completed. 14 completed double-blind, while 19 additional subjects received open label D-serine. There were no significant between design (open label *vs* double-blind D-serine) differences across ERP, and results were combined across design. Comparisons between the D-serine and placebo groups found significant, moderate/large effect size difference in MMN to frequency deviants and a non-significant, but moderate effect size improvement in Visual P1. For cognitive outcomes, across all subjects, a moderate effect size, significant drug effect was seen for the MCCB composite and Visual learning domains. D-serine also induced a significant, moderate effect-size reduction in PANSS total symptoms. While no significant drug effect was seen across any individual factors, moderate effect size differences were seen for the negative and depressive factors that favored D-serine *vs* placebo *NMDA + cognitive remediation* ERP analysis is ongoing. 13 patients have completed, and after controlling for baseline pitch processing, significant drug by order effect was seen. A trend towards significance was seen for a drug effect for within visit improvement ($p = 0.063$), primarily in subjects receiving d-serineon day 1. *NMDA-Bipolar* Enrollment is ongoing. 3 patients have received open label ketamine followed by NMDAR antagonist dose (1000 mg/day) DCS. All three patients remitted with ketamine no significant side effects. As of this writing, remission was maintained in all subjects (3–6 weeks), with no significant treatment emergent side effects.

Conclusions: The development of treatments targeted at the glutamatergic system remains novel. These findings represent the first double-blind data with 60 mg/kg D-serine in schizophrenia, and the first data on DCS in bipolar depression. No significant treatment-related side effects were observed, supporting viability of the NMDAR treatment approach in schizophrenia and affective illnesses.

Keywords: NMDA, clinical trial, ERP, cognition, bipolar.

Disclosures: J. Kantrowitz, **Part 1:** Dr Kantrowitz reports having received consulting payments within the last 2 years from Otsuka Pharmaceuticals, Quadrant Health, RTI Health solutions, the Healthcare Advisory Board, Vindico Medical Education, Health Advances, LLC, Strategic Edge Communications. He owns a small number of shares of common stock in GlaxoSmithKline., **Part 2:** NYS OMH, Columbia, RFMH, St Luke's-Roosevelt, **Part 4:** He has conducted clinical research supported by the NIMH, the Stanley Foundation, Roche-Genetech, EnVivo, Psychogenics, Sunovion, Novartis, Pfizer, Lilly and GlaxoSmithKline.; M. Epstein, Nothing to Disclose; O. Beggel, Nothing to Disclose; N. Scaramello, Nothing to Disclose; G. Silipo, Nothing to Disclose; E. Dias, Nothing to Disclose; S. Rohrig, Nothing to Disclose; B. Halberstam, Nothing to Disclose; M. Carlson, Nothing to Disclose; D. Javitt, **Part 1:** Honoraria from Sunovion, BMS, Eli Lilly, Takeda, Omeros, Otsuka, Consensus Medical Communications, Guidepoint global, American Capital, Clearpoint communications, Vindico Medical Communication, and Clearview Healthcare. Research support from Pfizer and Roche; equity in, Glytech, Inc. and AASI; intellectual property rights for use of glycine, D-serine and glycine transport inhibitors in schizophrenia, and serves, on the advisory board of Promentis., **Part 2:** Columbia, NYS OMH, Glytech, **Part 4:** Pfizer, Roche.

T157. Randomized Comparison of the Acute Effects of Olanzapine and Ziprasidone on Tissue-specific Insulin Sensitivity in Healthy Volunteers

John W Newcomer*, Karen Flavin, Michael D Yingling, Julia A Schweiger, Angie Stevens, Ginger E Nicol

Florida Atlantic University, Boca Raton, Florida

Background: The primary aim of the present study was to evaluate, using a within-subject placebo-controlled comparison, the acute effects of olanzapine or ziprasidone administration on whole-body as well as tissue-specific insulin sensitivity (SI) in antipsychotic-naïve healthy young men. We hypothesized that olanzapine, but not ziprasidone, would result in acute decreases in SI compared to placebo.

Methods: Sedentary healthy males were randomized in a cross-over design to IM olanzapine, ziprasidone, or placebo. Acute treatment effects on SI were assessed using hyperinsulinemic-euglycemic clamps with stable isotopomer tracing. Body composition was assessed with Dual Energy X-ray Absorptiometry (DEXA). SI at adipose tissue was measured by evaluating the rate of appearance (R_a) of labeled glycerol; SI at liver was measured by evaluating rate of appearance (R_a) of labeled glucose; SI at muscle was measured by evaluating the rate of disappearance (R_d) of labeled glucose. Main effects of time, time × order of exposure (drug *vs* placebo first) and treatment condition were assessed with ANCOVA, covarying baseline independent (DEXA-measured total body fat) and dependent variables (D_{20} infusion rate, % change in glucose R_a, glucose R_d, and glycerol R_a).

Results: In a sample of 37 healthy males (mean age: 33.5 + 8.5 years) participated in the study. In the olanzapine group, 14 participants received active drug first followed by placebo; 5 participants received placebo first followed by active drug. In the ziprasidone group, 12 participants received active drug first followed by placebo; 6 participants received placebo followed by active drug. The mean length of time between clamps was 56.1 + 38.3 days. Acute antipsychotic treatment, compared to placebo, was associated with significant reductions in glucose disposal (measured as % change in glucose R_d) and whole body insulin sensitivity. No significant difference in the magnitude of this effect was observed between olanzapine and ziprasidone. A significant time × order effect was observed for glucose R_d ($F[1, 31] = 10.90$, $p = 0.002$) and whole body SI (D_{20} infusion rate in mg/kg/min, $F[1, 31] = 13.96$, $p = 0.001$). No statistically significant effect of antipsychotic exposure was observed for glucose R_a or glycerol R_a. During clamped, fixed-dose insulin infusion conditions, olanzapine treatment compared to ziprasidone was associated with a significant increase in mean plasma insulin concentration, suggesting reductions in insulin clearance.

Conclusions: Antipsychotic treatment, including both olanzapine and ziprasidone, was associated with acute onset reductions in insulin sensitivity. The magnitude of the observed treatment effect on whole body insulin sensitivity in the present study; approximately 1 mg/kg/min, can be

compared to well-established effects of adiposity on clamp-measured insulin sensitivity (eg, a 2-unit increase in BMI). These results suggest that there are adiposity-independent, acute-onset effects of antipsychotic treatment on glucose regulation. However, it remains unclear how long this effect may last, and how it interacts, if at all, with well-established effects of adiposity on insulin sensitivity. Supported by Pfizer, Inc. This research was also made possible by P30DK056341 and UL1RR024992. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of Pfizer Inc., the NIH.
Keywords: obesity, antipsychotic, insulin, glucose, metabolism.
Disclosures: J. Newcomer, **Part 1:** Grant/Research Support:, The National Institute of Mental Health (NIMH), Consultant Fee/Data Safety Monitoring Committee:, Bristol-Myers Squibb, Merck, Boehringer-Ingelheim, VIVUS, Royalties/Patents/Other Income: Jones and Bartlett Publishing, Salaried Positions:, N/A, Honoraria:, American Physician Institute, CME Outfitters, CMEology, American Psychiatric Association, Stock Shareholder:, None, **Part 2:** Florida Atlantic University, University of Miami, BMS, VIVUS, Merck, **Part 3:** N/A, **Part 4:** Pfizer Inc., **Part 5:** N/A; K. Flavin, Nothing to Disclose; M. Yingling, Nothing to Disclose; J. Schweiger, Nothing to Disclose; A. Stevens, Nothing to Disclose; G. Nicol, **Part 1:** N/A, **Part 2:** N/A, **Part 3:** N/A, **Part 4:** NIMH, NARSAD (Brain and Behavior Foundation, Sidney R. Baer, Jr. Foundation, **Part 5:** N/A.

T158. Efficacy and Safety of Vilazodone in Major Depressive Disorder: A Randomized, Double-blind, Placebo-controlled Trial

Nunzio Pomara*, Carl Gommoll, Dalei Chen, Rene Nunez, Maju Mathews, Harry A Croft

Nathan Kline Institute/NYU School of Medicine, Orangeburg, New York

Background: Major depressive disorder (MDD) is a chronic and frequently recurrent illness that is associated with considerable psychiatric and medical morbidity. Although several antidepressant medications are available to treat MDD, many patients do not achieve full symptom resolution with currently available SSRIs and SNRIs. Vilazodone, a serotonin reuptake inhibitor and 5-HT_{1A} receptor partial agonist, is approved by the US Food and Drug Administration for the treatment of MDD in adults. The efficacy and safety of vilazodone in MDD were demonstrated in 2 positive placebo-controlled Phase III trials. The current study was conducted to further characterize symptom improvement, safety, and tolerability of vilazodone in patients with moderate to severe MDD.
Methods: A multicenter, 1 : 1 randomized, double-blind, placebo-controlled, parallel-group, fixed-dose study comparing vilazodone 40 mg/day with placebo; outpatients aged 18–70 years with DSM-IV-TR–defined MDD and a baseline total score $\geqslant$26 on the Montgomery-Asberg Depression Rating Scale (MADRS) were included. The study had 3 phases: 1- to 4-week no-drug screening, 8-week double-blind treatment, and 1-week double-blind down-taper. Patients randomized to vilazodone received 10 mg/day for Week 1, 20 mg/day for Week 2, and 40 mg/day for Weeks 3–8; all study drug was taken once daily with food. The primary efficacy outcome was MADRS total score change from baseline to Week 8; the secondary efficacy outcomes were Clinical Global Impressions-Severity (CGI-S) score change from baseline to Week 8 and MADRS sustained response rate (MADRS total score $\leqslant$12 for at least the last 2 consecutive visits during double-blind treatment). Safety assessments included adverse event (AE) recording, clinical laboratory and vital sign measures, electrocardiograms (ECGs), and the Columbia-Suicide Severity Rating Scale (C-SSRS). MADRS and CGI-S change from baseline were analyzed using a mixed-effects model for repeated measures (MMRM); between-group comparison for MADRS sustained response rate was analyzed using the Cochran-Mantel-Haenszel (CMH) test controlling for study center. Safety and efficacy analyses were based on the Safety Population (all patients who received $\geqslant$1 dose of study drug) and the Intent-to-Treat Population (patients in the Safety Population who had baseline and $\geqslant$1 postbaseline MADRS assessment), respectively.
Results: Of patients in the Safety Population (placebo = 253; vilazodone = 255), 83% completed the study; there were no statistically significant between-group differences in discontinuation rates overall or for any reason. The least squares (LS) mean decrease in MADRS score from baseline to Week 8 was significantly greater for vilazodone (−16.1) *vs* placebo (−11.0); the LS mean difference (LSMD) and 95% confidence interval (95% CI) was −5.1 (−6.9, −3.3) ($P<0.00001$). The treatment effect in favor of vilazodone was statistically significant at Week 2 ($P=0.0027$) and it increased throughout the study (Week 4: $P=0.0006$; Week 6: $P<0.00001$; Week 8: $P<0.00001$). The LS mean decrease from baseline to Week 8 in CGI-S was significantly greater for vilazodone (−1.8) *vs* placebo (−1.2); the LSMD (95% CI) *vs* placebo was −0.62 (−0.85, −0.40) ($P<0.00001$). CGI-S change was significantly greater for vilazodone *vs* placebo beginning at Week 2 ($P=0.0142$) and differences remained significant throughout the study. The percent of patients meeting criteria for sustained response was significantly greater for vilazodone (27.3%) *vs* placebo (17.1%, $P=0.0047$). Sensitivity analyses confirmed all primary and secondary outcomes.
Double-blind treatment-emergent AEs (TEAEs) were reported in 62 and 77% of placebo and vilazodone patients, respectively; the overall rate of discontinuation due to AEs was similar for placebo and vilazodone (5 and 6%). Most AEs were mild/moderate in severity (placebo = 97%; vilazodone = 99%) and considered possibly related to study drug (placebo = 64%; vilazodone = 79%). TEAEs that occurred in $\geqslant$5% of vilazodone patients and $\geqslant$2 times as frequently as placebo were diarrhea (33 *vs* 10%), nausea (25 *vs* 8%), dizziness (7 *vs* 3%), and insomnia (6 *vs* 1%). During double-blind treatment, serious AEs (SAEs) were reported in 1 placebo (breast cancer) and 2 vilazodone patients (noncardiac chest pain and suicidal ideation); no SAE was considered related to study drug. During double-blind down-taper, SAEs occurred in 1 placebo (intentional overdose and suicide attempt; considered treatment-related) and 1 vilazodone patient (myocardial infarction; not treatment-related). Mean changes in clinical laboratory values, liver enzymes, and vital signs (including weight changes) were low and similar between groups; no patient had a clinically

significant ECG or QTc interval >500 msec. C-SSRS results were similar overall between the 2 treatment groups.
Conclusions: Statistically significant improvements for vilazodone-treated patients compared with placebo-treated patients were observed on the primary and secondary efficacy outcomes. Efficacy advantages for vilazodone *vs* placebo emerged early and increased over time throughout the study. Vilazodone was generally well tolerated. These results support the efficacy, safety, and tolerability of vilazodone observed in earlier Phase III studies.
Keywords: vilazodone, major depressive disorder, antidepressant.
Disclosures: N. Pomara, Nothing to Disclose; C. Gommoll, **Part 5:** Forest Research Institute; D. Chen, **Part 5:** Forest Research Institute; R. Nunez, **Part 5:** Forest Research Institute; M. Mathews, **Part 5:** Maju Mathews; H. Croft, **Part 1:** Research Grants: Astra Zeneca, Boehringer-Ingleheim, Clinical Data, Bristol Myers Squibb, Eli Lilly, Pfizer, Forest, Takeda, Labopharm, Glaxo Smith Kline (GSK), Lundbeck, Otsuka, Sanofi, Trimel; Consultant: Forest, Clinical Data, GSK; Speaker's Bureau: Astra Zeneca, Boehringer-Ingelheim, Forest, GSK, Pfizer, Sanofi, **Part 4:** Research Grants: Astra Zeneca, Boehringer-Ingleheim, Clinical Data, Bristol Myers Squibb, Eli Lilly, Pfizer, Forest, Takeda, Labopharm, Glaxo Smith Kline (GSK), Lundbeck, Otsuka, Sanofi, Trimel.

T159. Efficacy of Cariprazine Across Schizophrenia Symptoms: A *Post Hoc* Analysis of PANSS Data from a Phase III, Double-blind, Placebo- and Active-controlled Trial

Stephen R Zukin*, Jeffrey A Lieberman, Andrew J Cutler, Kaifeng Lu, Raffaele Migliore, István Laszlovszky, György Németh, Suresh Durgam

Forest Research Institute, Jersey City, New Jersey

Background: Schizophrenia is a multidimensional disorder comprising positive, negative, cognitive, and mood symptoms. Antipsychotics are generally effective for reducing positive symptoms, but have shown limited efficacy for managing negative and/or cognitive symptoms. Compounds with activity at other receptors and new mechanisms of action may be needed to more effectively manage the broad range of symptoms associated with schizophrenia. Cariprazine is an orally active and potent dopamine D_3 and D_2 receptor partial agonist antipsychotic candidate with preferential binding to D_3 receptors. Unlike other antipsychotics, cariprazine showed high and balanced occupancy of D_2 and D_3 receptors *in vivo*. In animal models, cariprazine demonstrated D_3 receptor-mediated efficacy in paradigms of social interaction, cognitive impairment, and stress-induced anhedonia, suggesting potential utility in treating the negative and cognitive symptoms of schizophrenia. A randomized, double-blind, placebo- and active-controlled Phase III study (NCT01104766) evaluated the efficacy and safety of cariprazine in patients with acute exacerbation of schizophrenia. *Post hoc* analysis of PANSS data from this study was conducted to characterize the effects of cariprazine across the broad range of schizophrenia symptoms.
Methods: Patients were randomized to 6 weeks of double-blind treatment with placebo, cariprazine 3 mg/d, cariprazine 6 mg/d, or aripiprazole 10 mg/d. The primary efficacy parameter was change from baseline to Week 6 in Positive and Negative Syndrome Scale (PANSS) total score, analyzed using an MMRM approach adjusting for multiple comparisons. *Post hoc* analyses evaluated efficacy on PANSS-derived Marder factor groupings (negative symptoms, positive symptoms, disorganized thought, uncontrolled hostility/excitement, anxiety/depression) and PANSS single items; *post hoc* analyses did not adjust for multiple comparisons.
Results: A total of 617 patients were randomized and received double-blind treatment (placebo, 153; cariprazine 3 mg/d, 155; cariprazine 6 mg/d, 157; aripiprazole, 152). Approximately 66% of patients completed the study. Baseline PANSS scores were similar among groups (placebo, 96.5; cariprazine 3 mg/d, 96.1; cariprazine 6 mg/d, 95.7; aripiprazole, 95.6). PANSS score total change from baseline to the end of Week 6 was significantly greater for both cariprazine groups and aripiprazole compared with placebo (least squares mean difference [LSMD]: cariprazine 3 mg/d = −6.0, $P = 0.0044$; cariprazine 6 mg/d = −8.8, $P < 0.0001$; aripiprazole 10 mg/d −7.0, $P = 0.0008$). Cariprazine 6 mg/d demonstrated statistically significant improvements *vs* placebo and the largest LSMD of any treatment group on all 5 PANSS Marder factor groupings at Week 6; cariprazine 3 mg/d showed significant advantage *vs* placebo on 3 of 5 Marder factors. Aripiprazole showed significant improvements *vs* placebo on all 5 Marder factors. The largest numerical difference (LSMD) between cariprazine 6 mg/d and aripiprazole 10 mg/d was observed on the negative symptoms factor (cariprazine 3 mg/d: −1.44; $P = 0.0081$; cariprazine 6 mg/d: −2.14, $P < 0.0001$; aripiprazole 10 mg/d: −1.33, $P = 0.012$). Cariprazine 6 mg/d demonstrated statistically significant separation from placebo ($P < 0.05$) on 6 of the 7 individual items associated with the negative symptoms factor; aripiprazole demonstrated significant differences from placebo on 4 of 7 items. Both cariprazine doses and aripiprazole demonstrated significant advantage *vs* placebo (LSMD) on the positive symptom factor (cariprazine 3 mg/d: −1.60, $P = 0.0153$; cariprazine 6 mg/d: −2.35, $P = 0.0004$; aripiprazole 10 mg/d: −1.84, $P = 0.0044$) and the disorganized thought factor (cariprazine 3 mg/d: −1.60, $P = 0.0009$; cariprazine 6 mg/d: −2.07, $P < 0.0001$; aripiprazole 10 mg/d: −1.52, $P = 0.0012$). LSMD *vs* placebo was significantly greater for cariprazine 6 mg/d and aripiprazole 10 mg/d but not cariprazine 3 mg/d on the uncontrolled hostility/excitement factor (cariprazine 3 mg/d: −0.67, $P = 0.0795$; cariprazine 6 mg/d: −1.02, $P = 0.0076$; aripiprazole 10 mg/d: −0.93, $P = 0.0125$) and the anxiety/depression factor (cariprazine 3 mg/d: −0.51, $P = 0.1483$; cariprazine 6 mg/d: −1.08, $P = 0.0024$; aripiprazole 10 mg/d: −1.07, $P = 0.0020$). Analysis of PANSS individual items further supported the broad efficacy of cariprazine, with significant LSMD *vs* placebo ($P < 0.05$) on 20 of the 30 overall items for cariprazine 6 mg/d; cariprazine 3 mg/d and aripiprazole 10 mg/d showed significant advantage *vs* placebo on 11 of 30 and 17 of 30 items, respectively.
Conclusions: Cariprazine was significantly superior to placebo on PANSS total score, across Marder factors, and on many PANSS single items, suggesting broad efficacy in the treatment of schizophrenia. The cariprazine effect

appeared to be dose related, with the higher cariprazine dose (6 mg/d) resulting in greater numerical improvements than cariprazine 3 mg/d or aripiprazole on all 5 factors. Cariprazine 6 mg/d also demonstrated robust improvements in negative symptoms, with statistical separation from placebo on 6 of 7 items corresponding to the negative symptom factor. These results suggest that cariprazine is broadly effective in the treatment of schizophrenia.

Keywords: cariprazine, schizophrenia, antipsychotic.

Disclosures: S. Zukin, **Part 5:** Forest Research Institute; J. Lieberman, **Part 1:** Grant/research support from Allon, Biomarin, Genentech, GlaxoSmithKline, Intracellular Therapies, Lilly, Novartis, Psychogenics, Repligen, Sunovion, and Targacept; advisory board member for Intracellular Therapies; hold a patent from Repligen., **Part 4:** Grant/research support from Allon, Biomarin, Genentech, GlaxoSmithKline, Intracellular Therapies, Lilly, Novartis, Psychogenics, Repligen, Sunovion, and Targacept; A. Cutler, **Part 1:** Research grants from Abbott, Acadia, Alkermes, AstraZeneca, Bristol-Myers-Squibb, Forest Research Institute, Genentech, GlaxoSmithKline, Johnson & Johnson, Lilly, Lundbeck, Merck, Novartis, Ortho-McNeil Janssen, Otsuka, Pfizer, Sanofi, Shire, Sunovion, Targacept, and Vanda; consulting/speaking fees from Abbott, Alkermes, AstraZeneca, Bristol-Myers Squibb, Forest Research Institute, GlaxoSmithKline, Johnson & Johnson, Lilly, Lundbeck, Merck, Novartis, Ortho-McNeil Janssen, Otsuka, Pfizer, Sanofi, Shire, Sunovion, Targacept, and Vanda, **Part 4:** Research grants from Abbott, Acadia, Alkermes, AstraZeneca, Bristol-Myers-Squibb, Forest Research Institute, Genentech, GlaxoSmithKline, Johnson & Johnson, Lilly, Lundbeck, Merck, Novartis, Ortho-McNeil Janssen, Otsuka, Pfizer, Sanofi, Shire, Sunovion, Targacept, and Vanda; K. Lu, **Part 5:** Forest Research Institute; R. Migliore, **Part 5:** Forest Research Institute; I. Laszlovszky, **Part 5:** Gedeon Richter Plc; G. Németh, **Part 5:** Gedeon Richter Plc; S. Durgam, **Part 5:** Forest Research Institute.

T160. Randomized, Double-blind, Placebo-controlled Study of the Efficacy of Vortioxetine on Cognitive Dysfunction in Adult Patients with Major Depressive Disorder (MDD)

Roger S McIntyre*, Soren Lophaven, Christina K Olsen

University Health Network, Toronto, Ontario, Canada

Background: The investigative antidepressant vortioxetine is a 5-HT_3, 5-HT_7 and 5-HT_{1D} receptor antagonist, 5-HT_{1B} receptor partial agonist, 5-HT_{1A} receptor agonist and inhibitor of the 5-HT transporter *in vitro*. The primary objective of this study was to evaluate the efficacy of acute treatment of vortioxetine (VOR) 10 and 20 mg/day *vs* placebo on cognitive dysfunction in MDD.

Methods: Patients aged >18 and <65 years with recurrent MDD according to DSM-IV-TR, a current major depressive episode $\geqslant 3$ months, and a MADRS total score $\geqslant 26$ at both screening and baseline were eligible for this multi-national, randomized, double-blind, placebo-controlled study (FOCUS: NCT01422213). After screening, subjects were randomized to receive either VOR 10 or 20 mg/day or placebo for 8 weeks. Cognition assessments were made at Weeks 1 and 8 and depressive symptom assessments at Weeks 1, 4, and 8. The primary efficacy endpoint assessed cognitive function and was a composite z-score defined as the weighted sum of the z-scores in the objective neuropsychological tests Digit Symbol Substitution Test (DSST) [speed of processing, executive functioning, attention; (correct symbols)] and the Rey Auditory Verbal Learning Test (RAVLT) [learning; (acquisition), memory; (delayed recall)]. The primary analysis was the comparison of the composite *z*-score for VOR *vs* that for placebo at Week 8 using a mixed model for repeated measurements (MMRM). The primary and the sequentially ordered key secondary endpoints were tested separately in parallel for each VOR dose at a Bonferroni-corrected significance level of 0.025. Additional neuropsychological tests included the Trail Making Test (TMT) A (speed of processing), the TMT B (executive functioning), the choice reaction time task (CRT, attention), the STROOP test (executive functioning) and the simple reaction time task (SRT, motor speed). Depressive symptom severity was assessed as secondary endpoints, including change from baseline to Week 8 in the MADRS total score, MADRS response, remission (MADRS $\leqslant 10$), the CGI-S and the CGI-I score. The patient-rated Perceived Deficits Questionnaire (PDQ), which assesses cognitive function from the patient's perspective, was also administered.

Results: On the primary efficacy endpoint, both VOR doses were statistically significantly superior to placebo, with a mean difference to placebo ($n=194$) in the composite z-score of 0.36 (VOR10, $p<0.0001$, $n=193$) and 0.33 (VOR20, $p<0.0001$, $n=204$). Both doses of VOR were statistically significantly superior to placebo in the next predefined key secondary efficacy analysis [DSST (correct symbols), $p<0.0001$]. Differences to placebo ($p<0.05$) were seen for all measures of cognition function, with the exception of VOR20 on RAVLT (learning) and CRT. The clinical relevance of the significant effect on the neuropsychological tests was supported by the magnitude of the standardized effect sizes [ranging from 0.23 to 0.52 (Cohen's *d*)] and the patient-reported cognitive function for which statistically significant differences in favor of both VOR doses were found for both the PDQ total score and subscale scores. On the depression symptom assessments, the difference to placebo in mean change from baseline to Week 8 in the MADRS total score (FAS, MMRM) was -4.7 ($p<0.0001$) for VOR10 and -6.7 ($p<0.0001$) for VOR20. Statistically significant differences in favor of both VOR doses were found for all of the other depressive symptoms variables, including response and remission. The protocol-specified path analysis showed that VOR10 had a 64% (95% CI: 47–82%) direct effect on the primary cognition efficacy endpoint ($p=0.0007$) and VOR20 had a 48% (95% CI: 23–73%) direct effect ($p=0.0246$) after correcting for the effect on MADRS. *In post-hoc* analyses, VOR statistically significantly improved cognitive performance in patients who were non-responders, with a mean difference to placebo in the composite z-score at Week 8 of 0.20 for VOR10 ($n=92$; $p<0.05$) and 0.28 for VOR20 ($n=68$; $p<0.01$). For non-remitters, the corresponding improvement was 0.26 for VOR10 ($n=123$; $p<0.01$) and 0.28 for VOR20 ($n=110$; $p<0.01$). These results support a positive effect of VOR on cognitive dysfunction independent of the improvement in MADRS score. Overall, the most frequent primary reason for withdrawal was adverse events (AE) for placebo (4.1%),

VOR10 (3.6%) and VOR20 (5.3%). Common AEs (incidence $\geqslant$5% for VOR) were nausea (4.1%, 16.4%, 20.8%) and headache (7.1%, 8.2%, 12.6%) for placebo, VOR10 and VOR20, respectively. No clinically relevant changes over time or differences between treatment groups were seen in clinical laboratory test results, vital signs, weight, or ECG parameters.
Conclusions: In this randomized controlled study of MDD in adult patients (18–65 years), VOR 10 and 20 mg/day was statistically significantly superior to placebo on the primary cognitive function measure, comprising executive functioning, processing speed, attention, and memory. The improvement in cognitive performance was shown to include a direct effect of vortioxetine and was not solely due to improvement in depressive symptoms (MADRS score). The positive effect on cognitive function was supported by statistically significant improvements in performance on all objective neuropsychological tests, as well as subjective patient-reported cognitive function. In addition, statistically significant differences in favor of VOR were found for all depressive symptom variables.
Commercial support: This study was sponsored by H Lundbeck A/S and the Takeda Pharmaceutical Company, Ltd.
Keywords: 1. vortioxetine 2. cognitive dysfunction 3. major depressive disorder 4. neuropsychological tests 5. executive function.
Disclosures: R. McIntyre, **Part 1:** Astra Zeneca, Bristol-Myers Squibb, Janssen-Ortho, Eli Lilly, Lundbeck, Pfizer, Shire, Merck, Sepracor, Otsuka, Physicians' Postgraduate Press, CME Outfitters, **Part 4:** Eli Lilly, Janssen-Ortho, Shire, Astra-Zeneca, Pfizer, Lundbeck, Forest, Sepracor, BMS, ; S. Lophaven, **Part 5:** Lundbeck; C. Olsen, **Part 5:** Lundbeck.

T161. Cognitive Remediation in Bipolar Disorder: Efficacy and Neural Correlates of Treatment

Kathryn E Lewandowski*, Matcheri Keshavan, Bruce M Cohen, Sarah H Sperry, Dost Ongur

McLean Hospital, Belmont, Massachusetts

Background: Cognitive dysfunction is a major, lifelong feature of Bipolar Disorder (especially BD I with psychosis; PBD)—present by illness onset, persistent into euthymia, and strongly associated with functional outcomes. Cognitive remediation treatments have shown promise in improving cognitive and functional outcomes in patients with schizophrenia (SZ). Neuroplasticity-based CR treatments are designed to drive neurobiological and behavioral change through carefully-designed, repetitive activities that modulate basic perceptual and pre-attentive processing first as fundamental building blocks of a healthy neural system, and then build on these gains toward improving higher-order processes (eg memory, executive functioning). This model suggests the possibility for widespread and durable changes after training. The present study is the first we are aware of to employ a neuroplasticity-based CR paradigm in patients with Bipolar Disorder with psychosis in a randomized, double-blind, placebo controlled trial. *We aimed to*: (a) evaluate the efficacy of a 70-h, 24 week CR paradigm compared to a dose matched active computer control and (b) examine preliminary fMRI evidence of mechanisms of change after CR.
Methods: PBD patients ($n=30$) were randomized to either a 70 session a CR paradigm ($n=18$) or a dose-matched active computer control paradigm ($n=12$). The control condition was developed to mirror the treatment condition in format, and subjects and assessment staff were blind to group membership. Subjects in both groups completed 3 weekly CR sessions; 2 were completed remotely via the internet and 1 session was completed on site with study staff for weekly updates, trouble shooting and (in the CR condition only) bridging sessions. Pre- and Post-treatment assessments were completed using the MATRICS Consensus Cognitive Battery (MCCB). Pilot data were collected using task-based and resting state fMRI to evaluate changes in brain activation and functional connectivity after CR. For the task-based activation study, subjects in the CR ($n=5$) and control ($n=5$) conditions were administered the Multisource Interference Task (MSIT) in a 3T Seimens's scanner and anterior cingulate (ACC) activation was studied. Resting state functional connectivity of an ACC seed region with the rest of the salience network in CR subjects ($n=5$) and controls ($n=5$) was examined pre- and post- treatment or control.
Results: Subjects in the CR group showed significantly greater improvement in most MCCB domains including Attention, Processing Speed, Working Memory, Visual Learning and Memory, and Reasoning and Problem Solving than control subjects ($p<0.05$—$p<0.01$). Effects for the CR group were in the medium to large range (Cohen's $d=0.60$–0.91). Group-level analyses revealed increased ACC activation after CR during the MSIT in CR subjects but not controls, particularly in the anterior cingulate gyrus (Z (Gaussianised T/F) statistic images were thresholded using clusters determined by $Z>2.3$ and a (corrected) cluster significance threshold of $P=0.05$). Data suggest increased functional connectivity of the ACC to salience network regions after CR but not control.
Conclusions: These preliminary findings from a double-blind, placebo-controlled CR treatment suggest that neuroplasticity-based CR produces broad cognitive improvement in patients with BD. Additionally, CR but not an active control may increase BOLD activation in ACC during task, and may increase functional connectivity of the Salience Network, of which ACC is a key node. The generalizability of these changes to life activities is being studied.
Keywords: bipolar disorder; cogntitive remediation; cognitive training; anterior cingulate; psychosis.
Disclosures: K. Lewandowski, Nothing to Disclose; M. Keshavan, **Part 4:** grant from Sonovion; B. Cohen, Nothing to Disclose; S. Sperry, Nothing to Disclose; D. Ongur, **Part 1:** Scientific advisory board for Lily and Research support from Roche-Genentech.

T163. Protocol Complexity and Enrollment Eligibility Exclusivity: Are Today's Depression Study Volunteers Truly Representative?

Charles S Wilcox*, Judy L Morrissey, Nader Oskooilar, Mellissa M Henry, Daniel E Grosz, My-Linh Tong, Don F De Francisco

Pharmacology Research Institute, Los Alamitos, California

Background: In 2006 it was reported that up to 50% of depressed patients treated with first-line antidepressant

monotherapy do not reach full remission and as many as 33% become treatment resistant. In spite of a wide array of currently marketed antidepressants, including many available as low-cost generics, the need for more efficacious and/or more rapidly-acting antidepressants remains a strong one, as well as a viable commercial opportunity. The need to clinically and commercially differentiate new or forthcoming antidepressant candidates has resulted in an exponentially increasing trend toward heightened protocol complexity, extremely time-consuming clinical trial visits for study subjects and an ever-narrowing pool of potential clinical trial participants. Moreover, the unintended exclusivity associated with being truly eligible, sufficiently available, and willing to commit to the required time, raises the question of, just how truly representative are today's study volunteers?.

Methods: To strengthen the objectivity of our investigation, we chose to look at the most recent (circa: 2013—still blinded to their assigned 'active' *vs* 'placebo' treatment group) double-blind placebo-controlled outpatient antidepressant study participants. The study encompassed eight visits over a nine-to-thirteen week participation period, consisting of a one-to-four week washout phase, an eight-week double-blind treatment phase, a 50/50 randomization design, followed by a one-week double-blind down-taper period. One hundred two (102) subjects were screened for entry across the three Pharmacology Research Institute (PRI) outpatient clinics and eighty-two subjects (82) were randomized into the double-blind treatment phase. All of our statistical analyses pertained [only] to the randomized study subjects. Bivariate statistical analyses were performed on nine (9) baseline variables, including age, gender, race, Body Mass Index (BMI), prior antidepressant medication history, family psychiatric history, baseline Montgomery-Åsberg Depression Rating Scale (MADRS) scores, smoking status and single *vs* recurrent depressive episode.

Results: The 'top line' blinded study results indicated that 73 of the 82, or 89%, of the randomized subjects completed the study; this completion rate was slightly higher than anticipated. Also, 51.2% ($n = 42$) of the subjects were categorized as responders at endpoint, as defined by at least a 50% reduction from baseline to endpoint on their MADRS scores. Notably, 63.4% of the study participants were female; the female cohort had a slightly higher treatment response rate, 53.8 *vs* 46.7% amongst the males. There was a statistical trend toward higher response rates amongst Hispanic, 66.7% *vs* non-Hispanic 49.0% subjects ($p = 0.18$) and a similarly modest trend amongst smokers 63.6% *vs* non-smokers 46.7% ($p = 0.18$). In our statistical analysis of BMI as a baseline potential predictor, there was no correlation with compliance, retention or clinical outcomes; however, when changes in BMI were compared between 'responders' and 'non-responders' at endpoint, even after such a relatively short treatment period, it was evident that responders definitely gained weight with a statistically significant frequency ($p = 0.04$). A younger age at baseline was also statistically significantly correlated with a higher probability ($p = 0.02$) of a successfully therapeutic treatment response. The mean age for responders was 37 years *vs* 43 years of age for the non-responders.

Conclusions: From a retention and study completion perspective, outpatient depression study participants in 2013 are comparatively compliant with respect to keeping and completing their relatively frequent and time-consuming visits. While the weight gain experienced by the responders was both clinically and statistically significant, we certainly do not believe it is a function of the protocol eligibility criteria or 'representativeness' of the patient population. The fact that older aged participants demonstrated a lower response rate underscores the historical record of placebo-controlled 'geriatric antidepressant studies' rarely-if-ever demonstrating statistically significant results. Conversely, the fact younger aged participants demonstrated a more robust response rate clearly has potential placebo-response rate implications as well. The fact that our single center results included statistical trends relevant to differential response rates amongst Hispanics *vs* non-Hispanics, and smokers *vs* non-smokers, may be clinically relevant or an aberration; nonetheless, we believe these findings warrant further analyses within much larger sample sizes. In still blinded objective overview, our data analyses do not identify or highlight any alarming trends indicating that today's study participants are dangerously un-representative; however, we do believe that the protocol-mandated complexity and resultant exclusivity has been a key contributor to the soaring patient recruitment costs.

Keywords: depression, complexity, antidepressants, placebo-response.

Disclosures: C. Wilcox, **Part 1:** Calendar Years 2011 through Present, Pharmacology Research Institute has conducted clinical trials funded by the following pharmaceutical companies: Arteaus, Bristol-Myers Squibb, Eisai, Elan Pharmaceuticals, Forest Research, Genentech, Nabi Pharmaceuticals, Naurex, Pfizer, Shire, Sunovion, Takeda Global Research & Development and Vanda Pharmaceuticals.; J. Morrissey, Nothing to Disclose; N. Oskooilar, Nothing to Disclose; M. Henry, Nothing to Disclose; D. Grosz, Nothing to Disclose; M. Tong, Nothing to Disclose; D. De Francisco, Nothing to Disclose.

T164. Auditory Steady State Evoked Potential Abnormalities in Schizophrenia are Normalized by an mGluR2 Positive Allosteric Modulator

Bruce Turetsky*, Daniel Wolf, Christian Kohler, Mary March, Alan Cross, Mark Smith, Stephen R Zukin, Raquel E Gur

University of Pennsylvania Department of Psychiatry, Philadelphia

Background: High-frequency gamma oscillations (~30–60 Hz) represent the coordinated and integrated firing of local neural circuits. These are thought to underlie a host of important brain functions, including sensory afferent processing, perceptual integration and memory. Stimulus-evoked gamma synchrony is known to be disrupted in schizophrenia, and this has been related to abnormalities in working memory, cognitive control, and negative symptomatology. Mechanistically, gamma oscillations are generated by fast-spiking GABAergic interneurons, which produce rhythmic inhibitory postsynaptic potentials in associated pyramidal neurons. These GABAergic interneurons receive excitatory inputs through NMDA glutamate

receptors, suggesting that altered glutamate transmission may underlie the abnormalities in gamma activity commonly observed in schizophrenia. Agents designed to ameliorate the putative deficit in NMDA signaling may therefore also be expected to have an impact on aberrant gamma oscillations in schizophrenia patients. As part of a Phase I pilot study (NCT00986531) we examined the effects of an mGluR2 positive allosteric modulator (PAM) AZD8529 on auditory steady-state evoked potential measures of gamma synchrony. We hypothesized that enhancement of glutamate signaling would attenuate this schizophrenia deficit.

Methods: Subjects with complete and usable electrophysiology data included 25 patients (9 female; mean age 41.8 yrs) with DSM-IV diagnosed schizophrenia on stable dosages of antipsychotic medications, stably treated with antipsychotics. In a double-blind, placebo-controlled, counterbalanced within-subject crossover design, patients received three days of treatment with either AZD8529 (80 mg once-daily) or placebo, after which they were clinically assessed and underwent both EEG and cognitive neuropsychological testing. The washout period between the drug and placebo phases of the study was 14 days. A comparison sample of 25 healthy individuals (9 female; mean age 38.4 yrs) underwent an equivalent single EEG test session without any pharmacologic intervention. As part of a more comprehensive EEG test battery, subjects listened to auditory click stimuli presented at 20, 30 and 40 Hz in three separate blocks. Each block was 3.5 min long and consisted of 150 70 dB SPL auditory click trains, each lasting 500 ms with an inter-train interval of 800 ms. The order of the 3 blocks was randomized across subjects and sessions. EEG data for the 500 ms stimulation intervals were averaged for each block and subjected to a Fast Fourier Transformation (FFT). EEG power at the Fz electrode for the resonant frequency synchronized to auditory stimulation rate was the dependent measure. Drug effects were examined within patients and patient-control differences were assessed for patients on both active drug and placebo.

Results: As expected, 40 Hz auditory stimulation produced a greater synchronized EEG response that 20 or 30 Hz stimulation. Comparing patients on placebo to healthy controls, there was a significant group X stimulation frequency interaction [$F(2, 96) = 4.13$, $p = 0.04$]. The patients exhibited relatively less power at 40 Hz and more power at 20 Hz. Within the patient sample, there was a significant drug X frequency interaction [$F(2, 46) = 4.93$, $p = 0.02$]. AZD8529 increased 40 Hz power and decreased 20 Hz power relative to placebo. Consistent with this within-patients drug effect, when patients on the active agent were compared to controls, the previously observed group X frequency interaction was no longer significant [$F(2, 96) = 0.39$, $p = 0.63$]. The medication-induced change in the SSEP response was not related to the plasma concentration of AZD8529 or to baseline levels of clinical symptomatology. There were no significant differences in either clinical symptomatology or cognitive performance between placebo and active drug sessions, and no significant correlations between the change in SSEP response and change in clinical or cognitive measures.

Conclusions: A brief 3-day treatment of the mGlurR2 PAM produced no significant improvement in either clinical symptomatology or cognitive performance. However, it did effectively normalize schizophrenia patients' aberrant auditory steady-state responses. Specifically, it enhanced their ability to synchronize their EEG to 40 Hz stimulation, while reducing their tendency to synchronize more at an 'every other cycle' stimulation rate (20 Hz). This is consistent with more optimal 'tuning' of the neural circuitry underlying 40 Hz gamma band oscillations. It indicates that AZD8529 had a positive neurophysiological effect even if, in this very short trial, it did not result in more overt behavioral change. Further investigation of mGluR2 PAMs as potential adjunctive treatments in schizophrenia may be warranted. This study also demonstrates, more broadly, that EEG measures can serve as sensitive indicators of target engagement within the CNS.

Keywords: Gamma Oscillations, mGluR2 positive allosteric modulator, schizophrenia, auditory steady-state evoked potentials.

Disclosures: B. Turetsky, **Part 4:** Research grant support from AstraZeneca and Pfizer Pharmaceuticals.; D. Wolf, **Part 4:** Research grant support from AstraZeneca and Pfizer Pharmaceuticals.; C. Kohler, **Part 4:** Research grant support from AstraZeneca and Pfizer Pharmaceuticals.; M. March, **Part 4:** Research grant support from AstraZeneca and Pfizer Pharmaceuticals.; A. Cross, **Part 5:** AstraZeneca Pharmaceuticals; M. Smith, **Part 5:** Former employer: AstraZeneca Pharmaceuticals, Current employer: Shire Pharmaceuticals; S. Zukin, **Part 5:** Former employer: AstraZeneca Pharmaceuticals, Current employer: Forest Research Institute; R. Gur, **Part 4:** Research grant support from AstraZeneca and Pfizer Pharmaceuticals.

T165. Effects of Neurokinin 1 Receptor Antagonism on Brain Response to Emotional Visual Stimuli in Co-morbid Alcohol Dependence and Posttraumatic Stress Disorder

Primavera Spagnolo*, Laura Kwako, Reza Momenan, Melanie L Schwandt, Vijay A Ramchandani, Daniel W Hommer, David T George, Markus Heilig

NIH, Bethesda, Maryland

Background: Alcoholism is highly co-morbid with posttraumatic stress disorder (PTSD), suggesting the possibility that they share overlapping neural substrates. Specifically, functional neuroimaging studies have implicated the amygdala, hippocampus, and medial prefrontal cortex (mPFC) in the psychopathology associated with PTSD (Bremner, 2007). Similar to PTSD, excessive voluntary alcohol consumption and stress-induced relapse to alcohol seeking are also associated with up-regulated amygdala function and altered mPFC function. Previously, we have reported that neurokinin 1 receptor (NK1R) antagonism significantly decreased insula activation in response to negative sensory input, and also attenuated alcohol cravings and cortisol response to stress in alcohol-dependent patient (George *et al*, 2008). Since Substance P (SP), together with its preferred NK1R, has been implicated in both stress- and alcohol-related behaviors, we carried out the present study to examine the effects of aprepitant, an oral, brain-penetrant NK1R antagonist on PTSD symptoms, stress-

induced alcohol craving and brain response to emotional visual stimuli in alcohol dependent patients with PTSD.
Methods: Fifty-three patients with PTSD and alcoholism were admitted for 4 weeks to an experimental medicine unit at the NIH Clinical Center, and randomized to double-blind aprepitant (125 mg/day; based on PET studies reporting >90% central receptor occupancy [RO] at this dose) or placebo. After reaching steady state, subjects were assessed for PTSD symptom severity; behavioral and neuroendocrine responses to stress and alcohol cues; and fMRI responses to stimuli with positive or negative emotional valence.
Results: Aprepitant treatment had no effect on PTSD symptoms or subjective or physiological responses to stress or alcohol cues. However, this treatment robustly potentiated ventromedial prefrontal cortex (vmPFC) fMRI responses to aversive visual stimuli. In particular, there was a significant main treatment effect on an area including both right and left vmPFC BOLD activation in response to negative IAPS pictures (negative—neutral). The aprepitant group showed significantly higher bilateral activation than the placebo group (whole brain corrected $p = 0.01$; cluster volume: $8 \times 275/1000$ ml; cluster size: 5). There were no significant effects of treatment on neural activation in response to fearful *vs* neutral faces, or alcoholic *vs* non-alcoholic beverages.
Conclusions: Neuroimaging studies have generally shown that PTSD patients compared with non-PTSD subjects showed decreased Ventral mPFC activation in response to negative stimuli. Here, we discovered an unexpected effect of NK1 antagonism to activate the vmPFC during exposure to aversive visual stimuli. Because this brain area is critically important for extinction of fear memories and in alcohol craving and relapse risk, our finding suggests that NK1 antagonism, rather than acutely attenuating stress responses, might be a useful pharmacological treatment to enhance the effect of extinction-based therapy. In spite of the activation of vmPFC, we did not detect any signal for a treatment effect on PTSD symptoms and stress-induced alcohol craving. It is possible that pathophysiology of patients with co-morbid PTSD and alcoholism differs from that of patients with alcoholism only, and that stress-induced cravings are induced through different mechanisms in these two populations. Alternatively, the negative findings may be related to the use of an insufficient drug dose. Higher aprepitant doses or more potent NK1 antagonists may merit further evaluation.
Keywords: PTSD, alcoholism, NK1 antagonism, vmPFC.
Disclosures: P. Spagnolo, Nothing to Disclose; L. Kwako, Nothing to Disclose; R. Momenan, Nothing to Disclose; M. Schwandt, Nothing to Disclose; V. Ramchandani, Nothing to Disclose; D. Hommer, Nothing to Disclose; D. George, Nothing to Disclose; M. Heilig, Nothing to Disclose.

T166. Baseline Characteristics that Result in Higher Placebo on the MCCB Using Regression Analysis

George Haig*, Earle Bain

AbbVie Inc., North Chicago, Illinois

Background: The MATRICS Consensus Cognition Battery (MCCB) is the regulatory-endorsed standard endpoint measure used in clinical trials of medications for the treatment of Cognitive Impairment Associated with Schizophrenia (CIAS). Performance scores of the MCCB in the schizophrenia population are normally distributed and scoring is based on a t-distribution. The average score on the MCCB in the normal population is 50, with a standard deviation of 10. Average scores for patients with schizophrenia are around 28. Previous large clinical trials assessing the procognitive effect of medications in CIAS reported change scores in the placebo group ranging from 1 to 2 points. The objective of this report is to identify baseline characteristics in a patient population that predict a higher placebo response (or practice effects).
Methods: Two multicenter trials in stable subjects with schizophrenia were conducted at 45 sites in the United States. Both investigated the procognitive effects of novel investigational compounds as add-on therapy to antipsychotics. The same design was used in both trials, with 1 placebo and 2 active dose groups (planned $N = 70$/group; total $N = 420$) in each. Study medication was administered once daily. Trial duration was 12 weeks; clinic visits were every 2 weeks. The MCCB was administered 4 times throughout the trial: Screening (−4 weeks), Baseline, Week 6, and Week 12. Descriptive statistics were used for univariate analysis of the effect of baseline and demographic variables on the placebo response. Step-wise logistic regression analysis was used to evaluate a number of baseline and demographic variables on the placebo response.
Results: Between two studies, 421 subjects were randomized. The completion rate was approximately 80%. The dataset included 132 subjects in placebo groups. The mean change in MCCB score in placebo patients at last assessment was 0.9 and 2.0 points in Trial 1 and Trial 2, respectively, with a pooled placebo response of 1.4. Baseline characteristics with a 1 point or more difference in placebo response were: race (white 0.9, black 1.8), caregiver (parent 0.5 *vs* group home 1.6 or roommate 5.1), living situation (lives with family 0.9 *vs* lives independently 1.9), site as the primary care provider (yes 0.6 *vs* no 1.8). None of these differences were statistically significant. The placebo response was not correlated with age, age of diagnosis, baseline NSA score, baseline PANSS score or baseline MCCB (rho < 0.1 for each).
Conclusions: The placebo response on the MCCB was fairly consistent across a wide range of patient baseline characteristics, symptom scores, and cognition level. None of the factors examined predicted differences.
Keywords: cognitive impairment associated with schizophrenia; clinical trials; MCCB.
Disclosures: G. Haig, **Part 1:** Employee of AbbVie Inc.; owns AbbVie stock, **Part 2:** Employee of AbbVie Inc.; owns AbbVie stock, **Part 3:** Employee of AbbVie Inc.; owns AbbVie stock, **Part 5:** AbbVie Inc.; E. Bain, **Part 1:** Employee of AbbVie Inc.; owns AbbVie stock, **Part 2:** Employee of AbbVie Inc.; owns AbbVie stock, **Part 3:** Employee of AbbVie Inc.; owns AbbVie stock, **Part 5:** AbbVie Inc.

T167. Long-term Followup of Gamma Capsulotomy for Intractable OCD

Steven Rasmussen*, Benjamin Greenberg

Brown University, Providence, Rhode Island

Background: We report the results of the first systematic prospective twenty year followup study of the efficacy and

safety of ventral capsular/ventral striatal (VC/VS) gamma capsulotomy in the treatment of 55 severely ill intractable obsessive-compulsive patients, collected over a ten year period. In the intitial phase, a single bilateral lesion in the anterior limb of the internal capsule that carried the greatest number of afferent and efferent fibers connecting the medial and orbital frontal cortex and the midline thalamus was made in fifteen patients (Single-Shot). Thirteen of these patients went on to have a second stage lesion at an average of nine months from the first surgery due to lack of efficacy of the first procedure (Single-Shot Repeated). Forty additional patients were subsequently treated with two shots bilaterally on one day (Double-Shot). Probands were followed up at 3, 6, 12 and 24, 60, 120 and 240 months following their initial procedure. Clinician ratings were obtained from patients and corroborated with interviews with family members. Followup assessment included a psychiatric assessment, neuropsychologic testing, neurologic exam, followup MRI, as well as the YBOCS, CGI, HDRS, HARS, GAF, SIP and SAFTEE measures.

Methods: The Single-Shot group received 180 Gray to bilateral targets defined by points that were in the center of the capsule one-third of the way up the length of capsule from its most ventral extension in the coronal plane. The second stage lesion (Single-Shot Repeated) was placed immediately ventral to the first stage lesion and in the center of the capsule in the coronal plane bilaterally. The lesion was consistently placed 8–10 mm from the posterior border of the anterior commissure.

Results: At an average followup of 9months after the first stage single-shot procedure, only one out of fifteen patients met our criteria as a responder or as a partial responder. As a group, these 15 patients did not improve significantly on the YBOCS, CGI or GAF either at six month followup or at endpoint of the first stage procedure. There was also no significant improvement in measures of anxiety (HARS) or depression (HDRS). Thirteen of fifteen patients went on to have a second stage procedure. At one year followup of the single-shot repeated cohort 5 of 13 met criteria for being a responder and two additional patients were partial responders. The 13 patients who had failed to respond to the single stage procedure improved significantly from baseline at the 6, 12 and 24, 60 120 and 240 month timepoints on the YBOCS. The average reduction in YBOCS score was 9.1 ± 1.8 leaving the average patient in the moderately symptomatic range (1–3 h of symptoms per day with moderate impairment in function). There was a significant improvement in global function at the 12, 24,60.120 1nd 240 month followup. The double-shot group also showed significant improvements compared to the single-shot repeated lesions at all timepoints. Categorical analysis of the second shot group revealed that 13 (59%) met the responder criteria at one year with an additional five patients (23%) meeting criteria for partial response. These gains were maintained at all subsequent time points with 15 (69%) meeting criteria for full responders and five (23%) meeting criteria as partial responder at two years. Three patients suffered depressive episodes during the followup period requiring hospitalization but there were no relapses of OCD. Patients completed neuropsychological assessments before and after gamma ventral capsulotomy. There were no patterns of pervasive cognitive decline in any patient. Preliminary within-subjects analyses for initial (approximately 9 month) and long-term follow-up showed no group decline. Brain cysts have developed after a long delay (3–5 years post radiosurgery) in three of a total of 55 patients after gamma ventral capsulotomy. Two patients were asymptomatic. In the third, neurological symptoms including headache, dizziness, and visual changes required surgical cyst drainage, on two occasions. All three cysts occurred after double-shot radiosurgery with a newer-generation gamma knife. Other side effects included cerebral edema and headache that necessitated short term treatment with dexamethasone in 18% of the patients and a frontal syndrome with apathy and amotivation in one patient.

Conclusions: The overall results demonstrate that gamma ventral capsulotomy is highly effective in reducing OC symptoms of severely ill treatment refractory patients as measured by either continuous or categorical measures of the YBOCS at 6, 12 and 24 month followup *vs* baseline for those patients who received two bilateral lesions in the ventral capsule/ventral striatum. Highly significant reductions in comorbid depression and anxiety were also observed. Global function and quality of life were also significantly improved compared to baseline. There were no patients in either the Single-Shot or Double-Shot cohort who failed to respond at one year who went on to respond subsequently at two-year followup. Although not the subject of this report, we have now had the opportunity of following all of the patients in the Single-Shot Repeated group and 15 of the patients in the Double-Shot group out to five years. None of the responders have relapsed during the two to five year followup period. Instead, we have seen continued gradual improvement in symptoms and function in all responders.

Keywords: OCD neurosurgery safety efficacy follow-up.

Disclosures: S. Rasmussen, Nothing to Disclose; B. Greenberg, Nothing to Disclose.

T168. N-Acetylcysteine for the Treatment of Non-suicidal Self-injurious Behavior in Adolescents: A Preliminary Study

Kathryn R Cullen*, Bonnie Klimes-Dougan, Lori LaRiviere, Alaa Houri, Melinda Westlund, Bernard Lim, Ana Bortnova, Katharine Nelson, Michael J Miller, S Charles Schulz, Bryon Mueller, Lynn Eberly, Kelvin O Lim

University of Minnesota Medical School, Minneapolis, Minnesota

Background: Non-suicidal self-injury (NSSI) is a serious problem that has been increasing in prevalence in adolescents world-wide. Very few treatment options are available for this difficult problem. N-acetylcysteine (NAC), a derivative of the amino acid cysteine, is a safe, widely-available compound that has been tested as a treatment for several psychiatric disorders. In particular, NAC has been shown to be helpful in disorders that involve impulsive and compulsive behaviors such as hair-pulling, skin picking and addiction. Conceptualizing NSSI as a related, habitual behavior, this study sought to test the efficacy of NAC in

reducing NSSI in adolescents in an 8-week, open-label, pilot clinical trial.

Methods: *Oversight*. The research protocol was approved by the University of Minnesota institutional review board for protection of human subjects and the Federal Drug Administration (IND# 10345) and is registered under ClinicalTrials.Gov. *Participants*. Adolescents aged 13–21 with NSSI (at least 4 lifetime episodes, most recent episode occurring in the past 2 months) were recruited into the study. No psychiatric diagnoses were required for study entrance, but a diagnosis of autism, schizophrenia, bipolar disorder or mental retardation was exclusionary. Age- and sex-matched healthy controls with no history of NSSI or any psychiatric history were also recruited. *Clinical evaluation* included the Kiddie Schedule for Affective Disorders and Schizophrenia (ages 17 and under) or the Structured Interview for DSM-IV (ages 18–21). Assessment of NSSI included the Deliberate Self Harm Inventory and the Deliberate Self Harm Questionnaire: Part III Mood (DSHQ-M) and the Inventory of Statements About Self-Injury (ISAS). Other clinical measures include the Difficulties in Emotion Regulation Scale (DERS), the Toronto Alexithymia-Scale (TAS), the Barratt Impulsivity Scale (BIS), the Symptom Checklist-90 (SCL-90), and the Personality Assessment Inventory (PAI). *Treatment Schedule*: NAC was initiated at a dose of 600 mg taken orally twice daily for 2 weeks, then was increased to 1200 mg twice daily, and finally increased to 1800 mg twice daily for the remaining 4 weeks. *Visit Schedule*: Participants were seen every 2 weeks throughout the study, at which time they completed the Deliberate Self Harm Inventory, Clinical Change Version. The *primary outcome measure* was defined as the number of episodes of NSSI per 2 weeks as measured by the Deliberate Self Harm Inventory. Two baselines were defined: the average number of episodes per 2 weeks in the 2 months prior to the study, and the average number of NSSI episodes per 2 weeks in the past year. *Statistical Analysis*: Between-group differences in demographic and clinical characteristics were conducted using independent *t*-tests and chi-squared tests. To measure change in NSSI due to treatment, paired-sample *t*-tests were conducted comparing the average number of injuries per 2 weeks in the 2 months prior to the study with the number of injuries in the 2 final weeks of the study. Additionally, paired *t*-tests were conducted on all available pre-post clinical measures.

Results: Participants included 17 adolescents (16 female) with NSSI (mean age = 17.6, S.D. = 2.3) and 7 controls (all female; mean age = 18.1, S.D. = 2.5). In patients, the average number of self-injury episodes in the 2 months prior to the study was 19. Ten participants completed NAC treatment. Among those, 3 were medication-free at the start of the study and 7 were taking a stable dose of one or more psychiatric medication prior to starting the study. Compared to controls, the NSSI group consistently showed greater levels of symptomatology. NSSI adolescents were significantly different from controls on total BDI scores ($p < 0.001$), BIS total (0.02) and motor (0.008) impulsivity, DERS total ($p < 0.001$), the anxious, depressed, paranoid and borderline scales from the PAI ($p < 0.05$), all SCL-90 measures ($p < 0.01$), and TAS total ($p < 0.001$). Paired *t*-tests to assess for change in rate of NSSI episodes revealed a significant decrease from an average of 4.5 episodes per 2 weeks in the two months prior to the study to an average of 0.5 episodes in the 2 weeks at the end of the study ($p = 0.03$). Paired sample *t*-tests on clinical measures did not show a significant difference in overall BDI scores ($p = 0.2$); however, exploratory tests of the BDI subscales showed significant improvement in sense of past-failure ($p = 0.02$), loss of pleasure, ($p = 0.05$), and suicidal thoughts ($p = 0.08$). Among SCL-90 measures, the only significant change was a decrease in somatization ($p = 0.04$). There were no significant changes in impulsivity as measured by the BIS.

Conclusions: We report preliminary evidence that NAC may be effective in treating NSSI in adolescents. Limitations of this study include the small sample and the open-label design. Additionally, in this sample, NAC was used both as mono-therapy and as an add-on supplementary therapy. Future work should incorporate a larger sample and a randomized, placebo-controlled design, with incorporation of subgroups to test NAC efficacy as a mono-therapy and as an adjunct therapy.

Keywords: adolescent self-injury self-harm N-acetylcysteine treatment.

Disclosures: K. Cullen, Nothing to Disclose; B. Klimes-Dougan, Nothing to Disclose; L. LaRiviere, Nothing to Disclose; A. Houri, Nothing to Disclose; M. Westlund, Nothing to Disclose; B. Lim, Nothing to Disclose; A. Bortnova, Nothing to Disclose; K. Nelson, Nothing to Disclose; M. Miller, Nothing to Disclose; S. Schulz, **Part 1:** Genentech, Eli Lilly, **Part 4:** Astro Zeneca, Myriad/RBM, NIMH, Otsuka; B. Mueller, Nothing to Disclose; L. Eberly, Nothing to Disclose; K. Lim, Nothing to Disclose.

T169. Phase 2 Evaluation of ITI-007, a Novel Approach to the Treatment of Schizophrenia

Kimberly E Vanover*, Sharon Mates, Paul Greengard, Robert E Davis

Intra-Cellular Therapies, Inc., New York, New York

Background: Through synergistic actions via serotonergic, dopaminergic and glutamatergic systems, ITI-007 represents a novel approach to the treatment of schizophrenia and other neuropsychiatric disorders. ITI-007 is a potent antagonist at 5-HT_{2A} receptors, a mesolimbic/mesocortical dopamine phosophoprotein modulator (DPPM) with activity as a pre-synaptic partial agonist and post-synaptic antagonist at dopamine D_2 receptors, a mesolimbic glutamate GluN2B receptor phosphoprotein modulator and a serotonin reuptake inhibitor. This unique pharmacological profile is predicted to translate clinically, in a dose dependent manner, to broad antipsychotic efficacy for the treatment of positive and negative symptoms with improved cognition, affective symptoms, and sleep. Previous clinical data have shown that low doses of ITI-007 improve sleep maintenance while high doses elicit clinical signs consistent with improvement in the symptoms of schizophrenia. ITI-007 also is safe across a broad range of doses in healthy volunteers, patients with primary insomnia and patients with schizophrenia. The present randomized, double-blind,placebo- and active-controlled clinical study was designed to evaluate the efficacy and safety of ITI-007 in patients with acute schizophrenia.

Methods: Patients with an acutely exacerbated episode of schizophrenia were randomized to receive one of four treatments: 60 mg ITI-007, 120 mg ITI-007, 4 mg risperidone (positive control) or placebo in a 1 : 1 : 1 : 1 ratio. Patients received study treatment orally once daily in the morning for 28 days. The primary endpoint was change from baseline on the total Positive and Negative Syndrome Scale (PANSS) on study Day 28. Secondaryendpoints included weekly assessments of the total PANSS as well as its subscales and five factors. Safety and tolerability were assessed. An interim analysis of the data was planned *a priori* to be conducted after approximately 30 patients per treatment arm had completed 28 days of treatment. The goal of the interim was to validate the study assumptions on treatment effect of ITI-007 compared to placebo. Based on the results of the interim analysis, the trial may have proceeded as planned, been terminated prematurely or modified (eg, terminating one of the treatment arms).
Results: The interim analysis was conducted with 126 patients included in the intent-to-treat analysis. The results of the interim analysis indicated that the study assumptions were validated and the study was continued as planned. Demonstrating assay sensitivity, risperidone exhibited an effect size of 0.4 on total PANSS. ITI-007 exhibited an effect size on the total PANSS ranging from 0.4 to 0.5 depending on dose. Both ITI-007 and risperidone exhibited effect sizes on improving the positive symptom subscale in the 0.4–0.5 range. In contrast, only ITI-007, especially at the 60 mg dose, improved negative symptoms with an effect size of 0.4 on the negative symptom subscale and 0.63 on the negative symptom factor, whereas risperidone did not improve negative symptoms with an effect size of 0.02 on negative symptom subscale and 0.19 on negative factor. At the interim, ITI-007 was safe and well-tolerated in patients with acute schizophrenia; there were no drug-related serious adverse events. Approximately 80 patients per treatment arm are planned for the final analysis. Clinical conduct has been completed and final results will be available in December 2013.
Conclusions: The results from the interim analysis of a Phase 2 study in acute schizophrenia demonstrate the potential for broad antipsychotic efficacy of ITI-007. Moreover, the data suggest differential response on positive and negative symptoms by ITI-007 compared to risperidone. ITI-007 represents a novel approach to the treatment of schizophrenia and other neuropsychiatric and neurological disorders.
Keywords: ITI-007, schizophrenia, antipsychotic, positive symptoms, negative symptoms.
Disclosures: K. Vanover, **Part 1:** Intra-Cellular Therapies, Inc., **Part 2:** Intra-Cellular Therapies, Inc., **Part 3:** Intra-Cellular Therapies, Inc., **Part 5:** Intra-Cellular Therapies, Inc.; S. Mates, **Part 1:** Intra-Cellular Therapies, Inc., **Part 2:** Intra-Cellular Therapies, Inc., **Part 3:** Intra-Cellular Therapies, Inc., **Part 5:** Intra-Cellular Therapies, Inc.; P. Greengard, **Part 1:** Intra-Cellular Therapies, Inc., **Part 2:** Intra-Cellular Therapies, Inc., **Part 3:** Intra-Cellular Therapies, Inc.; R. Davis, **Part 1:** Intra-Cellular Therapies, Inc., **Part 2:** Intra-Cellular Therapies, Inc., **Part 3:** Intra-Cellular Therapies, Inc.

T170. Modafinil for the Treatment of Cocaine Dependence

Kyle M Kampman*, Jennifer G Plebani, Kevin G Lynch, Helen M Pettinati, Elizabeth Mahoney, Mary Slome, Margo Hendrickson, Charles P O'Brien

University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

Background: Background: Modafinil is a medication approved for narcolepsy and shift work sleep disorder. It has both dopaminergic and glutamatergic activity that could be useful for the treatment of cocaine dependence. Modafinil has reduced cocaine subjective effects in human laboratory trials, reduced cocaine self-administration in human laboratory trials and has reduced cocaine use in cocaine dependent patients in some clinical trials.
Methods: Methods: This was an 8-week, double blind, placebo controlled parallel group clinical trial involving 94 DSM IV cocaine dependent subjects without concurrent alcohol dependence. Subjects received 300 mg of modafinil or identical placebo each day along with weekly individual cognitive behavioral relapse prevention psychotherapy. The primary outcome measure was cocaine use measured by self-report, and confirmed by twice weekly urine drug screens. Additional outcome measures included cocaine craving measured by the Brief Substance Craving Scale (BSCS), cocaine withdrawal symptoms measured by the Cocaine Selective Severity Assessment (CSSA) and global improvement measured by the Clinical Global Impression Scale (CGI). Urine drug screen results, CGI improvement and BSCS scores were analyzed using generalized estimating equations with treatment and study week as covariates.
Results: Results: The odds ratio (OR) in favor of abstinence for modafinil *vs* placebo was 2.28 ($p = 0.038$) and modafinil-treated subjects were significantly more likely than placebo-treated subjects to be abstinent from cocaine during the last 3 weeks of the trial, 23 *vs* 9%, $\chi^2 = 3.9$, $p < 0.05$. Cocaine craving frequency scores from the BSCS were lower in modafinil-treated subjects compared to placebo-treated subjects (OR = 1.28, $p = 0.03$) and modafinil-treated subjects were significantly more likely than placebo-treated subjects to report no craving (OR = 2.09, $p = 0.025$). Modafinil–treated subjects were also more likely than placebo-treated subjects to rate themselves as 'very much improved' on the CGI (OR = 2.15, $p = 0.02$). Subjects who entered the trial with more severe cocaine withdrawal symptoms (higher CSSA scores) appeared to benefit more from modafinil. Among subjects who started study medication with CSSA scores in the highest tertile (above 13), the odds ratio favoring abstinence in modafinil-treated subjects *vs* placebo-treated subjects during the trial was 6.9, $p < 0.001$.
Conclusions: Discussion: In this trial, modafinil treatment was associated with reduced cocaine use, reduced cocaine craving and greater self-reported overall improvement in cocaine dependent subjects. Cocaine dependent subjects with more severe cocaine withdrawal symptoms appeared to have benefited more from modafinil. This trial is the third of four placebo controlled clinical trials in which modafinil was associated with reduced cocaine use in cocaine dependent subjects without concurrent alcohol dependence.

Keywords: cocaine, clinical trial, modafinil, placebo.
Disclosures: K. Kampman, Nothing to Disclose; J. Plebani, Nothing to Disclose; K. Lynch, Nothing to Disclose; H. Pettinati, Nothing to Disclose; E. Mahoney, Nothing to Disclose; M. Slome, Nothing to Disclose; M. Hendrickson, Nothing to Disclose; C. O'Brien, Nothing to Disclose.

T171. Personality Predicts Dropout and Placebo Response Risk in Patients with Bipolar Depression

Gary S Sachs*, Cynthia Siu, Josephine Cucchiaro, Robert Silva, Fred Grossman, Jay Hsu, Amir Kalali, Antony Loebel

Massachusetts General Hospital, Lincoln, Massachusetts

Background: Early dropouts and placebo response present major challenges to CNS drug development and likely contribute to the increased risk of failure in psychopharmacological trials. Previous research has demonstrated a consistent relationship between personality measures of neuroticism and treatment outcomes in anxiety and depression trials (Barnett *et al*, 2012; Costa and McCrae, 1992). The objective of this post-hoc analysis was to identify personality and clinical factors predictive of dropouts and placebo response in a multiregional, randomized, 6-week, double-blind, placebo-controlled study of lurasidone for the treatment of bipolar I depression.
Methods: Subjects meeting DSM-IV-TR criteria for bipolar I depression, with or without rapid cycling, a Montgomery-Asberg Depression Rating Scale (MADRS) score $\geqslant 20$ and a Young Mania Rating Scale (YMRS) score $\leqslant 12$, were randomized to 6 weeks of once-daily, double-blind treatment with lurasidone 20–60 mg (LUR20–60), lurasidone 80–120 mg (LUR80–120), or placebo (PBO). Personality traits were assessed by the 60-item NEO Five-Factor Inventory (NEO-FFI) which includes Neuroticism (N), Extraversion (E), Openness to experience (O), Agreeableness (A), and Conscientiousness (C). Other predictors considered were age, gender, duration of illness, baseline symptom severity, and study countries/regions. Placebo response was evaluated by the mean change from baseline to week 6 (LOCF) in MADRS total score and responder analysis. Logistic regression was applied to investigate the predictive relationships.
Results: Baseline distributions of the personality T-scores showed a majority of patients with bipolar I depression had high or very high Neuroticism (N, 86%), but low or very low T-scores in Extraversion (E, 81%), Openness to experience (O, 51%), Agreeableness (A, 84%), or Conscientiousness (C, 86%). A high level of Neuroticism combined with low levels of Extraversion, Openness, Agreeableness or Conscientiousness were associated with favorable outcomes of lurasidone monotherapy ($p<0.05$). Dropout rates for lurasidone 20–60 mg (26%), lurasidone 80–120 mg (27%), and placebo (25%) were comparable. Higher dropout was predicted by shorter illness duration ($p<0.05$), country ($p<0.05$), lower Agreeableness ($p<0.05$) especially 'hard-headed in attitude' ($p<0.05$; NEO item 44), higher Extraversion ($p<0.05$), 'don't like to be where the action is' ($p<0.05$; NEO item 22), and 'often feel tense and irritable' (NEO item 21, a Neuroticism item). Predictive accuracy associated with the prediction model was 0.74 (AUC ROC). Gender, baseline MADRS total score, Neuroticism and Openness factor scores were not significant predictors for dropout. The proportions of subjects responding to placebo were 47 and 30%, respectively, for 30 and 50% reduction in MADRS total score from baseline to Week 6 (LOCF). Higher Conscientiousness (C) level, less Agreeableness (NEO items 14 for 'high egotistical' and 49 for 'less consideration'), low energy (NEO item 32), and country were significant predictors for both MADRS response criteria. Placebo response based on 30% cutoff was more likely to occur for higher Openness (ie disagree or strongly disagree NEO item 8 'Once I find the right way to do something, I stick to it' in the questionnaire). For the more stringent 50% response criterion, higher baseline Neuroticism was a significant predictor of placebo response. Predictive accuracy was 0.74 and 0.73, respectively, for 30 and 50% placebo response prediction model.
Conclusions: Our findings suggest the personality measures of lower Agreeableness (A) and higher Extraversion (E), tense and irritable, less chronically ill and country were significant predictors of early dropouts. Personality factors of higher Conscientiousness (C), less Agreeableness ('high egotistical' and 'less consideration'), low energy, and country were significantly associated with a higher placebo response. These results indicate that patient profiles based on the NEO Personality Inventory may be useful to screen out subjects susceptible to high dropout and/or placebo response, and thus improve the likelihood of detecting a treatment signal in bipolar depression trials.
Keywords: bipolar depression, personality NEO factors, dropouts, placebo response.
Disclosures: G. Sachs, **Part 1:** Grant: NIMH, Repligen, Honoraria: AstraZeneca, BMS, Otsuka, Pfizer, Shares: Express Scripts, Paid positions: bracket, Massachusetts General Hospital, Advisory Boards: AstraZeneca, BMS, Otsuka, Pfizer, Janssen, Merck, Sunovion, Takeda, Teva, **Part 4:** Grant: NIMH, Repligen; C. Siu, **Part 1:** Consulting: Pfizer, Sunovion Pharmaceuticals, **Part 2:** Sunovion Pharmaceuticals; J. Cucchiaro, **Part 5:** Sunovion Pharmaceuticals; R. Silva, **Part 5:** Sunovion Pharmaceuticals; F. Grossman, **Part 5:** Sunovion Pharmaceuticals; J. Hsu, **Part 5:** Sunovion Pharmaceuticals; A. Kalali, ; A. Loebel,.

T172. Sleep Architecture Abnormalities as a Risk Factor for Elevated Suicidal Ideation: A Polysomnographic Investigation of Sleep in Treatment Resistant Unipolar and Bipolar Depression

Rebecca Bernert*, David Luckenbaugh, Wallace C Duncan, Carlos A Zarate

Stanford University School of Medicine, Stanford, California

Background: Suicide is a preventable public health problem and global disease burden, currently accounting for 57% of all violent deaths worldwide annually. Sleep complaints are listed among the top 10 warning signs of suicide by SAMHSA, and emerging findings indicate that disturbed sleep is an evidence-based risk factor for suicide outcomes. Across investigations—diverse in design, samples, and

methodologies—research indicates that subjectively-assessed sleep complaints confer elevated risk for suicidal ideation, suicide attempts, and death by suicide. Despite support for its utility as a treatment target and possible biomarker of antidepressant treatment response, electroencephalogram (EEG) sleep parameters have yet to be evaluated in association with suicide risk in treatment resistant depression (TRD).
Methods: The present EEG sleep substudy was conducted in the pretreatment period within a larger investigation of treatment maintenance following ketamine infusion for TRD. 54 patients were studied who met criteria for treatment resistant unipolar ($N=30$ MDD) and bipolar ($N=24$ BD) depression. All patients completed a 2–5 week washout period, and were free of psychotropic drugs prior to sleep assessments, with the exception of mood stabilizers among BD patients. Following a 1-night adaptation, polysomnography (PSG) sleep recordings assessed the following sleep statistics: sleep efficiency (SE), wake after sleep onset (WASO), REM Percent, REM Latency, REM Onset, and NREM Sleep Stage (1–4) Percent. Sleep staging and scoring was performed according to established criteria by trained reviewers who were blind to the protocol design and randomization procedures. Suicidal ideation (SI) symptoms were assessed according to HDRS Item 3 (Scores 0–4). Independent Samples t-Tests were performed to test the exploratory hypothesis that EEG sleep parameters would distinguish patients with *vs* without SI, with greater sleep architecture abnormalities expected among those endorsing current suicidal symptoms.
Results: Mean age among patients was 45.9 (SD = 12.5) years, and 50% of the sample was male. Descriptive statistics revealed indices of SE within the normal range (M = 87.3%), yet highly restricted sleep among both diagnostic groups (TST M = 6.0 h for BD, 6.25 h for MDD patients). For both groups, REM latency appeared increased among those with current SI. Correlations between HDRS SI and sleep variables appeared somewhat distinct by diagnosis, with the exception of reduced NREM Stage 4 Percent, which predicted greater SI among both groups ($r=-0.31$, $p=0.02$). Within BD, elevated SI was associated with greater NREM Stage 1 Percent ($r=0.47$, $p=0.02$) and increased WASO ($r=0.65$, $p<0.01$). Within MDD, NREM Stage 1 Percent was negatively correlated with SI ($r=-0.38$, $p=0.03$). In support of exploratory hypotheses, EEG sleep parameters distinguished patients with *vs* without current SI; however, this effect was limited to Slow Wave Sleep (SWS), with significantly reduced time spent in NREM Stage 4 observed among those endorsing current SI compared to those without SI ($t(52)=2.14$, $p<0.05$). When analyzed by diagnostic subgroups, effects appeared similar for MDD; but BD patients with SI showed significantly lower SE ($p<0.02$), increased WASO ($p<0.01$), and higher NREM Stage 1 Percent ($p=0.01$).
Conclusions: Findings are consistent with sleep architecture abnormalities in suicidal *vs* nonsuicidal TRD, with increased sleep fragmentation/light sleep and decreased SWS among those endorsing elevated suicidal symptoms. While preliminary, this is the first known report to evaluate EEG sleep parameters in treatment resistant unipolar and bipolar depression in association with suicide risk. Previous work shows that specific elements of EEG SWS (eg slow wave amplitude and slope) may serve as central biomarkers of enhanced synaptic plasticity, predicting ketamine-induced rapid antidepressant treatment effects, as well as an association with neurotrophic factor, BDNF. Compared to other suicide risk factors, disturbances in sleep are modifiable, non-stigmatizing, easily treatable, and visible to friends and family members in the weeks and months preceding death. Based on the present findings, sleep warrants further investigation as a neurobiological factor and intervention tool in the pathogenesis of risk for suicidal behaviors, particularly in TRD, where risk for suicide is heightened. Objective sleep parameters may present a novel therapeutic target for drug development in the treatment of mood disorders and in the prevention of suicide.
Keywords: suicidal ideation; suicide risk; sleep; polysomnography; depression.
Disclosures: R. Bernert, Nothing to Disclose; D. Luckenbaugh, Nothing to Disclose; W. Duncan, Nothing to Disclose; C. Zarate, **Part 4:** Dr Zarate is listed as a coinventor on a patent application for the use of ketamine and its metabolites in major depression. Dr Zarate has assigned his rights in the patent to the US government but will share a percentage of any royalties that may be received by the government.

T173. Benzoate, a D-Amino Acid Oxidase Inhibitor, for Treatment of Early-phase Alzheimer's Disease: A Randomized, Double-blind, Placebo-controlled Trial

Guochuan Emil Tsai*

UCLA School of Medicine, Pasadena, California

Background: N-methyl-D-aspartate receptor (NMDAR)-mediated neurotransmission is vital for learning and memory. Hypofunction of NMDAR has been reported to play a role in the pathophysiology of Alzheimer's disease (AD), particularly in the early phase. Enhancing NMDAR activation can be a novel treatment approach. One of the methods to enhance NMDAR activity is to raise the levels of NMDA coagonists (such as D-serine) by blocking their metabolism. This study examined the efficacy and safety of sodium benzoate, a D-amino acid oxidase (DAAO) inhibitor, for the treatment of amnestic mild cognitive impairment (aMCI) and mild AD.
Methods: A randomized, double-blind, placebo-controlled trial was conducted in four major medical centers in Taiwan. Sixty patients with aMCI or mild AD were treated with 250–750 mg/day of sodium benzoate or placebo for 24 weeks. Alzheimer's disease assessment scale-cognitive subscale (ADAS-cog, the primary outcome in this trial) and global function (assessed by Clinician Interview Based Impression of Change plus Caregiver Input [CIBIC-plus]) were measured every eight weeks. Additional cognition composite, including speed of processing (Category Fluency), working memory (WMS-III, Spatial Span) and verbal learning and memory tests (WMS-III, Word Listing), was measured at baseline and endpoint.
Results: Sodium benzoate produced better improvement than placebo in ADAS-cog ($p=0.0021$, 0.0116 and 0.0031 at week 16, week 24 and endpoint, respectively), with effect size of 0.86, additional cognition composite ($p=0.007$ at

endpoint) and CIBIC-plus ($p = 0.015$, 0.016 and 0.012 at week 16, week 24 and endpoint, with effect size of 0.73, respectively). Sodium benzoate was well tolerated without evident side-effects. The dropout rate (3.3%) of sodium benzoate group tended to be lower than that (16.7%) of the placebo group, yet insignificantly ($p = 0.195$). Sodium benzoate provided better efficacy than placebo at week 16 and week 24, with the mean dose of 525 and 716 mg/day respectively. Sodium benzoate was well tolerated.

Conclusions: It is critical to identify and treat AD as early as possible. The current study is the first to apply a DAAO inhibitor, sodium benzoate herein, as a novel treatment for the early stage of cognitive decline. Sodium benzoate substantially improved cognitive and overall functions in patients with early-phase AD. The preliminary results show promise for DAAO inhibition as a novel approach for new drug development for early dementing processes. While it is important to develop novel compounds for the early phase of dementia, cognitive deficits (the core symptoms of schizophrenia) can be improved by sodium benzoate in patients with schizophrenia. The potential of NMDA-enhancing agents in improving cognitive function for patients with other CNS disorders or for general populations deserves further investigation.

Keywords: NMDA, D-serine, D-amino acid oxidase, early dementia, MCI.

Disclosures: G. Tsai, **Part 1:** Dr Tsai is a director and shares holder of SyneuRx International Corp. which plans to develop D-amino acid oxidase inhibitors, including sodium benzoate, for the treatment of CNS disorders.

T174. Early Changes in Apathy Predicts Response to Adjunctive Oral Methylphenidate in Depressed Patients

Sidney Kennedy*, Sakina Rizvi, Joseph Geraci, Arun Ravindran

University Health Network, Toronto, Ontario, Canada

Background: The majority of patients with Major Depressive Disorder (MDD) do not remit with antidepressant monotherapy, prompting the increased use of augmentation therapies to target specific symptom clusters (eg anhedonia). However, it is unclear whether baseline levels or early alleviation of depressive symptoms, or symptom clusters, predict positive outcome with augmentation therapy. Methylphenidate is a common adjunctive treatment, which may preferentially affect depressive symptoms such as anhedonia, fatigue and sexual function that are modulated by the dopamine system. The aim of this study was to conduct a secondary analysis investigating predictors of response and remission to osmotic-release oral system (OROS) methylphenidate in MDD patients.

Methods: MDD subjects ($n = 144$) who had inadequate response to standard SSRI and SNRI therapy were enrolled into a 5-week randomized placebo controlled trial of adjunctive OROS methylphenidate. Apathy and anhedonia were measured using the Apathy Evaluation Scale (AES), and fatigue was measured using the Multidimensional Assessment of Fatigue (MAF).

Results: While the overall depression scores on the Montgomery Asberg Depression Scale (MADRS) did not differ between the placebo and active drug conditions (Ravindran *et al*, 2008), AES scores significantly improved over 5 weeks in the OROS group compared to placebo with group differences manifesting from day 5 onwards (40.3 *vs* 44.1, $p = 0.018$). Early change in apathy from baseline to week 2 was a predictor of percent MADRS change ($\beta = 1.71$, $p = 0.002$), response (% correctly predicted: 68.2%, $p = 0.005$) and remission (% correctly predicted: 69.7%, $p = 0.008$). Sexual function and fatigue were not predictors of response.

Conclusions: Early symptom changes in dopaminergic symptoms predict depression outcomes when patients are receiving a dopaminergic drug. Considering group effects were not observed at the global depression level, when evaluating augmentation therapies, specific symptom clusters should be assessed.

Keywords: major depressive disorder, methylphenidate, prediction, apathy, response.

Disclosures: S. Kennedy, **Part 1:** AstraZeneca, Clera, Boehringer-Ingelheim, Bristol-Myers Squibb, Eli Lilly, GlaxoSmithKline, Janssen-Ortho, Lundbeck, Merck Frost, Pfizer, Servier, and St. Jude Medical., **Part 4:** Clera, Bristol-Myers Squibb, GlaxoSmithKline, Lundbeck, and St. Jude Medical.; S. Rizvi, Nothing to Disclose; J. Geraci, Nothing to Disclose; A. Ravindran, **Part 1:** Astra-Zeneca, GlaxoSmithKline, Hoffman La Roche, Janssen, Lilly, Lundbeck, Novartis, Pfizer, Servier, **Part 4:** Astra-Zeneca, GlaxoSmithKline, Hoffman La Roche, Janssen, Lilly, Lundbeck, Novartis, Pfizer, Servier.

T175. CONSORT-NP: Guidelines for Reporting of Neuropsyhological Test Results in Clinical Trials

Robert M Bilder*

UCLA, Los Angeles, California

Background: Evidence based medicine (EMB) is defined as 'the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients' {Sackett, 1996 #4} and increasingly, is recognized as critical for the future of clinical neuropsychology, and more importantly neuropsychological research {Bilder, 2011 #5; Kaufman, 2013 #6}. Across the medical sciences, the evidence upon which best practices are determined is derived from the peer-reviewed, empirical literature base, frequently randomized controlled trials (RCT). In order to facilitate the conduct, dissemination and evaluation of research findings, reporting standards have been introduced and widely adopted throughout medicine including the Standards for the Reporting of Diagnostic accuracy studies (STARD), Strengthening the Reporting of Observational studies in Epidemiology (STROBE) and Consolidated Standards of Reporting Trials (CONSORT). In the health sciences literature, the RCT is typically regarded as the gold standard assessment of treatment efficacy and with the increasing prevalence of neuropsychology in RCTs, it is imperative that the field follow suit and adopt similar standards for reporting research findings if we are to remain at the forefront of behavioral medicine. Two primary research factors that are important for neuropsychology in which reporting stan-

dards are particularly relevant include neuropsychological endpoints and neuropsychological manipulation. In some studies, neuropsychological measures and assessment of cognition represent the primary endpoint and method of evaluation (eg, Alzheimer's disease, TBI, Stroke), however, in other studies, cognition may be secondary to other endpoints such as improvement of clinical symptomatology (eg, alleviation of psychotic symptoms in schizophrenia; improved emotional functioning in depression). Regardless of the role of neuropsychological variables within the literature, standardizing the reporting of neuropsychological research, including the operational definitions of cognitive constructs as well as measurement of cognitive endpoints is an essential step towards strengthening our evidence base. This paper parallels several others related to transportation of reporting standards to neuropsychology {eg, Loring, 2013}[add Bowden 2013] and considers unique features of the CONSORT statement as these pertain to neuropsychological research.
Methods: We develooped novel criteria for grading the credibility of NP findings in clinical trials, expanding upon existing criteria for grading RCT's using other measures as the key endpoints. We applied these criteria to grade 30 studies selected to represent clinical trials in psychiatry and neurological disorders.
Results: We find the new criteria are easy to employ and can be rated with reasonable reliability.
Conclusions: CONSORT-NP guidelines may be a useful complement to existing standards for reporting of results from RCT's and augment the existing CONSORT statement in ways that may be useful in all neuropsychiatric research that uses cognitive measures.
Keywords: cognition, randomized controlled trials, neuropsychology.
Disclosures: R. Bilder, Nothing to Disclose.

T176. Effects of Antidepressant Medication on Emotion Regulation in Major Depressive Disorder: An iSPOT-D Report

Leanne Williams*, Kateri McRae, William Rekshan, James Gross

Stanford University School of Medicine, Stanford, California

Background: Antidepressant medication (ADM) is thought to reduce depressive symptoms by regulating key neurotransmitters and altering core emotion-generative brain systems, but it is unknown whether antidepressants have any effect on the psychological and behavioral strategies that patients use to regulate their emotions. We examined the effect of antidepressants on the strategies individuals use to regulate their emotions in the international Study to Predict Optimized Treatment in Depression (iSPOT-D)—a multi-site, multi-phase realworld practical trial. Our aims were to test whether: (i) Emotion Regulation changes with antidepressant treatment, when regulation is defined by both emotional suppression, usually associated with maladaptive outcomes, and by reappraisal, usually associated with adaptive outcomes.
(ii) changes in emotion regulation are associated with changes in depressive symptoms following treatment.
(iii) changes in emotion regulation relate to clinically defined treatment outcomes.
Methods: 1008 outpatients with major depressive disorder from the first wave of iSPOT-D were randomly assigned to an 8-week course of one of three antidepressant medications: escitalopram, sertraline, or venlafaxine-XR. Emotion regulation was assessed by a well established self-report measure of both supression and reappraisal. Assessments were completed pre-treatment and 8 weeks following treatment. To assess clinical significance, we examined whether change in suppression and reappraisal was related to treatment response ($\geqslant$50% reduction in depressive symptoms on the Hamilton Rating Scale for Depression-17 item, HRSD17) and remission ($\leqslant$7 on the HRSD17).
Results: Antidepressant medication led to more adaptive emotion regulation: decreased use of suppression and increased use of reappraisal at week 8. In turn, the largest improvements in emotion regulation (larger decreases in suppression and increases in reappraisal) were associated with better treatment outcomes. The change in suppression was related to both response ($\geqslant$%50 reduction on the HRSD17), OR = 0.60, $p < 0.001$ and remission ($\leqslant$7 on the week 8 HRSD17), OR = 0.52, $p < 0.001$. The change in reappraisal also related to response, OR = 1.54, $p < 0.001$, and remission, OR = 1.39, $p < 0.01$.
Conclusions: Antidepressant medications are associated with adaptive changes in the psychological and behavioral strategies that patients use to regulate their emotions. The findings provide behavioral, psychosocial evidence that is consistent with neurochemical evidence that the antidepressants we examined influence serotonin, norepinephrine and dopamine systems that cut across subcortical and cortical circuits.
Keywords: depressive disorder, antidepressants, emotion regulation, practical biomarker trial, psychology.
Disclosures: L. Williams, **Part 1:** Consultant, Brain Resource, **Part 2:** Consultant, Brain Resource, **Part 4:** iSPOT-D sponsor, Brain Resource; K. McRae, **Part 4:** iSPOT-D sponsor, Brain Resource; W. Rekshan, **Part 1:** Employee, Brain Resource, **Part 2:** Employee, Brain Resource, **Part 4:** iSPOT-D sponsor, Brain Resource, **Part 5:** Brain Resource; J. Gross, **Part 4:** iSPOT-D sponsor, Brain Resource.

T177. Trichuris Suis Ova (TSO) as an Immune-inflammatory Treatment for Repetitive Behaviors in ASD

Eric Hollander*, Casara J Ferretti, Bonnie P Taylor, Rachel Noone, Jonathan Kirsch, Emma Racine

Albert Einstein College of Medicine, Bronx, New York

Background: Inflammatory mechanisms have been implicated in Autism Spectrum Disorders (ASD) and immunomodulatory interventions, such as Trichuris Suis Ova (TSO) may be an experimental therapeutic option for some individuals with ASD. In particular, ASD patients are observed to have dampened Th2 anti-inflammatory cytokine response, and an increased Th1 proinflammatory cytokine response. Furthermore, the observation that some

ASD patients manifest clinical improvement in response to fever suggests that ASD symptoms may be modulated by immune-inflammatory factors. The study of helminth worms, specifically TSO, for the treatment of autoimmune disorders emerged in part from the hygiene hypothesis, which suggests that simulation of the immune system by microbes is protective against the development of inflammatory diseases, and that arise in hygiene in urban settings has been associated with less protective microbes in humans and an increase in autoimmune inflammatory disorders such as multiple sclerosis, inflammatory bowel disease, asthma, allergic rhinitis and possibly ASD. The porcine whipworm TSO is proposed to work through multiple mechanisms, including interference with antigen presentation, cell proliferation, activation and antibody production. The interaction of the developing immune system with microorganisms, including helminthes, may be an important component of normal immune system maturation. Helminthes, including TSO, are well known to induce tolerance in their hosts via differential modulation of anti-inflammatory Th2 cytokine (IL-4, IL-5, IL-10, IL-13) and proinflammatory Th1 and Th17 cytokine (IL-1, IL-12, IFN-γ, TNF-α, IL-6) response. Th2 cell induction leads to strong IgE, mast cell and eosinophil response, while cytokines IL-4 and IL-13 trigger intestinal mucous secretion, enhance smooth muscle contractibility, and stimulate fluid secretion in the intestinal lumen. Additional studies have shown that a similar exposure to TSO results in the augmentation of the anti-inflammatory Th2 response, and a dampening of the toll-like receptor (TLR)-induced proinflammatory Th1 and Th17 responses, and an increased presence of myeloid and plasmacytoid dendritic cells, which are antigen producing cells that stimulate T-cells. Currently, TSO is being studied in multiple clinical trials of other immune-inflammatory disorders, with much success; however, we are the first to study this treatment in the ASD population.

Methods: A 28 week double-blinded, randomized crossover study of TSO *vs* placebo in 10 adults, aged 17.5–35, with Autism Spectrum Disorder was completed. ASD diagnosis was confirmed by DSM-IV criteria supported by the Autism Diagnostic and Observation Schedule (ADOS). Subjects had a personal or family history of allergies, a baseline YBOCS score of $\geqslant 6$ and an IQ greater than 70. Subjects eligible after the screening visit, returned to the Autism and Obsessive Compulsive Spectrum Program every two weeks to complete subject (DANVA-2, Affective Speech Recognition Task, Reading the Mind in the Eyes Test), parent (ABC, SRS, RBS-R) and clinician (YBOCS, Vineland-II) assessments in addition to safety monitoring. Subjects also completed clinical labs, including stools samples every 6 weeks. After the first 12 week phase of TSO or placebo subjects entered a 4 week washout before beginning the second 12 week phase; assessments and safety measures were continued throughout.

Results: This exploratory safety and efficacy study included young adults with high functioning ASD, normal intelligence and good verbal skills. The irritability scores on the ABC-I were low at baseline. Of interest, measures of rigidity, restricted interests, flexibility, and ability to transition were noted to improve in patients on TSO. Patients also demonstrated reduced discomfort and protest associated with interruption of restricted interests or deviation from expectations. These changes of rigidity and compulsivity were observed on the clinician rated YBOCS (YBOCS compulsions and rigidity subscale) and parent rated RBS-R (restricted interests subscale). As in previous studies of TSO in other populations, the side effect profile is low. Throughout the study only minimal gastrointestinal distress was observed in some patients.

Conclusions: This is the first placebo controlled trial of TSO in an ASD population. The preliminary analyses from our exploratory pilot study demonstrate feasibility of completing a 28 week study of TSO in an ASD population, safety of TSO in this population, and potential efficacy for repetitive behaviors/restricted interests in some patients. Limitations of the trial included a small sample size, lack of irritability at baseline, and use of parent outcomes measures in this population that potentially impact our understanding of the results. Future studies are needed in a younger population stratified for higher baseline irritability, and further exploration of target engagement with the immune system and relationship to clinical improvement in ASD. Funding provided by the Simons Foundation. Drug/Placebo provided by Coronado Biosciences.

Keywords: treatment, autism, immune, inflammation, repetitive behaviors.

Disclosures: E. Hollander, **Part 1:** Consultant to Coronado Biosciences Inc., **Part 4:** Funded by The Simons Foundation. Drug/Placebo provided by Coronado Biosciences Inc.; C. Ferretti, Nothing to Disclose; B. Taylor, Nothing to Disclose; R. Noone, Nothing to Disclose; J. Kirsch, Nothing to Disclose; E. Racine, Nothing to Disclose.

T178. Safety and Efficacy of Short-term Treatment with the Acetylcholinesterase Inhibitors Rivastigmine and Huperzine a to Reduce the Subjective and Reinforcing Effects Produced by Acute Cocaine Exposure

Christopher D Verrico*, James J Mahoney, Kimberly N Cooper, Tabish Iqbal, Thomas F Newton, Richard De La Garza

Baylor College of Medicine, Houston, Texas

Background: In the USA, estimates suggest there were 1.4 million current users, 670 000 new users, and 821 000 dependent users of cocaine during 2011. However, treatments for cocaine dependence are limited to behavioral interventions, as there are no FDA-approved medications. Preclinical and clinical studies suggest that cholinergic interneurons constitute an integral component of the mesocorticolimbic system, contributing an important role in the reinforcing effects produced by cocaine, and the cognitive processes implicated in the pathophysiology of cocaine dependence. Rivastigmine inhibits acetylcholinesterase but more potently inhibits butyrylcholinesterase. Huperzine A is a selective acetylcholinesterase inhibitor that also antagonizes NMDA receptors. We hypothesized rivastigmine and huperzine A would independently attenuate the subjective and reinforcing effects produced by acute cocaine exposure in a clinical laboratory setting.

Methods: We recently completed a double-blind, placebo-controlled, human laboratory-based study using a between-

groups design. Cocaine-dependent individuals, who were not seeking treatment, were randomly assigned to receive placebo ($n=16$), rivastigmine 3 mg ($n=13$), rivastigmine 6 mg ($n=13$), huperzine A 0.4 mg ($n=16$), or huperzine A 0.8 mg ($n=14$). On day 1 and on day 9, participants received both 0 and 40 mg of cocaine intravenously each day during two sessions separated by 4 h (cocaine dose randomized within each daily session). Subjective effects, measured on a visual analog scale digitized from 0–100 (strongest), were assessed before (−15 min) and after (5, 10, 15, 20, 30, 45, 60, 90, and 120 min) infusions. On day 10 during two sessions separated by 4 h, participants first received non-contingent cocaine infusions (0 or 20 mg; randomized within the daily session) before making five choices to receive either another infusion or money. Cardiovascular data were collected during all infusion sessions and safety was measured by summing frequencies of adverse events reported under each treatment condition across the 10-day study.

Results: Enrolled participants ($n=72$) were predominately Black (90%), 43 year old (range 27–54) males (79%) with 13 years of education (range 9–16). On average, they smoked (99%) 2.1 grams of cocaine per day, 4 days per week, for 16 years. In addition, the majority also currently smoked cigarettes (90%), drank alcohol (89%), and smoked cannabis (69%). Our preliminary data reveal the safety and tolerability of these medications in an inpatient setting, and the data on subjective, reinforcing, and cardiovascular effects will be presented in detail.

Conclusions: Preclinical studies suggest a critical role for acetylcholine in the reinforcing effects produce by cocaine. For example, a study integrating optogenetics with electrophysiology demonstrated that selectively inhibiting cholinergic interneurons in the nucleus accumbens with high spatial and temporal precision has the overall effect of increasing cholinergic activity and blocking cocaine conditioning in freely moving mice. Moreover, acetylcholinesterase inhibitors block cocaine self-administration in monkeys and block cocaine place preference and locomotor sensitization in mice. These, and other, preclinical studies demonstrate the powerful modulatory role cholinergic interneurons have in controlling activity of the neuronal circuits that mediate the reinforcing effects produced by cocaine, suggesting potential therapeutic efficacy of acetylcholinesterase inhibitors for cocaine dependence. The reversible acetylcholinesterase inhibitor rivastigmine binds at multiple sites on the cholinesterase molecule, prolonging inhibition. Of marketed inhibitors, rivastigmine is a more potent inhibitor of acetylcholinesterase in the cortex and hippocampus, regions strongly implicated in regulating the activity of the nucleus accumbens. In fact, we recently reported that rivastigmine safely and significantly reduced craving produced by acute methamphetamine exposure in methamphetamine-dependent volunteers. On the other hand, huperzine A is a widely available, inexpensive, and selective acetylcholinesterase inhibitor that, relative to rivastigmine, has better penetration through the blood-brain barrier, higher oral bioavailability, and a longer duration of action. Huperzine A also increases brain dopamine levels, attenuates cognitive impairments in rodent and non-human primate models, and ameliorates learning and memory deficits associated with aging and Alzheimer's disease. Finally, the side effect profile for Huperzine A is comparable or better than other cholinesterase inhibitors, and with few exceptions, it has been safe and well tolerated. Thus, the current study will provide important initial clinical data concerning the safety and therapeutic efficacy of acetylcholinesterase inhibitors as potential pharmacotherapies for cocaine dependence.

Keywords: cocaine use disorder, acetylcholinesterase, rivastigmine, huperzine A, pharmacotherapy.

Disclosures: C. Verrico, Nothing to Disclose; J. Mahoney, Nothing to Disclose; K. Cooper, Nothing to Disclose; T. Iqbal, Nothing to Disclose; T. Newton, Nothing to Disclose; R. De La Garza, Nothing to Disclose.

T179. The Establishing Moderators and Biosignatures of Antidepressant Response for Clinical Care (EMBARC) Study: Rationale, Design and Progress

Madhukar Trivedi*, Patrick J McGrath, Maurizio Fava, Ramin V Parsey, Marisa Toups, Benji T Kurian, Mary L Phillips, Maria Oquendo, Gerard Bruder, Diego A Pizzagalli, Sarah Weyandt, Randy Buckner, Philips Adams, Thomas Carmody, Eva Petkova, Myrna M Weissman

UT Southwestern Medical Center, Dallas, Texas

Background: Remission rates for Major Depressive Disorder (MDD) over 12 weeks of treatment are low (~30%) and unpredictable for any given antidepressant. Due to the clinical and biological heterogeneity of depression, it is unlikely that a single marker can have the specificity to guide treatment selection; 'biosignatures' composed of a set of markers are needed. These may be either: (1) moderators that optimize initial treatment choice or (2) surrogate endpoints (mediators) that can identify treatment outcome prospectively. The Establishing Moderators and Biosignatures of Antidepressant Response for Clinical Care (EMBARC) study was designed to address the need for biosignatures of treatment outcome and will advance personalized care of MDD. The study links a range of imaging, electrophysiological, and blood markers and behavioral/cognitive tasks to treatment response. These markers are validated additionally by the inclusion of a healthy control sample, assessed twice over one week which also provides test-retest reliability of early marker measurements in the clinical sample, and comparability across the four clinical sites of the study. The primary outcome of EMBARC will be a biosignature called the Depression Treatment Response Index (DTRI) which will integrate the biomarkers assessed into a single score that can be used to predict treatment outcome.

Methods: EMBARC has a two phase, randomized, placebo-controlled design. Adults, 18–65 with a diagnosis of MDD of at least moderate severity, and onset not later than age 35, are recruited while not taking any psychotropic medications. Baseline assessments are: (1) a comprehensive clinical phenotype including Structured Clinical Interview for DSM-IV (SCID); (2) imaging: anatomical magnetic resonance imaging, diffusion tensor imaging of white matter tracts, and functional MRI during an emotional conflict and a reward-dependent learning task, and pulsed arterial spin

labeling; (3) quantitative electroencephalography (qEEG) to assess cortical and subcortical brain activation patterns and cortical evoked EEG potentials; (4) behavioral neuropsychological tasks assessing variables such as reaction time and motor processing speed and (5) blood marker collection including DNA, RNA, and plasma. Subjects are then randomized 1 : 1 to placebo or sertraline. After one week of treatment baseline assessments (with the exception of SCID and other diagnostic phenotyping) are repeated. Subjects then complete 8 weeks of treatment. At week 8, non-responder subject are switched in a blinded fashion to a second phase treatment (placebo → sertraline, or sertraline → bupropion XL) and responders continue taking their initial treatment. Blood markers are collected again at weeks 8, (9 in those who switch) and 16 and plasma level of medication is collected at weeks 1, 4, 8, 12 and 16 to control for medication adherence. Healthy subjects complete the baseline and week 1 assessments, but do not continue into treatment. Each marker will be analyzed for prediction of outcome both for the baseline assessment and the change between baseline and week 1. Markers showing predictive ability of outcome, including tolerance to medications will be integrated into the DTRI.

Results: As of mid-august 2013, 123 of 400 planned subjects have been randomized into the EMBARC trial. The healthy control sample has been completed at 52 subjects. Each biomarker category has had ongoing quality control procedures. We have completed test-retest reliability analyses on the healthy control cohort and applied them to the baseline data for the initial cohort of depressed subjects to control for across site differences. We are beginning analyze the first 100 subjects as compared to healthy controls as preliminary results of the study.

Conclusions: EMBARC advances the analysis of biomarkers for personalized care of depression by integrating a range of markers with excellent quality control in a well phenotyped sample with a placebo control. Results of the reliability assessments indicate that the use of rigorous quality control procedures across sites allows for larger multi-site trials to use complex procedures to assess the biological and psychological underpinnings of treatment response. This study is a first step in the development of biosignatures of treatment, and may also advance the understanding of the neurobiology of depression. The data will become available to the scientific community in a repository for further analyses to generate new findings.

Keywords: biomarkers, depression, neuroimaging, qEEG, antidepressants.

Disclosures: M. Trivedi, Nothing to Disclose; P. McGrath, Nothing to Disclose; M. Fava, **Part 2:** I can receive income from use of the Sexual Functioning Inventory (SFI) and Antidepressant Treatment Response Questionnaire (ATRQ).; R. Parsey, Nothing to Disclose; M. Toups, Nothing to Disclose; B. Kurian, Nothing to Disclose; M. Phillips, Nothing to Disclose; M. Oquendo, **Part 2:** I can receive income from use of the Columbia Suicide Severity Rating Scale.; G. Bruder, Nothing to Disclose; D. Pizzagalli, Nothing to Disclose; S. Weyandt, Nothing to Disclose; R. Buckner, Nothing to Disclose; P. Adams, Nothing to Disclose; T. Carmody, Nothing to Disclose; E. Petkova, Nothing to Disclose; M. Weissman, Nothing to Disclose.

T180. Safety and Effectiveness of Aripiprazole Once-monthly for the Treatment of Schizophrenia: A Pooled Analysis of Two Double-blind, Randomized, Controlled Trials (246 and 247)

W Wolfgang Fleischhacker*, Raymond Sanchez, Tsai Lan-Feng, Timothy Peters-Strickland, Ross A Baker, Anna Eramo, Dusan Kostic, John Kane

Medical University Innsbruck, Innsbruck, Austria

Background: The objective of this analysis was, based on data from two large randomized trials, to evaluate the initial (3 months) safety and effectiveness of aripiprazole once-monthly (AOM), an extended release injectable suspension of aripiprazole, stratified by previous treatment.

Methods: Patients with schizophrenia requiring chronic treatment with an antipsychotic were eligible. Patients receiving another antipsychotic were cross-titrated to oral aripiprazole monotherapy during a 4–6-week oral conversion phase (Phase 1). All patients completing Phase 1, patients not on medication (documented lapse in antipsychotic medication of 3 or more days) or those already on oral aripiprazole entered a 4–28-week oral aripiprazole stabilization phase (Phase 2) with 10–30 mg/day of oral aripiprazole. In the first trial (246, Kane *et al*), the phase 2 stabilization period was 4–12 weeks; those stabilized for 4 consecutive weeks entered a single-blind AOM stabilization phase (Phase 3), wherein they received AOM every 4 weeks (400 mg, single decrease to 300 mg permitted) for 12–36 weeks with co-administration of oral aripiprazole tablets in the first 2 weeks. In the second trial (247), the phase 2 oral aripiprazole stabilization period was 8–28 weeks; those stabilized for 8 consecutive weeks were randomized (2 : 2 : 1) to double-blind maintenance treatment of AOM 400 mg (AOM-400; option to decrease to 300 mg permitted), oral aripiprazole (10–30 mg/day), or AOM 50 mg (aripiprazole once-monthly-50; a sub-threshold therapeutic dose for assay sensitivity; option to decrease to 25 mg permitted) for 38 weeks. Aripiprazole once-monthly was administered into the gluteal muscle using a double-dummy design such that all patients, including those randomised to oral aripiprazole, received an injection. Evaluating the safety of the subpopulation of patients initiating treatment with aripiprazole once-monthly (AOM) is important since patients will be exposed to the drug for at least 1 month after injection, thus discontinuations due to AEs in the first 3 months after initiating AOM was the primary endpoint of this analysis. The analysis was conducted on the pooled population in the first 3 months after initiating AOM treatment (Phase 3) ie patients in the open-label AOM stabilization phase (246) and those from the double-blind maintenance phase (247), all of whom received at least one dose of AOM. For effectiveness, all-cause discontinuations (with the exception of those discontinued by the sponsor due to 246 being stopped early after pre-specified efficacy parameters were met) are reported, as well as common adverse events (AEs). All outcome measures are reported for groups stratified by prior treatment: (1) those on antipsychotics other than oral aripiprazole prior to the study (converted); (2) those on oral aripiprazole prior to the study and (3) those not on antipsychotic treatment (no-AP) prior to the study.

Results: Eight-hundred forty one patients received at least one AOM 400 mg injection. Discontinuations due to adverse events in the first 3 months were 2.5% (21/841) overall; 2.4% (14/581) for patients who converted from another antipsychotic, 1.6% (3/191) for patients entering the study on oral aripiprazole, and 5.8% (4/69) for those who were not on antipsychotic treatment. All-cause discontinuations in the first 3 months were 13.2% (111/841) overall, and 13.1% (76/581) for converted patients, 12.0% (23/191) for patients entering on oral aripiprazole, and 17.4% (12/69) for patients not on antipsychotic treatment. In the first three months, insomnia was the most common AE: 8.4% (71/841) overall, and ranged from 3.1% (6/191) for the oral aripiprazole group to 11.6% (8/69) for patients not on antipsychotic treatment. The frequency of akathisia was 7.0% (59/841) overall, and ranged from 6.7% (39/581) for the converted group to 7.9% (15/191) for the patients entering on oral aripiprazole.

Conclusions: Aripiprazole once-monthly 400 mg appeared equally safe and effective in the first 3 months after initiation, regardless of treatment prior to entering controlled clinical trials. Discontinuation due to adverse events was low, and adverse events rates were within the range expected from historical data with aripiprazole oral formulation.

Keywords: schizophrenia, antipsychotic.

Disclosures: W. Fleischhacker, **Part 1:** Consulting honoraria: Lundbeck, Roche, Bristol-Meyers-Squibb, Otsuka, Janssen, Pfizer, MedAvante, Merck. Speaker honoraria: Lundbeck, Janssen, Otsuka, Roche., Stock: MedAvante, **Part 4:** Otsuka, Janssen, Reckitt-Benckiser, Pfizer; R. Sanchez, **Part 5:** Otsuka Pharmacutical Development & Commercialization; T. Lan-Feng, **Part 1:** Employee at Otsuka Pharmaceutical Development & Commercialization (OPDC) starting March 2013 to present., **Part 2:** Regular salary from OPDC, **Part 5:** Otsuka Pharmaceutical Development & Commercialization (OPDC); T. Peters-Strickland, **Part 1:** Employed by Otsuka Pharmaceutical Development and Commercialization, **Part 3:** Employed by Otsuka Pharmaceutical Development and Commercialization, **Part 5:** Otsuka Pharmaceutical Development and Commercialization; R. Baker, **Part 1:** Employed by Otsuka Pharmaceutical Development and Commercialization, **Part 3:** Otsuka Pharmaceutical Development and Commercialization, **Part 5:** Otsuka Pharmaceutical Development and Commercialization; A. Eramo, **Part 5:** H. Lundbeck A/S; D. Kostic, **Part 5:** Otsuka Pharmaceutical Development and Commercialization; J. Kane, **Part 1:** Alkermes, Bristol-Myers Squibb, Cephalon, Esai, Boehringer Ingelheim, Eli Lilly, Intracellular Therapeutics, Janssen, Johnson and Johnson, Lundbeck, Merck, Novartis, Otsuka, Pfizer, Pierre Fabre, Proteus, Roche, Sunovion and Targacept. He is a shareholder of MedAvante., **Part 2:** BMS, Otsuka, **Part 4:** Genentech—pending.

T181. Neural Response in Visual Cortex to Emotional Stimuli Predicts Clinical Outcome Across Rapid Antidepressant Agents

Maura L Furey*, Joanna Szczepanik, Allison C Nugent, Nancy Brutsche, David Luckenbaugh, Carlos A Zarate

NIMH, Bethesda, Maryland

Background: Emotional processing biases to visual stimuli have been characterized behaviorally in patients with major depressive disorder (MDD), and these biases may be associated with alterations in neural processing in areas of visual cortex.[1,2] In a previous study we showed that neural responses in bilateral middle occipital cortex (MOC) selectively during stimulus processing components of an emotion working memory paradigm predicted subsequent response to the rapid antidepressant agent, scopolamine (a muscarinic antagonist).[3] as no such correlation was observed when the same images were processed based on face identify. Here we test the hypothesis that activity in the same regions of MOC, as defined in the earlier study, will predict treatment outcome to the rapid antidepressant agent, ketamine (an NMDA antagonist), in an independent cohort of patients with MDD using an explicit and implicit emotion processing task.

Methods: Fifteen unmedicated patients with MDD participated in a functional MRI study. Identical emotional faces were presented under 2 task conditions: (1) explicit emotion processing while judging emotional expressions as positive (happy/neutral) or as negative (sad/angry) and (2) implicit emotion processing while judging gender (thus ignoring the emotional component of the stimuli) as echo-panar imaging data were collected ($TR = 2.5$; voxel $= 3.75 \times 3.75 \times 3.5$). Subjects subsequently participated in a placebo-controlled, crossover trial with i.v. placebo and ketamine (.5 mg/kg). The Montgomery-Asberg Depression Rating Scale (MADRS) was used to assess symptoms of depression 60 min prior to and 230 min after each infusion, which occurred two weeks apart and in random order. Multiple regression was conducted using AFNI to estimate BOLD response (relative to baseline) when judging emotion (explicit positive/negative) and when judging gender (implicit positive/negative) in a voxel-by-voxel manner. An independent mask, based on previous findings (see above),[3] was used to restrict the extent of the area included in the analysis. BOLD estimates were averaged over voxels within the predefined MOC mask for each of the 4 task conditions; mean BOLD was correlated with subsequent treatment response to ketamine (percent change in MADRS), using baseline MADRS as a covariate to control for differences in symptom severity at baseline. Significance was defined as $p < 0.025$ to control for multiple comparisons under implicit and explicit processing conditions.

Results: The mean BOLD response in MOC correlated positively with the magnitude of change in MADRS during explicit processing of negative emotion (judging negative emotion) in both the left ($r = +0.62$, $p = 0.018$) and right ($r = +0.64$, $p = 0.014$) hemisphres. No correlation associated with other task conditions reached statistical significance.

Conclusions: Here we demonstrate that baseline activity in the MOC, a region where baseline neural response during emotional processing predicted antidepressant response to scopolamine, predicts treatment outcome to ketamine in an independent patient group. As in the previous results with scopolamine,[3] the effects here are task specific, depending on the emotional valence (negative) and implicit *vs* explicit nature of stimulus processing (explicit). Thus, the relation between baseline BOLD response and subsequent treatment outcome is specific to the judging negative emotion condition. These findings suggest that differential baseline activity associated with selective emotional stimulus fea-

tures in visual processing areas may prove to be a shared biomarker of rapid antidepressant response. Importantly, this shared biomarker predicts treatment response to agents that target different receptor systems, including the NMDA and muscarinic systems. To determine whether this potential biomarker of response will extend beyond these rapid antidepressant agents requires further attention.
Keywords: ketamine, scopolamine, biomarker.
Disclosures: M. Furey, **Part 1:** Dr Furey is listed as a co-investigator on a patent application for the use of scopolamine in the treatment of mood disorders. Dr Furey have assigned her rights in this patents to the U.S. government but will share a percentage of any royalties that may be received by the government. ; J. Szczepanik, Nothing to Disclose; A. Nugent, Nothing to Disclose; N. Brutsche, Nothing to Disclose; D. Luckenbaugh, Nothing to Disclose; C. Zarate, **Part 1:** Dr Zarate is listed as a co-investigator on a patent application for the use of ketamine in the treatment of mood disorders. Dr Zarate has assigned his rights in these patents to the U.S. government but will share a percentage of any royalties that may be received by the government.

T182. Feedforward and Feedback Control Abnormalities During Precision Grasping Implicate Cerebellar Dysfunction in Autism Spectrum Disorder

Matthew W Mosconi*, Suman Mohanty, Rachel K Greene, Lauren Schmitt, David E Vaillancourt, John A Sweeney

UT Southwestern Medical Center, Dallas, Texas

Background: Sensorimotor impairments are present in the majority of individuals with autism spectrum disorder (ASD). Yet, these deficits and their neurophysiological mechanisms have not been systematically assessed. In the present study, we examined visually guided fine motor control in individuals with ASD. The relative influence of the quality of sensory input on movement abnormalities in ASD was examined by varying the precision of visual feedback.
Methods: Twenty-eight individuals with ASD and 29 healthy controls matched on age (range: 6–35 years), IQ and handedness performed precision grip force tasks in which they viewed a white FORCE bar on a screen that moved upwards with increased manual force toward a fixed green TARGET bar. In the first experiment, subjects were instructed to reach a target force level as fast as they could, and to sustain the target force level for the duration of the trial (15 s). Target force levels were varied between 5–85% of each individual's maximum force across trials. To assess the integrity of feedforward control mechanisms, we examined the accuracy and force dynamics of initial, rapid force generation. To assess feedback control mechanisms, force accuracy and variability were measured during sustained force generation. The regularity of each subject's sustained force time series also was examined to determine the degree to which individuals made online adjustments to refine their performance. A second experiment was performed to assess the relative impact of changes in sensory input on sustained precision force generation. During this task, the vertical distance the FORCE bar moved per Newton of force was varied between .12–145 mm. Thus, in experiment 2, visual feedback precision was increased by moving the FORCE bar a greater distance for every Newton of force generated.
Results: Primary responses generated by feedforward mechanisms were hypermetric for individuals with ASD compared to controls. Individuals with ASD also showed increased rates of force increase during their primary movements that were associated with the degree to which they overshot target force levels. During sustained force generation in which subjects attempted to maintain alignment of the force and target bars, individuals with ASD demonstrated increased force error and variability. These deficits implicating feedback control alterations were more severe at larger force levels, and at the most and the least precise visual feedback conditions. Spectral analyses showed that sustained force deficits in individuals with ASD were associated with reduced power in the 0–1 Hz frequency range and increased power at higher frequencies (1–3 Hz). Across all force levels and all levels of visual feedback precision, the force time series of subjects with ASD was less complex, or more regular.
Conclusions: These studies identified three distinct sensorimotor deficits in individuals with ASD. First, individuals with ASD show reduced accuracy and alterations in their force dynamics during primary motor responses suggesting that forward control mechanisms are disrupted in this disorder. Second, reduced accuracy of sustained motor responses indicates that feedback control systems also are impaired in ASD. The severity of feedback control deficits covaried with changes in both the motor execution and sensory processing demands, implicating abnormalities in both input and output processes. Third, increased force variability and increased regularity in the time-dependent structure of sustained precision force suggest that individuals with ASD utilize fewer degrees of freedom to correct their force precision during goal-directed actions. Taken together, these behavioral findings are consistent with the hypothesis that dysfunctions within cerebellar circuitry in ASD lead to both hypermetric feedforward motor processes and more variable sensory feedback control of motor commands.
Keywords: autism, motor control, cerebellum, sensorimotor.
Disclosures: M. Mosconi, Nothing to Disclose; S. Mohanty, Nothing to Disclose; R. Greene, Nothing to Disclose; L. Schmitt, Nothing to Disclose; D. Vaillancourt, Nothing to Disclose; J. Sweeney, **Part 1:** Consultant to Lilly, Takeda, Roche and BMS.

T183. Elevated Maternal C-Reactive Protein and Schizophrenia in a National Birth Cohort

Alan Brown*, Sarah E Canetta, Helja-Marja Surcel, Susanna Hinkka-Yli-Salomäki, Andre Sourander

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

Background: Mounting evidence from epidemiologic and preclinical studies supports a prenatal infectious component to schizophrenia. The most parsimonious explanation

for the association is activation of the maternal immune response. There are, however, few previous studies that have examined the relationship between maternal immunologic markers during pregnancy and schizophrenia among offspring. In addition, the findings are not consistent and are based on modest sample sizes. We therefore sought to conduct a novel test of the hypothesis that elevated pregnancy levels of maternal C-reactive protein (CRP), a well-established biomarker of inflammation, is associated with schizophrenia in offspring from a large national birth cohort.
Methods: The study is based on the Finnish Prenatal Study of Schizophrenia (FiPS-S), which consists of virtually all pregnancies (over 1.5 million) in the country since 1983 with archived maternal prenatal serum specimens prospectively drawn during the first and early second trimesters. Cases were identified from a national psychiatric registry. Maternal CRP levels during pregnancy were quantified for 784 cases of schizophrenia or schizoaffective disorder and controls matched 1 : 1 on birthdate, sex, and residence in Finland at time of case diagnosis.
Results: There was a statistically significant increase in maternal CRP levels in pregnancies of case compared to control offspring (case offspring: median = 2.97 mg/l; control offspring: median = 2.66 mg/l, df = 1, $p = 0.004$), adjusting for maternal age, previous births, maternal education, maternal psychiatric disorders, urbanicity and province of birth. The association was statistically significant in males (df = 1, $p = 0.029$), but not females (df = 1, $p = 0.29$), though there was no sex by CRP interaction (df = 1, $p = 0.67$).
Conclusions: These findings provide the most robust evidence to date that maternal immune activation is related to risk of adult schizophrenia, which may lead to new insights into the pathogenic mechanisms that underlie the disorder and offer the potential for preventive approaches.
Keywords: infection, inflammation, schizophrenia, C-reactive protein, epidemiology.
Disclosures: A. Brown, Nothing to Disclose; S. Canetta, Nothing to Disclose; H. Surcel, Nothing to Disclose; S. Hinkka-Yli-Salomäki, Nothing to Disclose; A. Sourander, Nothing to Disclose.

T184. Childhood Maltreatment and the Structure of the Incidence of Psychiatric Disorders: A National Study

Carlos Blanco*, Melanie Wall, Chelsea Jin

Columbia University, New York, New York

Background: To date, there are no prospective studies that have examined whether the incidence of psychiatric disorders can be explained a limited number of underlying liabilities. Furthermore, it is unknown whether the effect of childhood maltreatment on the incidence of disorders occurs through these liabilities or on individual disorders. The goal of this study was to examine the structure of the incidence of psychiatric disorders, the structure of childhood maltreatment and the effect of different types of childhood maltreatment on the incidence of psychiatric disorders.
Methods: We drew on the data from the National Epidemiologic Survey on Alcohol and Related Conditions, large prospective study of a nationally representative sample of the US population. Participants ($n = 34\,653$) were interviewed face-to-face in 2001–2002 (wave 1) and reinterviewed in 2004–2005 (wave 2). We used structural equation models to examine the covariance structure of the incidence of psychiatric disorders, the structure of childhood maltreatment and the effectof childhood maltreatment on the incidence of psychiatric disorders. Model selection was based on interpretability and fit indices including the Comparative Fit Index (CFI), Tucker-Lewis Index (TLI) and Root Mean Square Error of Approximation (RMSEA).
Results: A model with two correlated factors (one representing the internalizing disorders and the other the externalizing disorders) provided a good fit to the structure of the incidence of psychiatric disorders for both men (CFI = 0.97, TLI = 0.96, RMSEA = 0.010) and women (CFI = 0.99, TLI = 0.98, RMSEA = 0.007). A second order confirmatory factor analysis of the five childhood maltreatment factors measured by a single common abuse factor provided an excellent fit to the data for men (CFI = 0.98, TLI = 0.98, RMSEA = 0.038) and women (CFI = 0.99, TLI = 0.98, RMSEA = 0.040). The latent variable representing the shared effect of childhood maltreatment increased the risk of both internalizing and externalizing disorders in men and women. Furthermore, in both men and women, the sexual abuse factor had a direct effect on the internalizing factor.
Conclusions: The incidence of psychiatric disorders is well described by two broad liabilities, one representing internalizing and the other externalizing disorders. Childhood maltreatment increases the risk of incidence of psychiatric disorders mainly through an increase in these broad liabilities. Preventing childhood maltreatment could help decrease the suffering of children, and decrease the burden of mental disease when they reach adulthood.
Keywords: childhood maltreatment Incidence of psychiatric disorders.
Disclosures: C. Blanco, Nothing to Disclose; M. Wall, Nothing to Disclose; C. Jin, Nothing to Disclose.

T185. Factors Impacting Functional Disability and PTSD Symptoms in OEF/OIF/OND Veterans with PTSD

F Andrew Kozel*, Jeffrey Spence, Christina Bass, Cassie Rae Morgan, Penelope Jones, Caitlin D Schraufnagel, Mary B Turner, John Hart

James A. Haley VA, Tampa, Florida

Background: A significant portion of veterans returning from OEF/OIF/OND experience post-traumatic stress disorder (PTSD) that results in significant impairment, morbidity, and mortality. As part of a randomized controlled trial of repetitive Transcranial Magnetic Stimulation (rTMS) to augment Cognitive Processing Therapy (CPT) in veterans with PTSD, a systematic evaluation of function and symptoms was performed at baseline. The participants could choose to have a summary of their evaluations sent to the Veterans Administration, but otherwise this study was performed outside of any disability determination. The purpose of this analysis was to

determine which factors are associated with the degree of disability and PTSD prior to treatment with CPT and rTMS.
Methods: Participants from OEF/OIF/OND were screened to determine eligibility and evaluated to determine if they met criteria for PTSD. Demographic information, self report scales, urine drug screens/pregnancy tests, and symptom ratings performed by trained evaluators were obtained. The variables of childhood adversity (Adverse Childhood Experience Questionnaire: ACE), level of combat exposure (Full Combat Exposure Scale: FCES), severity of PTSD (Clinician-Administered PTSD Scale for DSM_IV _TR: CAPS), and severity of depression (Quick Inventory of Depressive Symptomatology –Self Report: QIDS-SR) were investigated in multiple regression models to determine their contribution to degree of disability as measured by Inventory of Psychosocial Functioning (IPF). Similarly, to identify the factors that were significantly associated with severity of PTSD symptoms, as defined by CAPS score, the subset of variables mentioned above was also investigated by multiple regression. For both sets of models, a variable selection routine was utilized which incorporated combinations of forward addition and backward elimination of variables until there was no further improvement in model diagnostics. The Bayesian information criterion (BIC) was the chosen statistic for the determination of the best model.
Results: There were 55 participants (mean age 30 years (s.d. 5.6, range 22–47); 51 male; race—33 White, not Hispanic; 2 Black/African American, not Hispanic; 14 White, Hispanic; 2 American Indian/Alaska Native; 1 Native Hawaiian/Pacific Islander; 3 other) included for the assessment of PTSD severity, while 46 participants were included in the model assessing degree of functional disability, due to missing data. Only the depression self-report measure (QIDS) was selected in the final model that best predicted the degree of functional disability. A significant ($p = 0.0005$) positive association was found between QIDS and the IPF score. Similarly, in the final model that assessed the severity of PTSD symptoms, QIDS was a highly significant ($p < 0.0001$) predictor of CAPS. In addition, combat exposure also proved influential in the prediction of PTSD severity. A significant ($p = 0.02$) positive association was found between exposure to traumatic events, defined by FCES, and PTSD severity.
Conclusions: In veterans returning from OEF/OIF/OND with PTSD symptoms, depressive symptoms contribute most to functional disability compared to many other variables, including PTSD symptoms. Depression also contributes most to the severity of PTSD, though combat exposure is also an important factor in the determination of PTSD severity. Due to the strong association between depressive symptoms and both disability and PTSD symptoms, there should be an emphasis on the clinical management of depression in the care of these veterans.
Keywords: PTSD, disability, depression, combat exposure.
Disclosures: F. Kozel, **Part 4:** 03/2011—present DM090122, (Hart PI) Department of Defense 'Novel Treatment of Emotional Dysfunction in PTSD' Role: Co-investigator, 09/30/10—06/30/14 1 U01MH092221-01, (Trivedi PI), NIMH 'Establishing Moderators and Biosignatures of Antidepressant Response for Clinical Care (EMBARC)' Role: Co-investigator, no salary support since July 2011, Neuronetics (Kozel Site PI) -no salary support just research, 'An open-label study to evaluate the efficacy and safety of the Neuronetics Neurostar® TMS therapy system in patients with major depressive disorder (MDD) with postpartum onset.'; J. Spence, Nothing to Disclose; C. Bass, Nothing to Disclose; C. Morgan, Nothing to Disclose; P. Jones, Nothing to Disclose; C. Schraufnagel, Nothing to Disclose; M. Turner, Nothing to Disclose; J. Hart, Nothing to Disclose.

T186. Early-onset and Very-early-onset Bipolar Disorder: Distinct or Similar Clinical Conditions?

Lukas Propper*, Claire O'Donovan, Martina Ruzickova, Cynthia Calkin, Tomas Hajek, Abigail Ortiz, Claire Slaney, Julie Garnham, Martin Alda

Dalhousie University, Halifax, Nova Scotia, Canada

Background: There is considerable controversy regarding what constitutes early-onset bipolar disorder (BD), and little is known about the early clinical stages, onset, and early course of the illness. While some researchers observed a high rate of manic-like symptoms in clinical samples of children with attention deficit hyperactivity disorder (ADHD) and a high rate of ADHD in referred children with ultra-rapid cycling BD, other researchers believe that childhood ADHD is not part of the typical developmental trajectory of BD. Several previous studies found early-onset of BD associated with more chronic and severe clinical course, including increased rates of suicide, rapid cycling, psychosis, substance abuse, overall psychiatric comorbidity, higher prevalence of family history of mood disorders, more relatives with early onset of the illness, and lower rates of antimanic response to treatment with lithium. In this study we examined differences in the clinical presentation of very-early-onset and early-onset of BD which has not been explored in the previous studies.
Methods: Subjects were recruited through the Maritime Bipolar Registry, a community-based project in the Maritime Provinces of Canada. Diagnostic interviews followed the Schedule for Affective Disorders and Schizophrenia-Lifetime version (SADS-L) format; diagnoses were based on the Research Diagnostic Criteria (RDC) and DSM-IV criteria. Family history was obtained using the Family History-Research Diagnostic Criteria (FH-RDC) and SADS-L interviews with available relatives. Age of onset was defined as the age at which the patient experienced first episode of major depression or mania/hypomania, meeting the DSM-IV criteria. We selected two subgroups based on age at onset of first major mood episode (very-early-onset at age < 15 years and early-onset at age 15–18 years) and compared them with the rest of the sample as a reference group.
Results: Three hundred and sixty three subjects (236 women and 127 men) were included in the sample (mean age $44.2 + 12.8$ years), 240 with bipolar I and 123 with bipolar II disorder. There were 41 subjects in the very-early-onset BD (age < 15 years) and 95 in the early-onset group. Majority of patients had major depression as their first episode (88% in the very-early-onset group, compared to 61% in the early-onset and 54% in the reference groups). Comorbid ADHD was significantly more common in the very-early-onset probands (46%) in comparison to the

early-onset subjects (7.7%; $p = 0.0001$). Similarly, a history of rapid cycling was more frequent in the very-early-onset group (54%) than in the early-onset subgroup (34%; $p = 0.04$). Increased rates of suicide behaviour (attempts and completed suicide) were found in both early-onset groups (53 and 44%) in comparison with the reference group (25%; $p = 0.0001$). Comorbid generalized anxiety disorder was more prevalent in the very-early-onset subgroup (61%; $p = 0.03$) but there was no significant difference between the early-onset (39.8%) and the reference groups (37%; $p =$ ns). Comorbid panic disorder was also more prevalent in the very-early-onset group (42%) than in the early-onset subjects (28%; $p = 0.005$). Higher rates of mood disorders among first-degree relatives were present in both early-onset groups ($p = 0.01$). Overall, psychiatric comorbidity was higher in the very-early-onset subgroup ($p = 0.01$) with no difference between the early-onset probands and the reference group ($p =$ ns). In addition, the very-early onset patients had the lowest scores on the Global Assessment of Functioning scale in comparison with the early-onset and reference groups ($p = 0.04$).

Conclusions: Our results support the view that early-onset BD represents a more severe subtype of the illness associated with higher rates of psychiatric comorbidity and significant impairment of functioning. The majority of subjects in both early-onset groups developed major depression as an index episode of BD; an index episode of mania/hypomania was observed in only 9.8% of the very-early-onset subgroup, contrary to studies that reported a high rate of an early-onset manic like symptoms. On most measures the early-onset subgroup represented a continuum between the very-early-onset and reference groups. However, the very-early-onset subgroup differed from the early-onset and reference groups by increased rates of comorbid ADHD and rapid cycling. These findings may explain lower reported rates of antimanic response to treatment with lithium in the very-early-onset subtype of BD, but more studies are required to confirm these results.

Keywords: bipolar disorder, early onset, comorbidity, family history, outcome.

Disclosures: L. Propper, Nothing to Disclose; C. O'Donovan, Nothing to Disclose; M. Ruzickova, Nothing to Disclose; C. Calkin, Nothing to Disclose; T. Hajek, Nothing to Disclose; A. Ortiz, Nothing to Disclose; C. Slaney, Nothing to Disclose; J. Garnham, Nothing to Disclose; M. Alda, Nothing to Disclose.

T187. Obsessive-compulsive Symptom Dimensions in School Age Children and Their First Degree Relatives: Results from a Large Community-based Study

Pedro de Alvarenga*, Raony Cassab Cezar, Tais Moriyama, Marcelo Hoexter, Gisele Manfro, James Leckman, Euripedes Constantino Miguel, maria conceicao rosario

University of Sao Paulo, Sao Paulo, Brazil

Background: Obsessive-compulsive disorder can be expressed as various potentially overlapping obsessive-compulsive symptom (OCS) dimensions. In clinical samples, some dimensions are more familial and are associated with increased risk factors but not known whether this association is valid for non-clinical individuals.

Objectives I.To examine the frequence and distribution of OCS and its dimensions in a large sample of non-clinical school age children and their first degree realtives; II.To examine family aggregation of OCS and its dimensions, and; III. to determine the relationship of these phenomena to familial risk factors.

Methods: Data from 9937 children between 6 and 12 years and their first degree relatives were asessed. OCS and risk factors assessment were obtained from the Family History Screening. A 7- item-scale adapted from DYBOCS for four-model OCS dimensions (symmetry, contamination, sexual/aggressive/religious and hoarding) was used. Biological mothers were informants in 88%. Data analise included exact tests, logistic regression models and tetrachoric correlations.

Results: Prevalence of OCS in the hole sample [$n = 46\,148$, mean age: 17.6 (12.6); 49.2% females] was 16.2 and 14.7% in probands [$n = 9937$ mean age 8.9 (1.9), 47.9% females]. Familial OCS (OR = 3.96) and risk factors (OR = 1.49) predicted OCS in probands. The four studied dimensions aggregated in families; the magnitude of these associations tended to be greater for the father-proband dyads and siblings, mainly in the presence of contamination (OR: 9.96) and aggression symptoms (OR: 8.98).

Conclusions: These findings suggest that OCS is relative prevalent in non-clinical individuals. Familial OCS and risk factors predicts probands simptomatology. Contamination and sexual/aggressive dimensions exibited more strongly familial pattern.

Keywords: obsessive-compulsive symptoms, obsessive compulsive disorder, child and adolescent.

Disclosures: P. de Alvarenga, Nothing to Disclose; R. Cassab Cezar, Nothing to Disclose; T. Moriyama, Nothing to Disclose; M. Hoexter, Nothing to Disclose; G. Manfro, Nothing to Disclose; J. Leckman, Nothing to Disclose; E. Miguel, Nothing to Disclose; m. rosario, Nothing to Disclose.

T188. Premorbid Impairments in Childhood-onset Schizophrenia

David I Driver*, Deanna Greenstein, Madison Farmer, Judith L Rapoport, Nitin Gogtay

NIMH, Washington, District of Columbia

Background: The general model of schizophrenia as a neurodevelopmental disorder is widely accepted. This model is supported by research that has demonstrated aberrant motor, speech/language, and social development in individuals who later became schizophrenic. Childhood-onset schizophrenia (COS), a severe form of the illness that is clinically and neurobiologically continuous with the adult onset disorder, and requires onset of psychosis before age 13. Aberrant neurodevelopment may be more salient in cases of COS, presumably because the early onset cases are closer to the developmental roots of the disorder. Previous examination of premorbid developmental difficulties in 47 COS revealed abnormalities in the domains of speech/language (55%), motor (57%), and social (55%) development several years before the onset of psychotic symptoms.

This study attempts to confirm and extend these findings while providing a 12 year update on our expanded COS sample. We hypothesize that, consistent with the neurodevelopmental model of the schizophrenia and our previous findings, a significant portion of the cohort will have experienced premorbid impairments.

Methods: This study was approved by the Institutional Review Board of the National Institute of Mental Health and has been ongoing since 1991. The diagnosis of schizophrenia was made in a cohort of 118 patients according to DSM criteria with good reliability ($\hat{E} = 0.77$) using clinical and structured interviews, and prolonged inpatient observations by the research team. The cohort consisted of 49 males, 69 females; 52%—Caucasian, 28%—African American, and 20%—other. The mean onset of psychosis was 9 years 10 months. Premorbid development was assessed through review of previous medical records, including clinician notes, original pediatric, psychiatric, psychological, and educational reports and records. We also used information gleaned from clinical interviews with parents. Diagnosis of a pervasive developmental disorder was made using a clinical interview, the autism screening questionnaire, and observations by the research team.

Results: Of the 118 children in the cohort, 65 (55.08%) had premorbid academic impairments, 85 (72.03%) had premorbid social/behavioral impairments, 60 (50.85%) had premorbid language impairments, 52 (44.07%) had premorbid motor impairments, and 24 (20.34%) screened positive for pervasive developmental disorder. The average number of abnormalities (15 domains) in each child was 3.89 and 103 (87.29%) of the children had premorbid impairment in at least one domain.

Conclusions: Rates of premorbid developmental impairments were similar to those found in other studies of childhood-onset schizophrenia. Direct comparisons with studies of adult-onset schizophrenia are problematic because of variable methods but suggest that premorbid impairments are more common and severe among patients with childhood-onset than adult-onset schizophrenia. Although pronounced in children and adolescents who later develop schizophrenia, premorbid abnormalities are neither sensitive nor specific to childhood-onset schizophrenia. These impairments are seen in the early histories of patients who later develop a variety of other mental illnesses and the vast majority of patients with developmental impairments do not develop schizophrenia in adolescence or adulthood. However, given the high rate developmental disturbances in patients with schizophrenia and the high rate of the schizophrenia in a study of adults who had had severe language abnormalities as children, premorbid impairments may be an early manifestation of the neurodevelopmental abnormalities underlying schizophrenia. Limitations of this study include a lack of a healthy comparison group, sampling and recall biases, and variability in quality of premorbid records.

Keywords: childhood-onset schizophrenia, premorbid impairments.

Disclosures: D. Driver, Nothing to Disclose; D. Greenstein, Nothing to Disclose; M. Farmer, Nothing to Disclose; J. Rapoport, Nothing to Disclose; N. Gogtay, Nothing to Disclose.

T189. Replication and Refinement of the Predictive Value of Cognitive Markers in ADNI: Four Year Follow-up Data

Jesus J Gomar*, Concepcion Conejero-Goldberg, Peter Davies, Terry E Goldberg

Litwin-Zucker Alzheimer's Disease Research Center, Manhasset, New York

Background: Relatively few studies have directly compared the differential contribution of different kind of markers (biomarkers and cognitive markers) in their predictive utility for the conversion from Mild Cognitive Impairment (MCI) to Alzheimer's Disease (AD). In a previous study (Gomar JJ *et al*, Arch Gen Psychiatry 2011), we found that a combination of delayed verbal episodic memory measures and a middle temporal lobe cortical thickness measure were the strongest predictive factors of the conversion to AD from MCI in a follow-up period of two years, using a sample from the Alzheimer's disease Neuroimaging Initiative (ADNI). In the present study we intended to replicate and refine the predictive value of the combination of different classes of markers in the progression from MCI to Alzheimer's disease AD but now over 4 years of follow-up in the same ADNI sample.

Methods: 371 MCI patients were assessed on clinical, cognitive, morphometric MRI and CSF markers at baseline, and followed in a yearly basis for four years to ascertain progression to AD. For comparative purposes at baseline, 180 healthy control subjects were also assessed on the same variables. Logistic regression models were fitted in different clusters of variables including demographics, APOE genotype, cognition, CSF, and brain morphometry. The significant markers on each cluster were then combined in a 'winners' model. MCI patients who converted to AD were also compared according to the rate of progression, 'rapidly' *vs* 'slowly' progressing patients. Coefficient of determination in the form of pseudo-R2 was used as a measure of the relative predictive power of the models. Predictive accuracy of the model was calculated using receiver operating characteristic curve (ROC) analysis. Age, sex and education were forced in all analyses.

Results: Of the 371 MCI patients, 150 (40%) developed AD during follow-up (mean time until conversion 20.44 months; range 5.75–52.63), and 168 MCI patients remained stable at last follow-up (mean follow-up time 33.28 months; range 7.26–61.44). An additional sample of 180 healthy stable subjects during the same follow-up period was also included for comparative purposes on descriptive measures. At baseline, differences in almost all clinical staging variables, cognitive, brain morphometric variables, and CSF measures were found between each of the three groups compared to the other. First, we applied the best predictive model of conversion obtained at two years, including delayed episodic memory measures and middle temporal lobe thickness, to the four years data and demonstrated an Area Under the Curve (AUC) of 0.77, a sensitivity of 66%, a specificity of 70%, and a pseudo-R2 of 0.29. Second, 'Winners' analysis from the significant variables of the clustered regression models at four years revealed that an episodic memory measure (AVLT Trial 5) and two brain morphometric measures, left middle temporal cortex thickness and left hippocampus volume, were the best predictive factors of conversion to AD from MCI (AUC = 0.78, a sensitivity of

63%, a specificity of 71%, and a pseudo-R2 of 0.30). Finally, delayed episodic memory and left hippocampus volume significantly predicted imminent progression to AD as compared to delayed progression.
Conclusions: A combination of an episodic memory measure and two brain morphometric measures such as left middle temporal thickness and left hippocampus volume at baseline were found to better predict progression to AD from MCI over four years of follow-up. Consequently, when trying to differentiate between early and late MCI converters, delayed episodic memory and left hippocampus volume were the most informative measures in our study. These findings are consistent with the hypothetical model of temporally-ordered pathophysiological processes leading to AD, in which brain morphometric biomarkers (mainly hippocampal volume) and memory impairment are considered factors that immediately precede AD dementia onset. The empirical and clinical model that we proposed in the present study for the prediction of the development of AD in MCI patients is consistent to the previous model obtained at two years of follow-up, highlighting the importance of cognitive measures on the detection of preclinical AD and prediction of progression from MCI to AD.
Keywords: mild cognitive impairment; Alzheimer's disease; cognition; MRI; CSF.
Disclosures: J. Gomar, Nothing to Disclose; C. Conejero-Goldberg, Nothing to Disclose; P. Davies, **Part 1:** Dr Davies has received research support from and served as a consultant to Applied Neurosolutions.; T. Goldberg, **Part 1:** Dr Goldberg receives royalties for the use of the Brief Assessment of Cognition in Schizophrenia (BACS) in clinical trials.

T190. Frequency and Characteristics of Isolated Psychiatric Episodes in Anti-NMDA Receptor Encephalitis

Matthew Kayser*, Maarten Titulaer, Nuria Gresa-Arribas, Josep Dalmau

Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania

Background: Anti-N-methyl-D-aspartate (NMDA) receptor encephalitis is an autoimmune disorder in which IgG antibodies are directed against the NR1 subunit of the NMDA receptor (NMDAR). The disorder includes a range of psychotic symptoms early in the course of the disease followed by neurologic involvement, and ultimately protracted cognitive and behavioral symptoms. The occurrence of severe behavioral changes reminiscent of a schizophrenia-like illness has fueled speculation that this disorder might define a subset of patients misdiagnosed with a primary psychiatric disease. Hoewever, the frequency and type of isolated psychiatric episodes (pure psychiatric symptoms without neurological involvement) either as initial presentation of the disease or as relapse are unknown. This work aims to determine the frequency, symptoms, and outcome of isolated psychiatric episodes in a large cohort of patients with anti-NMDAR encephalitis.
Methods: This was an observational cohort of patients diagnosed over a 5 year period (median follow-up 2 years). 571 patients with IgG antibodies against the NR1 subunit of the NMDAR were included in the study. Antibody studies were performed at the Universities of Pennsylvania and Barcelona, and clinical information was obtained by the authors or referring physicians. We measured frequency, type of symptoms, and outcome of patients with anti-NMDAR encephalitis and isolated psychiatric manifestations. All patients had a detailed work up to rule out other disorders, including brain MRI, and blood and CSF studies. Isolated psychiatric presentations were defined as episodes (either initial presentation or relapse) that occurred in association with NMDAR antibodies in serum or CSF without neurological involvement. Relapse was defined by the new onset or worsening of symptoms at least two months after improvement or stabilization, without any other etiology involved, and persistent detection of NMDAR antibodies.
Results: 23/571 patients (4%) developed isolated psychiatric episodes, 5 at disease onset and 18 during relapses. For all 23 patients, age (median 20 years), gender (91% female), and tumor association (43%, ovarian teratoma) were similar to the population at large. Predominant symptoms included, delusional thinking (74%), mood disturbances (70%, usually manic), and aggression (57%). Brain MRI was abnormal in 10/22 (45%) and CSF showed pleocytosis in 17/22 (77%). Eighty three percent of the patients had full/substantial recovery after immunotherapy and tumor resection when appropriate. After relapse, 17/18 (94%) patients returned to a similar or better pre-relapse functional level.
Conclusions: We report 23 patients with anti-NMDAR encephalitis who developed isolated psychiatric symptoms either as initial episode of the disease (5 patients) or as relapse of encephalitis (18 patients). Predominant symptoms included delusional thinking, auditory or visual hallucinations, and manic and aggressive behavior. The fact that 5 patients had initial psychiatric presentations without neurologic symptoms or past history of encephalitis suggests that some cases of anti-NMDAR encephalitis can be mistaken for a primary psychiatric disorder. Therefore, isolated psychiatric episodes are rare but can occur as initial onset or relapse of anti-NMDAR encephalitis. Recognition of these episodes is important because they respond to immunotherapy. In patients with new onset psychosis, history of encephalitis, subtle neurological symptoms, and/or abnormal ancillary tests should prompt screening for NMDAR antibodies.
Keywords: psychosis, NMDA, autoimmune, encephalitis.
Disclosures: M. Kayser, Nothing to Disclose; M. Titulaer, Nothing to Disclose; N. Gresa-Arribas, Nothing to Disclose; J. Dalmau, **Part 1:** JD receives royalties from Athena Diagnostics for a patent for the use of Ma2 as an autoantibody test, and licensing fees from Euroimmun for a patent for the use of NMDAR as an autoantibody test.

T191. Rare Genetic Variants in VMAT1 (SLC18A1) Are Functional *In Vitro* and Associated with Bipolar Disorder

Falk W Lohoff*, Rachel Hodge, Sneha Narasimhan, Glenn Doyle

University of Pennsylvania, Philadelphia, Pennsylvania

Background: The gene encoding the vesicular monoamine transporter 1 (VMAT1) has recently emerged as a candidate

gene for bipolar disorder (BPD), schizophrenia and emotional behavior. We have shown that the common amino acid substitution polymorphism Thr136Ile leads to increased monoamine transport *in vitro* and affects negative emotion processing *in vivo*. In this study we conducted deep sequencing of patients with BPD in order to detect rare VMAT1 variants and to determine their function *in vitro*.

Methods: Sanger sequencing of all VMAT1 exons was carried out in 196 BPD individuals and 196 Caucasian controls. Novel rare variants that are likely to change protein function were tested for functional relevance using monoamine reuptake assays in CV-1 cells. Missense SNPs that were functional *in vitro* were then genotyped in a large cohort of BPD ($n = 4023$) and normal controls ($n = 3305$) of European descent from the NIMH Genetic Initiative using standard ABI TaqMan genotyping protocols.

Results: Sequencing of BPD patients identified several novel and rare variants. Comparison of sequencing results of rare variants in BPD individuals with normal controls from the 1000 Genome project shows that the global burden of rare variants was increased in the BPD group. Interestingly, several novel variants were only detected in the BPD group but were absent in the controls. Monoamine uptake *in vitro* was carried out for Gln10Arg, Phe84Ser, Ala101Pro, Arg138Leu and Leu392Val. Phe84Ser robustly increased monoamine uptake in particular for DA ($p < 0.001$) and the three variants, Ala101Pro, Arg138Leu and Leu392Val decreased uptake, with Arg138Leu showing the largest effect for DA ($P < 0.001$), although similar results were also obtained for 5-HT and NE. Because of the robust functional effects of Phe84Ser and Arg138Leu, we genotyped these rare variants in a large sample of BPD cases and controls. The Ser84 allele was absent in controls but present in seven BPD individuals, including one homozygote and six heterozygotes (Fisher exact test, $P = 0.009$). The Leu138 frequency did not differ statistically between cases and controls. Haplotype analysis of the individuals with the rare variant Phe84Ser showed that all subjects had almost exclusively the same halplotype Thr–Ser–Thr, indicative of a common origin and founder population effect.

Conclusions: Sequencing detected several rare and novel missense variants in BPD patients. *In vitro* results show that rare variants lead to 'hyper or hypo' transport of monoamines. Association analyses of the rare variants Phe84Ser and Arg138Leu show that the Ser84 allele was only present in BPD but not controls. Given that the common Thr136Ile was previously shown to increased monoamine transport and has an effect on interindividual responses to medial PFC activation of negative words and threat-related amygdala reactivity, the rare Phe84Ser variant may have similar effect on these brain circuits and may contribute to the pathophysiology of BPD. Future studies are needed to comprehensively investigate common and rare SNP-dosage effects on transporter function *in vivo* and risk for BPD.

Keywords: bipolar disorder sequencing SLC18A1 translational research monoamines.

Disclosures: F. Lohoff, Nothing to Disclose; R. Hodge, Nothing to Disclose; S. Narasimhan, Nothing to Disclose; G. Doyle, Nothing to Disclose.

T192. Can Serotonin Put Your Mind at Rest?

Alexander Schäfer, Inga Burmann, Ralf Regenthal, Katrin Arelin, Andre Pampel, Arno Villringer, Daniel Margulies, Julia Sacher*

Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany

Background: The serotonin transporter (5-HTT) is essential to maintaining adequate brain serotonin homeostasis, and alteration of its function has been linked to heightened susceptibility for depression and anxiety (Holmes *et al* 2003). Differences in the 5-HTT genotype have also been recently related to variation in intrinsic functional brain organization (Li *et al*, 2012). While preliminary evidence supports a connection between the serotonergic system and intrinsic brain activity, the precise role of serotonin in modulating its functional organization is not known. Here we demonstrate that a single dose of a selective serotonin reuptake inhibitor (SSRI) dramatically alters intrinsic functional connectivity throughout the human brain.

Methods: Degree centrality (DC) mapping of resting-state functional magnetic resonance imaging (rs-fMRI) data was applied to twenty-one individual data-sets of healthy, antidepressant naïve participants following a single oral dose of the selective serotonin reuptake inhibitor (SSRI) escitalopram in a randomized placebo-controlled design. Degree centrality measures connectivity by counting the number of connections of each specific node. This number is then assigned as a centrality value to the given node (a node is defined by each separate voxel in gray matter resulting in a network of ~63000 voxels). rs-fMRI data was acquired on a Siemens Verio 3 tesla scanner equipped with a 32-channel head coil (410 volumes, TR = 2000 ms). Standard image preprocessing was performed using FSL and AFNI (Biswal *et al*, 2010).

Results: DC-analysis revealed a widespread decrease in connectivity in most cortical and subcortical areas ($p = 0.01$, cluster corrected) following the oral intake of a single dose of 20 mg escitalopram. While the majority of functional connectivity decreased, localized increases were observed in cerebellar and thalamic regions. These connectivity changes could not be explained by alterations in the local signal properties, such as the amplitude of the resting state signal, and appeared to be specific to the correlation between regions which points towards an alteration in long range synchronization. It is noteworthy that these neural changes were also reflected in behavioral findings of significantly increased Visual Analogue Scores (VAS)-scores for concentration, alertness, attention and coordination ($p < 0.001$, post-hoc Bonferroni corrected).

Conclusions: The increase in connectivity found in the thalamus and cerebellum may be of particular relevance for the excitability of the many serotonergic projection neurons that terminate in the thalamus. By cerebellar modulation these neurons can turn from burst into tonic mode, a mechanism hypothesized to alert cortical networks. This conceptional framework is further supported by the reported increases in concentration, alertness, attention and coordination. Our findings are the first to directly link a single dose of an SSRI to such a substantial mechanism of modulating intrinsic functional connectivity in the human

brain. The evidence we present for an acute and global change in connectivity following a single dose of escitalopram is a first step towards identifying noninvasive neural biomarkers for individual responsivity of the human brain to serotonergic modulation.
Keywords: serotonin transporter, resting state MRI, connectivity, SSRI.
Disclosures: A. Schäfer, Nothing to Disclose; I. Burmann, Nothing to Disclose; R. Regenthal, Nothing to Disclose; K. Arelin, Nothing to Disclose; A. Pampel, Nothing to Disclose; A. Villringer, Nothing to Disclose; D. Margulies, Nothing to Disclose; J. Sacher, Nothing to Disclose.

T193. Poverty and the Past: The Relation between Hippocampus Function and Memory Performance Is Linked to Childhood Poverty

Elizabeth R Duval*, Sarah N Garfinkel, Chandra S Sripada, James E Swain, Gary W Evans, Israel Liberzon

University of Michigan Health System, Ann Arbor, Michigan

Background: Childhood poverty is a risk factor for poorer cognitive performance both among children and possibly in adulthood, although most of the adult studies rely on retrospective estimates of childhood SES. While connections between poverty and cognitive deficits have been accumulating, the underlying neural mechanisms are undetermined. In order to investigate the neurobiological link between childhood poverty and memory deficits, we examined neural activity and working memory in a prospective design among young adults with and without childhood history of poverty. We predicted that memory recall would differ between the two groups, and that these differences would be related to differences in hippocampal activation during encoding.
Methods: Fifty four right handed healthy adults between the ages of 20 and 27 were divided into two groups based on family income to need ratio at age nine. Twenty-eight came from middle income families, and 26 were from households falling below the poverty line. Within the context of a larger study, participants underwent fMRI scanning while encoding line drawings of common objects and animals, followed by a memory recall task. Signal detection (d-prime) was the measure of performance. D-prime was entered as a regressor into fMRI analyses, to examine brain activations during encoding that predicted memory recall performance. The effects of childhood poverty were also examined with respect to memory related activations.
Results: Adults who grew up in middle income families performed significantly better than the poverty group ($t(52) = 2.21$, $p < 0.05$). A d-prime regressor in fMRI analysis demonstrated a significant positive relationship between activation in left hippocampal regions during encoding and memory recall performance ($p < 0.005$, > 10 contiguous voxels). This relationship remained even after controlling for current income to need ratios. The relationship between left hippocampal activation during encoding was significant in the middle income group (Pearson's $r = 0.48$, $p < 0.01$) but not in the poverty group (Pearson's $r = 0.29$, $p > 0.05$).
Conclusions: Our prospective results confirm previous retrospective studies that childhood poverty is associated with poorer memory performance during adulthood. Our findings indicate left hippocampal activation during encoding is related to performance on a subsequent recall task. More specifically, the degree of left hippocampal activation during encoding was associated with better memory recall, but these relationships were demonstrated in the middle income group only. Future studies should continue to investigate mediators and moderators of these relations, including chronic stress, parenting, and other factors related to poverty.
Keywords: poverty encoding memory performance fMRI.
Disclosures: E. Duval, Nothing to Disclose; S. Garfinkel, Nothing to Disclose; C. Sripada, Nothing to Disclose; J. Swain, Nothing to Disclose; G. Evans, Nothing to Disclose; I. Liberzon, Nothing to Disclose.

T194. Therapeutic Benefits of Dutasteride, a 5 Alpha-reductase Type I Inhibitor, in PMDD: Results of a Pilot Study

Pedro Martinez*, Lynnette K Nieman, Leslie Morrow, Dahima Cintron, Karla Thompson, David Rubinow, Peter Schmidt

NIH/NIMH, Bethesda, Maryland

Background: There is ample evidence that premenstrual dysphoric disorder (PMDD) represents an abnormal affective response to progesterone (PROG). The exposure to or withdrawal from a PROG metabolite, allopregnanolone (ALLO), produces dramatic effects on GABA-A receptor subunit conformation, brain excitability and anxiety-like behavior. Despite mixed reports of plasma ALLO levels in PMDD, the onset of symptoms in this disorder could reflect a sensitivity to changing levels of ALLO during the normal luteal phase of the menstrual cycle. We tested this hypothesis by preventing the luteal phase increase in ALLO by administering dutasteride, a blocker of the 5 alpha reductase type I enzyme present in the brain and critical for the conversion of PROG to ALLO.
Methods: Women with prospectively confirmed diagnoses of PMDD ($n = 16$) and asymptomatic controls (ACs) ($n = 16$) (mean [SD] ages = 41.8 [6.1], 41.5 [4.8] years, respectively) participated in a double-blind, placebo-controlled, cross-over trial of dutasteride (DUT), an inhibitor of 5αR. (Type I& II). Two doses of DUT were employed: in the first study a low dose (LD) 0.5 mg/day and in study 2 a high dose (HD) 2.5 mg/day (to better ensure adequate suppression of 5αR). Double-blind DUT and placebo (PBO) were administered for one menstrual cycle each. Outcome measures included daily self-ratings assessing the severity of PMDD symptoms. In the HD group, serum levels of sex steroids and the corresponding 5 alpha- and 5 beta-reduced metabolites were assayed by GC/MS-MS (results pending). Results were analyzed with ANOVA-R and Bonferroni *t*-tests.
Results: In women with PMDD who received LD DUT ($n = 8$), we observed no significant effects of DUT on symptom severity compared with symptom scores during either baseline or PBO conditions, with all women

continuing to meet criteria for PMDD. In contrast, HD DUT in a separate group of women with PMDD ($n = 8$) resulted in significant decreases in symptom scores (ie, irritability, anxiety, sadness, breast pain, bloating, and food cravings) compared with both baseline and PBO conditions ($F_{6, 36}$ [range] = 4.8–8.7; p[range] = 0.001–0.03). Seven of eight women with PMDD met criteria for remission with the absence of significant pre- and post-menses symptom severity scores. Importantly, luteal phase plasma PROG levels during both HD DUT and PBO were consistent with ovulation and did not differ in women with PMDD on HD DUT compared with PBO. Both LD and HD doses of DUT were well-tolerated in both PMDD and AC groups. Plasma levels of 5 alpha- and 5 beta-reduced metabolites will be available by time of poster presentation.

Conclusions: The results of this pilot study suggest that DUT at a dose of 2.5 mg per day effectively treats a range of affective, physical, and behavioral symptoms in women with PMDD. These preliminary therapeutic results require replication in a larger RCT. Nonetheless, the elimination of PMDD symptoms in the context of ostensibly normal ovulation and despite the absence of differences in plasma PROG secretion between DUT and placebo suggests that the mechanism underlying DUT's effects in PMDD involves the prevention of a luteal phase increase in ring A-reduced metabolites of PROG. Alternate mechanisms of action underlying DUT's therapeutic effects could involve interference with the production of 5 alpha reduced metabolites of several other steroids (eg, testosterone), or direct binding of DUT to steroid hormone receptors (since DUT has a steroid-like ring structure). Our data suggest that the neurosteroid metabolites of PROG rather than the parent steroid are the proximate triggers of PMDD symptoms. Our results also suggest that reducing the conversion of PROG to its neurosteroid metabolites throughout the menstrual cycle can prevent the appearance of PMDD without the induction of dysphoric symptoms in ACs.

Keywords: premenstrual mood disorders (PMDD), 5-alpha reductase, allopregnanolone, progesterone, dutasteride.

Disclosures: P. Martinez, Nothing to Disclose; L. Nieman, Nothing to Disclose; L. Morrow, Nothing to Disclose; D. Cintron, Nothing to Disclose; K. Thompson, Nothing to Disclose; D. Rubinow, Nothing to Disclose; P. Schmidt, Nothing to Disclose.

T195. The Hypothalamic-Pituitary-Adrenal Axis, Reproductive Aging, and Depression: Results of Cortisol and ACTH Response to Dex/CRH testing in Women with and without Perimenopause-related Depression

Gioia Guerrieri*, Rivka Ben Dor, Leslie Smith, Karla Thompson, Pedro Martinez, David Rubinow, Peter Schmidt

NIH/NIMH, Bethesda, Maryland

Background: Depression is a leading cause of disease-related disability and the perimenopause represents a period of increased risk for both first onset and recurrent depression in women. Additionally, abnormalities in the hypothalamic pituitary-adrenal (HPA) axis are frequent accompaniments of depression, and HPA axis function is modulated by both aging and ovarian steroid secretion. To date, only a few studies have evaluated basal (but not stimulated) HPA axis function in women with perimenopause-related depression (PMD), with findings suggesting differences in adrenal androgen secretion but not basal cortisol secretion in PMD compared with controls. We examined HPA axis function in PMD and asymptomatic perimenopausal women (AC) using the combined dexamethasone-corticotropin releasing hormone (Dex/CRH) test.

Methods: Women with PMD met the following criteria: (1) onset of depression at midlife (ie, age 40–55 years) in association with menstrual cycle irregularity of at least six months duration but not greater than one year of amenorrhea; (2) the presence of either major or minor depression of moderate severity (confirmed by the Structured Clinical Interview for DSM IV [SCID]), associated with self-reported personal or occupational impairment (confirmed by the Global Assessment of Function [GAF] as well as a daily symptom self-rating form); and (3) elevated plasma follicle stimulating hormone (FSH) levels consistent with the perimenopause (>2 standard deviations above the average early follicular phase levels for normal women at peak reproductive age as recommended by the Stages of Reproductive Aging Workshop). AC's had no past or current history of depression, based on SCID interview, and were matched with the PMD women for age and reproductive state. Participants were medically well and were not on psychotropic medications. Outcome measures were plasma hormone levels (estradiol, progesterone, adrenocorticotropic hormone (ACTH) and cortisol) and cortisol binding globulin (CBG). 24-h urinary free cortisol (UFC) measures also were obtained. The Childhood Trauma questionnaire (CTQ) and standardized depression rating scales (including the Center for Epidemiology Depression Scale (CES-D) were administered to each woman prior to testing. Dex/CRH tests were performed during the early follicular phase (days 3–5) in women who were menstruating or randomly in those who were amenorrheic. Participants took oral dexamethasone, 1.5 mg, at 11 PM the night before the Dex/CRH test. Data were analyzed with independent-sample *t*-tests and ANOVA, using repeating measures (ANOVA-R) for the individual time points of ACTH and cortisol (baseline, 15–75 min) after CRH administration (100 micrograms).

Results: Thirty-one women (19 PMD and 12 ACs) ages 46–57 years (mean (SD): PMD 50.2 (3.1); AC 52.6 (2.9) years), have completed the Dex/CRH procedure to date. Average (SD) CES-D scores were as follows: PMD = 26.2 (2.9) and ACs = 0.8 (0.3). There were no significant differences between PMD and ACs in age or BMI, (p = ns), nor were baseline cortisol and ACTH different between groups. No significant main or interactive effects of diagnosis (Dx) or Dx by time were observed in the patterns of stimulated ACTH and cortisol secretion (ANOVA-R [Dx by time]:-$F_{5, 145} = 0.7$ and 0.8, p = ns, respectively). Similarly, no significant diagnostic differences were present in the ACTH or cortisol areas under the curve (AUC) or 24 h UFC levels (all comparisons, p = ns). Finally, plasma hormone levels of estradiol and progesterone did not differ in PMD compared with ACs (p = ns).

Conclusions: These preliminary results suggest that abnormalities of HPA axis function do not distinguish perimenopausal women with depression from those without depression. Thus, unlike depression in premenopausal women, HPA axis dysfunction is not a frequent accompaniment of depression during the perimenopause. Previous studies employing Dex/CRH testing in women with premenstrual dysphoric disorder (PMDD) showed similar findings with an absence of Dex/CRH stimulated HPA axis dysfunction. These findings suggest that reproductive endocrine-related mood disorders are not uniformly associated with the HPA dysregulation and could reflect underlying pathophysiologic processes that are distinct from those reported in women with non-reproductive-related depressions.
Keywords: perimenopause, depression, HPA-axis, cortisol, ACTH.
Disclosures: G. Guerrieri, Nothing to Disclose; R. Ben Dor, Nothing to Disclose; L. Smith, Nothing to Disclose; K. Thompson, Nothing to Disclose; P. Martinez, Nothing to Disclose; D. Rubinow, **Part 1:** Sage Pharmaceuticals Consultant; P. Schmidt, Nothing to Disclose.

T196. Impaired Glycemic Control in Urban African Americans with Type 2 Diabetes: Depression and Deficits in Functional Capacity

Dominique L Musselman*, David Ziemer, Julia Seay, Marcia McNutt, Erica Royster, Bridget Larsen, Terrika Barham, Angelo Brown, Octavia Vogel, Lawrence Phillips, Philip Harvey

University of Miami, Miami, Florida

Background: Diabetes has been long-recognized as a risk factor for dementia; increasingly recognized is that even hyperglycemia (without diabetes) can lead to cognitive deficits. Such deficits may, in turn, complicate the self-care and adherence of patients with type 2 diabetes. Given that African Americans (AAs) suffer from increased prevalence of type 2 diabetes and its complications, we: a) sought to determine the magnitude of deficits in performance of everyday living skills (ie functional capacity) among urban, AA patients with type 2 diabetes, and b) test whether these deficits were associated with poorer glycemic control.
Methods: At their initial visit to an inner-city diabetes clinic, 172 AA patients with type 2 diabetes were assessed with a variety of instruments, including the Mini International Neuropsychiatric Interview (MINI) and the UCSD Performance Skills Assessment-Brief (UPSA-B). They then entered a comprehensive diabetes management intervention, whose success was indexed by glycosylated hemoglobin (HbA1c) levels at up to four reassessments over a one-year period. A mixed-effects model repeated-measures method was used to predict HbA1c.
Results: The point prevalence of major depression was 19%. The mean UPSA-B score was 81 ± 17. After multivariate adjustment, increased HbA1c levels over time were predicted by the presence of major depression ($B = 0.911$, $p = 0.002$) and decreasing (ie worse) scores on the UPSA-B ($B = -0.016$, $p = 0.027$), respectively. There was no interaction between these two neuropsychiatric variables. Further adjustment for increasing the dosage of oral or insulin during the treatment eliminated the association between the UPSA score and HbA1c level ($B = -0.010$, $p = 0.115$).
Conclusions: Even in busy clinic settings, diabetes care providers can select patients (those with higher UPSA-B scores) who can adhere to more complex diabetes care regimens. Nevertheless, confirmation is needed regarding the adverse impact of deficits in functional capacity upon glycemic control. Future studies will elucidate the pathophysiology underlying these relationships, and determine whether interventions targeted at *both* depression and deficits in functional capacity can improve glycemic control.
Keywords: type 2 diabetes; functional capacity; HbA1c; UPSA-B; depression.
Disclosures: D. Musselman, Nothing to Disclose; D. Ziemer, Nothing to Disclose; J. Seay, Nothing to Disclose; M. McNutt, Nothing to Disclose; E. Royster, Nothing to Disclose; B. Larsen, Nothing to Disclose; T. Barham, Nothing to Disclose; A. Brown, Nothing to Disclose; O. Vogel, Nothing to Disclose; L. Phillips, **Part 1:** Advisory Panel—Boehringer Ingelheim Pharmaceuticals, Inc., Other Relationship—Expert Witness—Centers for Medicare and Medicaid Services, **Part 4:** Research Support—Novo Nordisk, Inc., Eli Lilly and Company, PhaseBio, Roche Pharmaceuticals, Amylin Pharmaceuticals, Inc., Sanofi, Merck, Cystic Fibrosis Foundation. ; P. Harvey, **Part 1:** Consultant: Abbvie; Boeheringer-Ingelheim; Forest Labs; Genentech; Otsuka-America; Roche Pharma; Sunovion Parma, **Part 4:** Contracted Research: Genentech.

T197. Mu Opioid Receptor A118G Polymorphism Alters Venous Plasma Cortisol Prolactin and Heart Rate During Stress

Edward F Domino*, Keeley Erhardt, Mika Fujita-Hirasawa

University of Michigan, Ann Abor, Michigan

Background: It is well known that tobacco smoking as well as stress increases venous plasma cortisol and prolactin levels. The present study examines single nucleotide polymorphism (SNP), of the mu opioid receptor OPRM1 A118G, differences in cortisol, prolactin, and heart rate responses to PET scanner stress.
Methods: Twenty normal healthy males with no history of substance abuse except tobacco smoking were recruited for this study. Subjects were between the ages of 20 and 36 years old (mean 25.8 ± 4.8) and smoked 15–40 cigarettes per day. After overnight tobacco abstinence the smokers underwent two separate 90 min PET scans at ~7:30 AM in which they lay in the scanner with their head and arms restrained for about 4 h. Venous plasma nicotine, cortisol, prolactin, and heart rate were measured periodically and expressed as mean ± SE. Ten ml of venous blood was taken from each subject prior to scanning to genotype OPRM1 A118G carriers. Genotyping was determined by pyrosequencing with blood samples. Subjects were divided into two groups based on whether they carried the G allele. The group without the G allele was homozygous for the A allele (AA group; $n = 15$), and the group containing

the G allele was either homozygous ($n = 1$) or heterozygous ($n = 4$).

Results: Similar increases in nicotine levels were observed in both the OPRM1 AA and *G smokers. Following denic tobacco smoking, nicotine levels were 3.60 ± 0.32 (AA) and 3.90 ± 0.94 (*G), and after avnic smoking 16.59 ± 2.12 (AA) and 17.13 ± 1.96 (*G) ng/ml. AA smokers had statistically significant higher cortisol levels throughout the study, before avnic smoking 7.93 ± 0.73 (AA) and 4.48 ± 0.67 (*G), and after 11.93 ± 0.95 (AA) and 8.43 ± 1.63 (*G) μg/dl. OPRM1 118AA carriers also exhibited significantly greater heart rates (beats/min) before (62.02 ± 1.81 (AA) and 54.78 ± 2.51 (*G)) and after (86.00 ± 2.02 (AA) and 76.50 ± 3.78 (*G)) avnic tobacco smoking. In contrast, prolactin levels before and after avnic smoking in the *G subjects showed a trend for an increase compared to the AA carriers but with marked variability. Mean prolactin levels between the genotypes were similar before and after avnic smoking and increased from 6.29 ± 0.32 (AA) and 6.92 ± 1.01 (*G) to 7.21 ± 0.54 (AA) and 9.50 ± 1.91 (*G) μg/dl.

Conclusions: It appears that an intervention like stress is needed to show a significant difference in venous cortisol and prolactin levels in OPRM1 A118G SNP carriers. A review of the literature reveals multiple studies related to the OPRM1 A118G effects especially on cortisol release and supports the role of stress or an intervention like naloxone in producing SNP differences.

Keywords: cortisol, prolactin, heart rate, genetics, stress.

Disclosures: E. Domino, Nothing to Disclose; K. Erhardt, Nothing to Disclose; M. Fujita-Hirasawa, Nothing to Disclose.

T198. Inflammation Is Heightened in Iraq and Afghanistan Veterans with Posttraumatic Stress Disorder

Aoife O'Donovan*, Beth Cohen, Karen Seal, Daniel Bertenthal, Shira Maguen, Mark Pacult, Thomas Neylan

University of California San Francisco, San Francisco, California

Background: Accumulating evidence indicates that inflammation could play a role in the pathogenesis of posttraumatic stress disorder (PTSD). For example, the administration of pro-inflammatory proteins or endotoxin elicits an inflammatory response and promotes symptoms similar to those seen in PTSD, including sleep disturbance, fatigue, difficulties concentrating, sadness, and anxiety. However, studies assessing levels of inflammation in PTSD have had mixed findings, with some reporting higher, some lower and some similar levels of inflammation in individuals with and without PTSD. Large-scale studies are necessary to clarify the relationship between PTSD and inflammation.

Methods: Our sample included 16 626 Iraq and Afghanistan veterans under the age of 55 who entered the Veterans Affairs (VA) healthcare system between October 1, 2005 and December 31st 2012. We included only veterans who received a measure of the inflammatory marker high sensitivity C-reactive protein (hsCRP) for any reason (15% women, M age = 35 ± 9 years) during their time in the VA system. Administrative data from the Department of VA were used to identify ICD-9 codes for mental health disorders and to obtain sociodemographic, military service, and health service utilization information. Generalized linear models to ascertain differences among groups with and without PTSD and other mental health disorders in log-transformed levels of hsCRP. We used generalized linear models with Poisson distribution and robust error variance to compare the adjusted relative risk (ARR) for clinically high levels of hsCRP (>3 mg/l) in individuals with PTSD and other mental health disorders compared to veterans with no mental health disorders. All models were adjusted for age, race, marital status, education, and BMI.

Results: Veterans with mental health disorders were overrepresented in this sample who had hsCRP tested for any reason; PTSD was diagnosed in 8973 (54%) veterans, and mental health disorders other than PTSD were diagnosed in an additional 4087 (25%) veterans. In continuous analyses, veterans with PTSD had significantly higher levels of hsCRP compared to veterans with no mental health disorders ($\beta = 0.07$, $p < 0.001$), and compared to veterans with mental health disorders other than PTSD ($\beta = 0.045$, $p < 0.001$). In gender-stratified analyses, the pattern of results remained the same. Compared to veterans with no mental health disorders, veterans with PTSD with or without other mental health disorders (ARR = 1.16, [1.07, 1.26], $p < 0.001$), and veterans with mental health disorders other than PTSD (ARR = 1.12 [1.02, 1.23], $p = 0.015$) had significantly increased risk for clinically high levels of hsCRP. However, there were no significant differences between veterans with PTSD and veterans with other mental health disorders in the risk for clinically high CRP (ARR = 1.04, [0.96, 1.11], $p = 0.331$).

Conclusions: In this large-scale study, veterans with PTSD had higher levels of the inflammatory marker hsCRP than veterans with no or other mental health disorders. Veterans with mental health disorders other than PTSD also had significantly higher levels of hsCRP than veterans with no mental health disorders. Veterans with PTSD and veterans with mental health disorders other than PTSD had similar levels of increased risk for clinically high levels of hsCRP compared to veterans with no mental health disorders. Inflammation was significantly elevated in veterans with mental health disorders even when adjusting for potential confounds and mediators including age, race, marital status, education, and BMI. Thus, inflammation may be a contributor to symptoms of PTSD and other mental health disorders. However, it also remains possible that unmeasured third variables account for the observations of high hsCRP in individuals with mental health disorders. Further research is necessary to clarify whether anti-inflammatory interventions could be an effective treatment for symptoms of mental health disorders, including symptoms of PTSD.

Keywords: PTSD, inflammation, C-reactive protein, psychoneuroimmunology, immune system.

Disclosures: A. O'Donovan, Nothing to Disclose; B. Cohen, Nothing to Disclose; K. Seal, Nothing to Disclose; D. Bertenthal, Nothing to Disclose; S. Maguen, Nothing to Disclose; M. Pacult, Nothing to Disclose; T. Neylan, Nothing to Disclose.

T199. Reduced Function of Cacna1c Encoded Cav1.2 During a Hormone Sensitive Period in Brain Development Leads to Sex-dependent Resilience to Despair in Adulthood

Michal Arad*, Margaret M McCarthy, Todd D Gould

University of Maryland School of Medicine, Baltimore, Maryland

Background: It is well documented that sex differences are a critical consideration to understanding the pathophysiology of mood disorders. While it is likely that susceptibility genes and hormones interact to modify the development of mood disorders, there exists limited current experimental evidence to support this. Genome-wide association studies have associated polymorphisms in the *CACNA1C* gene with a diagnosis of bipolar disorder and depression. *CACNA1C* codes for $Ca_v1.2$, which is an L-type voltage-gated calcium channel a1 subunit. We previously reported that *Cacna1c* haploinsufficiency in the mouse alters despair behaviors in a sex-dependent manner, and that specific human *CACNA1C* genotypes alter susceptibility to development of a mood disorder in women only. Given that male and female haploinsufficient mice share reduced levels of $Ca_v1.2$, but do differ in the behavioral effects of genotype, our aim was to assess the role of gonadal hormones in mediating this sex by genotype interaction. Organizational sex differences in the adult brain are established early in development as a result of an organizational process mediated by gonadal hormones.

Methods: All experiments were conducted on wild-type (WT) or *Cacna1c* haploinsufficient (HET) mice on a C57BL/6J background. We modeled the surge of brain testosterone levels that occurs in the male brain by subcutaneous injection of testosterone (100 μg/50 μl) or vehicle to PND-0 WT and HET female mice. In this experiment WT and HET females were assigned to one of three experimental conditions: intact; vehicle-treated (PND 0) and ovariectomized (PND 35); testosterone-treated (PND 0) and ovariectomized (PND 35). Treated mice were ovariectomized to avoid effects induced by neonatal testosterone treatment (eg ovarian hypertrophy), as well as to assess for the role of adult hormones in mediating the sex-specific effects of genotype. To assess a more precise interaction between L-type calcium channel function and the biological sex of the brain at the same sensitive period (PND 0) WT male and female mice received bilateral intracerebroventricular injection of the L-type calcium channel antagonist nimodipine (0.25 μg/0.25 μl/side) or vehicle. In this experiment males and females were assigned to one of three experimental conditions: intact; vehicle-injected; nimodipine-injected. In all experiments, at adulthood between postnatal day (PND) 70 and 98, locomotion was assessed with the Open Field Test (OFT; 100×100 cm; 10 min), and despair behavior was assessed using the Forced Swim Test (FST; recorded over a 6 min session, from which the last 4 min were analyzed) and Learned Helplessness (LH; training phase consisted of 60 inescapable 15-s shock sessions. 24 h following training, escape behavior was recorded over 45, 15-s shock, trials. P1-P5 are defined as Pre-test and trials 1–40 as Test) procedures.
Results: Sex dependent effects of genotype were observed in the OFT, LH, and FST procedures. Ovariectomy had no effect on the sex specific effects of genotype. The sex specific genetic effect on LH and OFT behavior in females was abolished by administration of testosterone at the early postnatal period. Acute blockade of L-type calcium channels with nimodipine on PND-0 phenocopied resilience to despair behavior observed in HET mice as tested both in the LH and FST in adult WT female mice, without affecting adult WT male mice despair behavior. Locomotor activity was not affected by either of the PND 0 manipulations.
Conclusions: These findings provide support for a sex-dependent role of L-type calcium channels in the development of mood disorders. The nature of the interaction between gonadal hormones and $Ca_v1.2$ L-type calcium channels is defined by a hormonally sensitive phase in neonatal life in which changes in the activity of L-type calcium channels alters trajectories for the development of a mood disorder in a sex-dependent manner. In early life there exists an interaction between L-type calcium channel function and biological sex, likely mediated by production of testosterone and leading to female-specific development of resilience. Our findings reveal a mechanism underlying sex-specific risk for mood disorders, and support the hypothesis that gonadal hormones and genetic risk factors interact at sensitive developmental time periods to modulate the risk to develop a mood disorder.
Keywords: CACNA1C; mood disorders; sex differences; animal models; neurodevelopment.
Disclosures: M. Arad, Nothing to Disclose; M. McCarthy, Nothing to Disclose; T. Gould, Nothing to Disclose.

T200. Fasting-induced Increase in Plasma Ghrelin is Blunted by Intravenous Alcohol Administration: A Within-subject Placebo-controlled Study

Lorenzo Leggio*, Melanie L Schwandt, Emily N Oot, Alexandra A Dias, Vijay A Ramchandani

NIAAA/NIDA, Bethesda, Maryland

Background: Ghrelin is a 28-amino acid peptide produced mainly by mucosal neuroendocrine cells lining the fundus of the stomach. Preclinical and clinical studies suggest that ghrelin plays a role in alcoholism. Furthermore, human laboratory studies indicate that acute oral administration of alcohol results in reduced circulating ghrelin. As ghrelin is primarily produced in the stomach, one question never previously explored is whether alcohol administered intravenously (IV) results in similar decrease in ghrelin levels.
Methods: This study analyzed the potential effects of IV alcohol administration on plasma ghrelin levels in healthy nonsmoking social drinkers ($n = 44$) who received either a 180-min IV infusion of 6% v / v alcohol or 0.9% normal saline in two separate counterbalanced sessions. At each session, participants arrived having fasted for ~ 7 h and received a light breakfast 60 min before the infusion.
Results: The percent change (%Δ) in ghrelin levels was 4.5-fold less in the alcohol condition than the saline condition. In fact, there was only a modest change in ghrelin levels from baseline in the IV alcohol condition (9.6%Δghrelin) while in the IV saline condition there was a robust change (43.4%Δghrelin). There was a trend toward significance in %Δghrelin in the alcohol condition compared to the

placebo condition (F[1, 33] = 3.3, $p = 0.07$; dz: 0.4).
Conclusions: While the exact mechanisms by which alcohol influences ghrelin levels are unclear, alcohol may act directly in the stomach by inhibiting ghrelin secretion and/or release, and may also attenuate ghrelin levels systemically. Although IV alcohol did not reduce circulating ghrelin levels, as seen in previous studies with oral alcohol administration, the present findings suggest that, despite bypassing the stomach, alcohol still attenuated circulating ghrelin levels, ie the fasting-induced increase in circulating ghrelin was blunted by IV alcohol administration. These findings lead us to hypothesize that alcohol might affect ghrelin signaling not only via a local effect on the stomach mucosa, but also via a systemic effect.
Keywords: alcohol, ghrelin, insulin, GLP-1, PYY, intravenous alcohol administration.
Disclosures: L. Leggio, Nothing to Disclose; M. Schwandt, Nothing to Disclose; E. Oot, Nothing to Disclose; A. Dias, Nothing to Disclose; V. Ramchandani, Nothing to Disclose.

T201. Early Life Adversity Increases Risk of New Onset Depression During the Menopause Transition

C Neill Epperson*, Mary Sammel, Stephanie Scalice, Sarah Conlin, Ellen Freeman

Penn Center for Women's Behavioral Wellness, Penn Center for the Study of Sex and Gender in Behavioral Health, Philadelphia, Pennsylvania

Background: There is an increased risk for new onset depression during the menopause transition, even in women without previous psychiatric history. Childhood adversity has lasting effects on neurotransmitter systems, which are also modulated by estrogen. We sought to determine whether early life adversity contributes to the increased risk of incident menopause depression.
Methods: In a 14-year longitudinal study of women undergoing a natural transition to menopause, participants ($n = 390$) completed the Clinical Epidemiologic Scale for Depression (CES-D) each year. A CES-D score of >16 was used to denote a probable major depressive episode. The Adverse Childhood Events (ACE) Questionnaire was utilized to assess the presence of abuse, neglect, and serious family dysfunction experienced prior to the age of 18. Of the 390 participants with at least 2 mood assessments, 206 of the women who remain in the study have been contacted to collect ACE information. The impact of number of ACEs (0, 1, >2) on first onset depression across the five stages of menopause (late premenopause, early transition, late transition, postmenopause) was determined.
Results: There were 1033 observations with each individual contributing on average 12 observations. Using generalized estimating equations and controlling for age, menopause stage was associated with a 2.7-fold risk ($p = 0.02$) of new onset depression as women progressed from the pre to postmenopause.
Conclusions: Childhood adversity, defined broadly, is a risk factor for first episode depression during the menopause transition when ovarian sources of estrogen are waning. These data suggest an environment by hormone interaction predicting depression risk in menopausal women.
Keywords: depression, menopause, childhood adversity.
Disclosures: C. Epperson, **Part 4:** Novartis; M. Sammel, Nothing to Disclose; S. Scalice, Nothing to Disclose; S. Conlin, Nothing to Disclose; E. Freeman, Nothing to Disclose.

T202. Cortisol Response to Psychosocial Stress During Depression and Remission

Uma Rao*, Matthew C Morris

Meharry Medical College, Nashville, Tennessee

Background: Disruptedhypothalamic-pituitary-adrenal (HPA) function often characterizes individualswith major depressive disorder (MDD). Psychosocial stress protocols elicit endogenousactivity of the entire HPA system and complement pharmacologic/neuroendocrinechallenges, which do not recruit suprahypothalamic circuits involved in the HPAresponse. It remains unclear whether cortisol responses to psychosocial stress,which appear to be altered in MDD, can be characterized as state-like ortrait-like risk markers.
Methods: We compared salivarycortisol responses to a standard psychosocial stressor (the Trier Social StressTest) during a major depressive episode (MDE) and again during remission inadolescents and young adults. Participants included 26 individuals with nopersonal or family history of a major psychiatric disorder (NC) and 24 individualswith MDD at Time 1. Time 2 stress protocol was conducted after the MDD groupremitted from the depressive episode for at least 3 months.
Results: NC showed a robust cortisol response to the psychosocial stressor at Time 1 but habituation to the repeated stressor, as evident in a flatter cortisol response profile at Time 2. In contrast to this, the MDD group showed robust cortisol responses during their index episode and after recovery. Within the MDD group, net peak cortisol during the first stress test was positively associated with the duration of the index MDE and negatively associated with the total duration of all MDEs. Whereas summary indices of cortisol responses were relatively stable across repeated stress tasks within the MDD group, this was not the case for NC.
Conclusions: To our knowledge, this study is the first to demonstrate that cortisol responses fail tohabituate to repeated psychosocial stress during recovery from an MDE and couldreflect a trait-like marker of risk for recurrence.
Keywords: depression, remission, stress, adolescent, risk.
Disclosures: u. rao, Nothing to Disclose; M. Morris, Nothing to Disclose.

T203. Salivary Cortisol Response to Trier Social Stress Test in Healthy Third Trimester Pregnant Women and Third Trimester Pregnant Women at Elevated Risk of Developing Postpartum Depression

Kristina M Deligiannidis*, Aimee R Kroll-Desrosiers, Bruce A Barton, Anthony J Rothschild

University of Massachusetts Medical School, Worcester, Massachusetts

Background: Postpartum depression (PPD) affects 1 in 8 women and negatively impacts infant attachment, cognitive development and behavior. The hypothalamic-

pituitary-adrenal (HPA) axis is the core endocrine axis which mediates the stress response and the development of depression in susceptible individuals. Dramatic physiologic endocrine changes during pregnancy/postpartum affect maternal HPA activity in healthy women. Normal pregnancy is associated with suppressed hypothalamic corticotropin releasing hormone (CRH) but increased plasma CRH, adenocorticotropin and cortisol concentrations while the early postpartum is associated with suppressed CRH and rapidly falling cortisol levels. HPA axis reactivity to stress is reduced during the postpartum period: abnormalities in HPA reactivity in the postpartum are associated with PPD. Preliminary data suggest HPA axis abnormalities may exist during pregnancy and contribute to the development of PPD. It is not known how stress impacts cortisol response in pregnant women at risk for developing PPD, eg with antenatal depressive/anxiety symptoms as measured by the Edinburgh Postnatal Depression Scale (EPDS) and/or a history of depression. This study tested the hypotheses: (1) third trimester pregnant women at increased risk of developing PPD would have a prolonged salivary cortisol response to a psychological stress test as compared to healthy comparison pregnant women (2) depression and anxiety scale scores would be correlated with salivary cortisol at baseline and over time.

Methods: 56 subjects were evaluated between 28–36 weeks gestation with the Structured Clinical Interview for DSM Disorders (SCID-IV), and mood and anxiety questionnaires (ie EPDS, Spielberger State-Trait Anxiety Inventory (STAI) and a mood reporting visual analog scale). Healthy comparison subjects (HCS: $n=24$, mean age in years: 31.8 ± 3.9, mean gestational age in weeks: 31.0 ± 2.8) had no history or current psychiatric illness and an EPDS total score $\leqslant 5$. Subjects at risk for developing PPD (AR-PPD: $n=32$, mean age in years: 33.4 ± 5.4, mean gestational age in weeks: 32.1 ± 2.6) had an EPDS total score $\geqslant 10$ and/or a history of depression. 46 subjects (21 HCR, 25 AR-PPD) completed the Trier Social Stress Test (TSST). Salivary cortisol was collected and STAI and mood visual analog scale for repeated measures were completed at time 0 (T0 = prior to TSST), T15 minutes (immediately post-TSST), T25 min, T35 min, T45 and T75 minutes. Salivary free cortisol concentration was analyzed using a time-resolved immunoassay with fluorescence detection (limit of detection = 0.5 nmol/liter). Baseline characteristics were compared based on case-control status using the likelihood ratio χ^2 test for categorical variables and Student's independent samples *t*-test for continuous variables. Pearson correlation coefficients were computed for comparisons between continuous variables of interest. We analyzed cortisol changes longitudinally using generalized estimating equation methods to control for the correlation among the data at the six time points. Main effects for group and EPDS score were modeled separately and then in combined models with risk group and one score variable at a time. *P*-values are either from a test that a single regression coefficient was equal to 0 (for main effects models), or from the overall Type 3 test that investigated a difference between the risk groups adjusting for other factors in the model.

Results: EPDS total score significantly differed between groups (HCS mean EPDS $= 2.6 \pm 2.0$; AR-PPD mean EPDS $= 8.9 \pm 5.2$; $p<0.0001$). There were no significant differences between groups in age, race, ethnicity, marital status, number of children in household or gestational age ($p>0.05$). Mean STAI total score significantly differed between groups, at all individual time points (each $p<0.05$) and over time (T0-T75; $\chi^2=9.23$, df $=1$, $p=0.0024$) with AR-PPD subjects reporting higher anxiety compared to HCS. STAI total score did not affect cortisol concentration over time ($\chi^2=1.81$, df $=1$, $p=0.1782$) and did not differ by group ($\chi^2=0.05$, df $=1$, $p=0.8250$). Reported calmness significantly affected cortisol by -0.0005 µg/dl for each one-unit change in calmness ($\chi^2=5.97$, df $=1$, $p=0.0145$) and this did not vary by group ($\chi^2=0.07$, df $=1$, $p=0.7908$). There were no significant differences between free salivary cortisol concentration at T0 (HCS: 0.29 ± 0.14; AR-PPD: 0.28 ± 0.1) and there was no correlation between EPDS and cortisol ($r=-0.009$, $p=0.9483$). Overall, each one-unit change in EPDS score non-significantly influenced cortisol by -0.001 ug/dl across time points ($z=-0.44$, $p=0.6594$) for all subjects, with no between group difference ($\chi^2=0.02$, df $=1$, $p=0.8901$). There was no difference in free cortisol concentration over time (T0-T75) between groups ($z=-0.13$, $p=0.8977$).

Conclusions: Understanding potential differences in HPA functioning in women with antenatal depressive/anxiety symptoms compared to healthy women is important since pregnancy alters HPA functioning. Our data show that healthy third trimester pregnant women and those with antenatal depressive/anxiety symptoms and/or a history of depression have a similar salivary cortisol response to the TSST. The TSST worsened reported stress/anxiety in both groups however women at risk for PPD experienced more anxiety to the psychosocial stressor.

Keywords: postpartum depression; cortisol.

Disclosures: K. Deligiannidis, **Part 1:** Dr Deligiannidis has received support from Elsevier & Society of Biological Psychiatry (Travel Award); Career Development Institute (NIMH funded award); National Network of Depression Centers (Travel Award). She is on the Board of Directors for the American Society of Clinical Psychopharmacology. Dr Deligiannidis receives royalties from an NIH employee invention., **Part 4:** Dr Deligiannidis has received research grant funding from Forest Research Institute, NIH, University of Massachusetts Medical School and the Worcester Foundation for Biomedical Research. Funding for this research abstract was provided by NIH-UL1TR000161.; A. Kroll-Desrosiers, Nothing to Disclose; B. Barton, Nothing to Disclose; A. Rothschild, **Part 1:** Dr Rothschild has consulted for Allergan, GlaxoSmithKline, Eli Lilly, Noven, Pfizer, Shire and Sunovion. Dr Rothschild has received royalties for the Rothschild Scale for Antidepressant Tachyphylaxis (RSAT); Clinical Manual for the Diagnosis and Treatment of Psychotic Depression, American Psychiatric Press, 2009; Evidence-Based Guide to Antipsychotic Medications, American Psychiatric Press, 2010; and Evidence-Based Guide to Antidepressant Medications, American Psychiatric Press, 2012., **Part 4:** Dr Rothschild has received grant/research support from NIMH, Cyberonics,Takeda, and St Jude Medical.

T204. Direct Comparison of the Psychometric Properties of Multiple Interview and Patient-Rated Assessments of Suicidal Ideation and Behavior in a Large Inpatient Sample

Eric Youngstrom*, Ahmad Hameed, Michael Mitchell, Andrew Freeman, Anna Van Meter, Guillermo Perez Algorta, Alan J Gelenberg, Roger Meyer

University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Background: In the past several years, reports that pharmacologic agents may contribute to an increase in suicidal thoughts and behaviors has led to a requirement from the FDA Division of Psychiatry Products that all participants in clinical trials of CNS-active drugs be evaluated for treatment emergent suicidal ideation and behavior using a scale that maps to the C-CASA algorithm. Multiple tools have been developed to map onto the C-CASA categories, with the FDA currently recognizing the Columbia Suicide Severity Rating Scale (C-SSRS) as an acceptable method. It is crucial to evaluate the comparative psychometrics of other methods, to calibrate results from trials that used other methods and to provide guidance about the acceptability of different methods.
The primary objective was to compare self-report and clinical interview-based versions of the older and current editions of the Sheehan Suicide Tracking Scale (S-STS) to the C-SSRS in the assessment of suicidal ideation/behavior in the context of the domains defined by the C-CASA and in relationship to the clinical evaluation of recently admitted psychiatric inpatients.
Methods: Participants were 199 adult inpatients (age 38.5 ± 12.4 years, 57% female) invited to participate shortly after admission, completing informed consent. Participants were stratified based on age and psychosis, and randomly assigned to one of two batteries with three counterbalanced administration sequences. Patients completed the C-SSRS as well as old and new versions of the Sheehan Suicide Tracking Scale (SSTS). Patients completed the SSTS as either a self-report or interview; CSSRS was always an interview. Interviews were recorded, and 60 were re-rated by independent reliable raters. Kappa quantified agreement about 4 C-CASA categories (suicidal ideation, preparatory acts, suicide attempt, non-suicidal self injury) for present and lifetime periods, comparing each method pairwise and also to a latent class analysis (recommended for 'missing or imperfect gold standard' applications; Pepe, 2003). Bootstrapping with 1000 resamples estimated bias-corrected accelerated confidence intervals.
Results: Agreement between assessment methods and the latent class for each C-CASA category ranged from 'good' (kappa 0.60 to 0.74) to 'excellent' (0.75 or higher). Pairwise agreement between assessments was in the 'good' or 'fair' (0.40 to 0.59) range, except for Old-STS and C-SSRS agreement about preparatory acts, which was 0.38. Re-rating of interviews from recordings produced good to excellent reliability estimates (kappa of 0.60 to 0.89 for lifetime ratings, and 0.57 to 0.96 for past month ratings). The C-SSRS took significantly longer than the other interviews to complete, but all interviews were well tolerated and rated favorably by patients on satisfaction measures.
Conclusions: Findings indicate that all three measures—C-SSRS, Old STS, and New S-STS, demonstrate strong inter-rater reliability (based on re-rating of recordings) and strong convergence as measures of suicidal ideation, preparatory acts, suicidal behavior, and non-suicidal self injury (the four C-CASA categories that make sense to evaluate in an inpatient interview). Reliability estimates were higher benchmarked against the latent class categories than head-to-head, because head-to-head comparisons are penalized due to errors in either measure. The lower agreement between some interview methods about preparatory acts was due to differences in item content, not due to inherent unreliability of the assessment. Patient satisfaction was high across all methods. The long length of some interviews was offset by patients appreciating the thoroughness of the evaluation. Limitations include that the use of an inpatient sample resulted in high rates of suicidal ideation, increasing the standard error of estimates (albeit all results still achieved high degrees of statistical significance). It also was not possible to examine associations between diagnoses and suicidal ideation and behavior.
Keywords: assessment, suicidal ideation, suicidal behavior, reliability, psychometrics.
Disclosures: E. Youngstrom, Part 1: Consultation with Lundbeck; A. Hameed, Nothing to Disclose; M. Mitchell, Nothing to Disclose; A. Freeman, Nothing to Disclose; A. Van Meter, Nothing to Disclose; G. Algorta, Nothing to Disclose; A. Gelenberg, Part 1: I own stock in Healthcare Technology Systems, Inc., which could profit from electronic versions of some of the rating scales designed to measure suicidality., Part 2: Only my salary from Hershey Medical Center., Part 3: none, Part 4: I was PI on an investigator-initiated grant from Pfizer, Inc., which funded the trial described in this poster.; R. Meyer, Nothing to Disclose.

T205. Methylation of the Leukocyte Glucocorticoid Receptor: Early Adversity and HPA Axis Function

Audrey R Tyrka*, Lawrence H Price, Carmen J Marsit, Noah S Philip, Linda L Carpenter

Brown Medical School, Providence, Rhode Island

Background: Early life stress exposure is a major risk factor for child and adult psychopathology. Epigenetic changes to genes that regulate stress reactivity have been proposed as a mechanism of this effect. Recent work in adults and infants has shown that of methylation of the promoter of the glucocorticoid receptor (GR) gene (*NR3C1*) is associated with early adverse exposures. We previously published preliminary findings showing associations of stressful early experiences with *NR3C1* methylation. This study aimed to examine the effect of childhood adversity on *NR3C1* methylation and the functional consequences of methylation in a separate sample of healthy adults.
Methods: Healthy adults, $N = 107$, without current major Axis I disorder participated in this study. The Childhood Trauma Questionnaire was used to assess experiences of childhood abuse and neglect, and childhood parental loss was determined ($N = 21$). DNA was extracted from frozen whole blood. Sodium bisulfite modification of DNA was

performed and methylation at the *NR3C1* promoter region was examined with a quantitative pyrosequencing approach. Plasma cortisol was measured during the dexamethasone/corticotropin-releasing hormone (Dex/CRH).
Results: Childhood maltreatment was significantly correlated with methylation of the *NR3C1* promoter, after controlling for age and sex ($p<0.05$), and childhood parental loss showed a trend-level association ($p<0.10$). At other CpG sites, methylation was significantly associated with cortisol response to the Dex/CRH test ($p<0.05$).
Conclusions: These findings suggest that adverse parenting experiences in childhood may lead to epigenetic modifications of the human GR gene which could partially underlie the associations between childhood adversity and risk for psychopathology.
Keywords: methylation, epigenetics, cortisol, abuse, early-life stress, dex/crh test.
Disclosures: A. Tyrka, Nothing to Disclose; L. Price, Nothing to Disclose; C. Marsit, Nothing to Disclose; N. Philip, Nothing to Disclose; L. Carpenter, Nothing to Disclose.

T206. Third Trimester Free Thyroxine and Thyroid Binding Globulin Predict Subsequent Perinatal Depression and Anxiety Symptoms as Well as Syndromal Depression

Cort Pedersen*, Jacqueline Johnson, Nacire Garcia, Melissa Stansbury, Jane Leserman

University of North Carolina, Chapel Hill, North Carolina

Background: The prevalence of syndromal depression (major or minor) is approximately 10–15% during pregnancy and postpartum; subsyndromal depressive and anxiety symptoms are also common. These mood disturbances can have profound negative effects on mother-infant and family relationships. Subtle dysregulation of the hypothalamic-pituitary-thyroid axis is associated with major depression, elevated depressive symptoms and anxiety disorders. High estrogen levels during pregnancy cause an approximately 150% increase in thyroid-binding globulin (TBG) concentrations. Recent studies have established that the normal range of FT4 levels is lower in pregnant compared to non-pregnant women. We previously reported a significant negative relationship between FT4 concentrations at pregnancy week 38 and depressive symptoms at that time point and over the first 6 weeks postpartum. The current study examined the relationships of late pregnancy TBG and FT4 (the thyroid variables that change the most during gestation) to depression and anxiety ratings as well as syndromal depression over the subsequent perinatal period.
Methods: We studied 199 euthyroid, pregnant women from a low income public health clinic in Raleigh, NC. Mothers were screened at 31–33 weeks of pregnancy at which time serum was collected for thyroid assays. Women with TSH concentrations outside the normal range or elevated thyroid antibody titers were excluded. Home visits were conducted at pregnancy weeks 35–36 and postpartum weeks 6 and 12. The MINI Neuropsychiatric Interview was administered during the 1st and 3rd home visits. A Structured Trauma Interview was completed at the 1st home visit. Depression and anxiety were rated at each visit (Edinburg Postnatal Depression Scale-EPDS, Montgomergy–Åsberg Depression Rating Scale- MADRS, Spielberger State/Trait Anxiety Inventory-STAI, and Hamilton Anxiety Rating Scale-HAM-A).
Results: The majority of our sample was Hispanic (63.8%), 21.6% was black, and mean education was 10.8 years (SD = 3.1). Mothers reported an average of 4.5 categories of traumatic events in their lifetimes (eg, sexual and physical abuse, childhood neglect and violence). Subjects became significantly less depressed and anxious over time ($p<0.001$) on all measures; however, 18.6% had syndromal major (DSM-IV) or minor (RDC) depression during the perinatal period. Because thyroid-by-time interactions were not significant, we examined the effects of screening thyroid values on depression and anxiety ratings on each instrument over the entire perinatal period. We fit repeated measures random coefficients models with screening FT4 or TBG concentrations and controls (eg, demographic, pregnancy or lactation-related variables, number of lifetime traumas, TSH and sex hormone levels at screening, BMI) to predict depression and anxiety over all pregnancy and postpartum time points. We found that FT4 and TBG were consistently and negatively related to depression and anxiety ratings as follows: EPDS ($p=0.029$; $p=0.026$), STAI ($p=0.134$; $p=0.035$), MADRS ($p=0.029$; $p=0.017$), and HAM-A ($p=0.006$; $p=0.022$). Number of categories of life-time trauma was strongly related to depression and anxiety ratings ($p<0.001$ in all cases). Logistic regression showed that women with low FT4 and TBG had greater risk of syndromal depression ($p=0.063$; $p=0.011$). Number of life-time traumas was also significantly predictive of syndromal depression ($p=0.024$). TSH and sex hormone concentrations at screening were not predictive of mood ratings or syndromal depression. TSH concentrations were within the euthyroid range at all time-points for all subjects. FT4 concentrations were below the normal non-pregnant range in 20 subjects at screening but rose into the normal range in all mothers by 12 weeks postpartum. In agreement with prior studies, the usual negative correlation between FT4 and TSH was lost during pregnancy but was re-established by postpartum week 12.
Conclusions: Our findings have important clinical implications. Pregnancy FT4 and TBG may be useful in identifying mothers at risk of developing greater perinatal depression and anxiety symptoms and, most importantly, syndromal depression. Additional studies are necessary to determine how early in pregnancy these thyroid variables predict subsequent mood disturbance. Our results also suggest that thyroxine supplementation of mothers with lower pregnancy FT4 concentrations may decrease their risk of developing perinatal mood disturbance. Although many studies have robustly linked trauma history to depression, screening FT4 and TBG (especially the latter), were comparable to lifetime trauma experiences as predictors of perinatal syndromal depression. We found no relationship between trauma history and screening FT4 or TBG. Our findings may also have pathophysiological significance. Mothers who had greater perinatal depression and anxiety appear to be less sensitive to up-regulation of TBG by the

elevated estrogen levels of pregnancy and their lower FT4 concentrations suggest that they secrete less thyroxine. Because pregnancy TSH was not related to perinatal depression/anxiety or negative feedback (pregnancy FT4 levels), less thyroxine secretion in mothers with greater perinatal depression and anxiety indicates that their thyroid glands may be less sensitive to TSH.
Keywords: thyroid perinatal depression perinatal anxiety trauma.
Disclosures: C. Pedersen, Nothing to Disclose; J. Johnson, Nothing to Disclose; N. Garcia, Nothing to Disclose; M. Stansbury, Nothing to Disclose; J. Leserman, Nothing to Disclose.

T207. Dexamethasone Attenuates Impaired Fear Inhibition in PTSD in a Double-blind Placebo-controlled Study

Tanja Jovanovic*, Seth D Norrholm, Jennifer Stevens, Karin M Nylocks, Kimberly Kerley, Bekh Bradley, Kerry J Ressler

Emory University School of Medicine, Atlanta, Georgia

Background: Our previous studies with traumatized adult populations showed that PTSD patients with the highest symptoms have impaired inhibition of fear-potentiated startle in the presence of safety cues (Jovanovic *et al*, 2010; Norrholm *et al*, 2010). Reported alterations in cortisol levels in PTSD subjects after dexamethasone administration (Yehuda, 2009) have been associated with dysregulated fear responses (Jovanovic *et al*, 2011). This study examined the effects of dexamethasone administration on hypothalamic-pituitary-adrenal (HPA) function and fear-potentiated startle in trauma-exposed individuals with and without PTSD in a placebo-controlled double-blind study.
Methods: The study sample ($N = 58$) was recruited from a highly traumatized civilian population at Grady hospital in Atlanta, GA. Presence of PTSD was assessed with the PTSD Symptom Scale (PSS, Foa & Tolin, 2000), based on DSM-IV criteria. We used a fear discrimination procedure, in which one visual conditioned stimulus (CS+, danger cue) was paired with aversive airblasts to the throat (US), and another conditioned stimulus (CS−, safety cue) was presented without airblasts. Fear-potentiated startle was measured during the fear conditioning task using electromyographic recordings of the eyeblink muscle contraction in response to an acoustic startle probe. We used a double-blind cross-over design in which participants either received placebo (PBO) or matched 0.5 mg of dexamethasone (DEX) at 11pm the night prior to the fear conditioning session. The subjects were randomized for the first treatment and then received the alternate pill one week later and repeated fear-potentiated startle.
Results: Both PTSD subjects ($F(1, 21) = 9.67$, $p = 0.005$) and trauma controls ($F(1, 35) = 40.54$, $p < 0.001$) showed significant increases in startle to the CS+ compared to baseline startle responses, with no interaction effects of dexamethasone. In the trauma controls startle was higher to the CS+ than CS− ($F(1, 35) = 35.46$, $p < 0.001$) regardless of dexamethasone condition. However, in the PTSD subjects there was a significant interaction of trial type and dexamethasone ($F(1, 21) = 4.33$, $p = 0.05$). In the PBO condition, the PTSD subjects had higher fear-potentiated startle to the CS− than the controls ($F(1, 56) = 6.18$, $p = 0.02$); this group difference was no longer present in the DEX condition, as both groups showed reduced levels of fear to the safety signal.
Conclusions: These preliminary results suggest that inhibition of fear-potentiated startle in the presence of safety cues may be associated with cortisol suppression after dexamethasone administration. The present study replicated our earlier findings of impaired fear inhibition in PTSD (Jovanovic *et al*, 2010), as well as HPA axis involvement in fear responses (Jovanovic *et al*, 2011). The addition of the experimental placebo control suggests that the administration of low dose dexamethasone may attenuate fear-related symptoms in PTSD.
Keywords: fear-potentiated startle, fear conditioning, PTSD, dexamethasone, HPA.
Disclosures: T. Jovanovic, Nothing to Disclose; S. Norrholm, Nothing to Disclose; J. Stevens, Nothing to Disclose; K. Nylocks, Nothing to Disclose; K. Kerley, Nothing to Disclose; B. Bradley, Nothing to Disclose; K. Ressler, Nothing to Disclose.

T208. Next-generation Psychiatric Drugs: Acetyl-L-carnitine

Carla Nasca*, Danielle Zelli, Per Svenningsson, Aleksander Mathé, Bruce S Mcewen

The Rockefeller University, New York, New York

Background: Depression, a growing problem worldwide that desperately needs new therapeutic approaches, is often triggered by stressful experiences, which induce epigenetic modifications leading to altered gene profiles. Current antidepressants require at least 2–3wk to significantly improve mood, leaving patients at risk for suicide in the early phases of treatment, and approximately 40% of patients are considered treatment resistant. Moreover, although anxiety and depression are higher risks of suicide, individuals may have a predisposition or tendency towards suicidal ideation, which is aggravated further by stressors, such as poverty, social isolation and childhood abuse. In the new era of *pharmacoepigenetics*, the reversible nature of epigenetic modificationshas prompted a widespread interest in histone deacetylase (HDAC) and DNA methyltransferase inhibitors, as well as in the promising findings on the fast acting, naturally occurring, antidepressant acetyl-L-carnitine (LAC), providing a hope in developing rapidly acting psychiatric drugs and in uncovering the limitations of current antidepressants.
Methods: C57bl mice were stressed for 2 h either acutely or chronically for 21 consecutive days; euthanasia occurred 45 min after acute stress and 24 h after chronic stress. Additional group of chronically restraint stressed mice were subjected to an acute novel stress on day 22. Transcript levels were analyzed by RT-PCR. For IHC, all animals were killed by deep anesthesia, followed by transcardially perfusion and specific histone mark antibodies were used. CORT levels were measured by RIA.

Results: Previously, we showed that LAC (Nasca *et al*, PNAS 2013; Comment in (i) Nature Research Highlight 2013, Flight MH.; (ii) PNAS Commentary 2013, Russo SJ and Charney DS., PNAS 2013) induces rapid and long lasting antidepressant effects on two rodent models of depression through the epigenetic regulation of the metabotropic glutamate receptor, mGlu2, in brain regions critically involved in the pathophysiology of depression: the hippocampus and prefrontal cortex. Because of the importance of stressors in triggering depression, we are currently exploring a stress challenge paradigm that allows us to evaluate acute and chronic stress effects and whether the brain responses to acute stressors is modified by a previous period of chronic stress in terms of chromatin assembly and its interplay with the glutamate transcripts, such as presynaptic mGlu2 and mGlu3 receptors, which inhibit glutamate release, and postsynaptic mGlu5 and NMDA receptors, which regulate mechanisms of synaptic plasticity. We have thus far found that the hippocampus and prefrontal cortex respond in different and complementary ways to acute and chronic stress and that an organism with a history of chronic stress loses the ability to respond appropriately to an acute stress, both in terms of epigenetic marks and glutamate transcripts, which appear to be causally connected. Remarkably, chronic stress altered the response to an acute stress inducing a complex, surprisingly rapid, and regionally specific pattern of chromatin remodeling within the DG, CA3 and CA1 hippocampal subregions and the medial orbital and prelimbic cortex, causing divergent changes in H3K27ac and H3K27me3 immunoreactivity that exactly parallel the stress challenge-induced reductions in glutamate transcripts. Finally, we discovered that the oral supplementation of the safe endogenous compound, LAC, prior to the stress exposure, prevents the stress induced epigenetic reprogramming of gene expression, blocking the stress effects on the histones coupled to the glutamate transcripts, therefore, abolishing the stress induced aberrant gene transcription. Moreover, we are comparing LAC efficacy with both HDAC inhibitors and current antidepressants, with the goal of finding better antidepressant treatments and providing a mechanistic understanding of SSRI action and how individuals sharing similar genes might show large differences in their response to treatment.
Conclusions: LAC prevention of stress effects on the glutamate synapse paves the way in understanding how epigenetic changes, induced by psychological stressors, alter brain plasticity, shaping individual vulnerability to stress through brain changes that resemble those observed in depressed patients. Since the hippocampus encodes memories related to spatial and stressful daily events and the PFC plays an important role in working memory and executive function as well as in self-regulatory behaviors, these findings are particularly relevant to the pathophysiology of anxiety and depression. Moreover, new ongoing results on effects of selective GR- and MR- antagonists support the fundamental role of chromatin remodeling in the brain responses to stress as a novel potential therapeutic target by which overcoming the limitations of common psychiatric medications and developing drugs with faster onset of effects and good profile of efficacy and tolerability; therefore reducing the probability of suicidal behavior. Being a natural substance, LAC represents a potential alternative to the use of HDAC inhibitors and may provide better therapeutic outcomes with fewer side effects.
Keywords: epigenetic, depression, stress, Acetyl-L-carnitine, glucocorticoids.
Disclosures: C. Nasca, Nothing to Disclose; D. Zelli, Nothing to Disclose; P. Svenningsson, Nothing to Disclose; A. Mathé, Nothing to Disclose; b. mcewen, Nothing to Disclose.

T209. Sex Steroid Receptor Gene Expression Correlates with the Expression of Neurodevelopmental Genes and Modulates Gray Matter Volume in the Human Brain

Tuong-Vi Nguyen*, Peter Schmidt, J Shane Kippenhan, Melanie Sottile, Victor Ekuta, Bhaskar S Kolachana, Beth A Verchinski, Venkata S Mattay, Joel E Kleinman, Barbara Lipska, Daniel R Weinberger, Karen F Berman

NIH, Bethesda, Maryland

Background: Biological effects of estrogen receptor alpha (*ESR1*), estrogen receptor beta (*ESR2*) and the progesterone receptor (*PGR*) have been documented in the animal brain, and variations in these sex steroid receptor (SSR) genes have been associated with psychiatric disorders in humans. Yet the mechanisms underlying these associations remain elusive. We hypothesized that SSRs may modulate the expression of neuronal and glial markers in the human brain, as they do in lower animals, and that morphological effects of SSR genes would be measurable in the adult human brain. To explore these hypotheses, we used a two-pronged approach: (1) in post-mortem human brains, we examined the expression of SSR genes across the lifespan, from in utero development through old age, and correlated these expression levels with those of candidate genes related to neuronal migration, dendritic remodeling, and glial proliferation; and (2) in structural MRIs of healthy living adults, we examined associations between gray matter volumes (GMV) and SSR genotypes.
Methods: To characterize SSR gene expression and correlations with the expression of genes related to neuronal/glial development across the lifespan, we studied 269 post-mortem prefrontal cortices ([PFC], fetal age 14 weeks to 80 years old, 92 females; http://braincloud.jhmi.edu/). Genes related to neuronal/glial development were selected according to two main criteria: (1) previous evidence of sex steroid-related modulation of these markers in human and animal cell lines, and (2) relevance to brain development across the lifespan. Neuronal markers included genes involved in prenatal neuronal migration and postnatal dendritic remodeling (ezrin, LIM domain kinases [*LIMK1/2*] genes). Glial markers included genes related to oligodendrocyte proliferation/myelination (myelin basic protein, oligodendrocyte myelin glycoprotein, myelin-associated glycoprotein), as well as a marker of astrocytic proliferation (glial fibrillary acidic protein). Two-color custom-spotted oligonucleotide microarrays were used to determine mRNA expression of each gene. A False Discovery Rate ($q < 0.05$) correction for multiple comparisons was applied to all analyses. To characterize adult brain phenotypes associated with developmental effects of SSRs, we tested for associations between SSR single nucleotide polymorphisms (SNPs)

and GMV in structural MRIs of healthy adults. We acquired 1.5T T1-weighted SPGR MRI scans in 289 subjects (18–55 years old, 157 females) and analyzed voxelwise Jacobian-modulated gray matter volumes (GMV) obtained from SPM8-based segmentation and DARTEL-based spatial normalization. Genotypes for *ESR1* (rs2234693, rs9340799), *ESR2* (rs1256049, rs4986938) and *PGR* (rs10895068) SNPs were determined with 5′exonuclease Taqman allelic discrimination assay. A correction for multiple comparisons using Montecarlo simulation was applied to all analyses ($p<0.05$ at the cluster level).
Results: In the post-mortem brain, we found *ESR2* expression to be higher prenatally than postnatally and to be correlated with the expression of genes related to neuronal migration and dendritic remodeling (see Methods, $q<0.05$). *ESR1* and *PGR* expression levels were higher postnatally and correlated with the expression of genes related to glial development (see Methods, $q<0.05$). Correlations between the expression of estrogen receptor genes and genes related to neuronal development differed across genders: compared to men, women showed more positive correlations between the expression of *ESR1* and *LIMK2* and more negative correlations between the expression of *ESR2* and *LIMK1*. In both men and women studied *in vivo* with MRI, we found that variation in SSR genes modulated GMV in the caudate nucleus (*ESR2*); in the PFC, temporal, parietal and occipital lobes (*ESR1*); and in the post-central/supramarginal gyrus (*PGR*). Variation in the estrogen receptor genes also modulated GMV in a sexually dimorphic manner: genotypes linked to higher estrogen receptor expression were associated in women with increased GMV in the frontal and parietal lobes (*ESR1*), and with decreased GMV in the posterior lobe of the cerebellum (*ESR2*), whereas the opposite patterns were observed in men. These sex differences in GMV parallel the female-specific positive correlation between the expression of *ESR1* and *LIMK2*, as well as the female-specific negative correlation between the expression of *ESR2* and *LIMK1* observed in the postmortem PFC. In contrast to other SSR genes, variation in the *ESR2* gene modulated subcortical GMV in the caudate and in the cerebellum. Since subcortical and cerebellar development predominantly occurs during the prenatal period, these findings are consistent with *ESR2*'s higher prenatal expression and with its correlation with the expression of genes involved in neuronal migration.
Conclusions: *ESR1*, *ESR2* and *PGR* show specific developmental trajectories of gene expression and correlations with selected neuronal and glial markers in the post-mortem brain, and variations in SSR genes translate into morphological variations in the adult brain studied *in vivo*. These findings highlight potential mechanisms through which hormones may affect brain structure in an enduring way and may lead to sex differences in the susceptibility to and course of neuropsychiatric disorders.
Keywords: estrogen receptor, progesterone receptor, estradiol, progesterone, development.
Disclosures: T. Nguyen, Nothing to Disclose; P. Schmidt, Nothing to Disclose; J. Kippenhan, Nothing to Disclose; M. Sottile, Nothing to Disclose; V. Ekuta, Nothing to Disclose; B. Kolachana, Nothing to Disclose; B. Verchinski, Nothing to Disclose; V. Mattay, Nothing to Disclose; J. Kleinman, Nothing to Disclose; B. Lipska, Nothing to Disclose; D. Weinberger, Nothing to Disclose; K. Berman, Nothing to Disclose.

T210. Disruption of Early Maternal Care Results in Epigenetic Regulation of the Oxytocin Receptor (OXTR) Gene in Rhesus Macaques

Maggie B Baker, Stephen Lindell, Qiaoping Yuan, Zhifeng Zhou, J Dee Higley, Stephen Suomi, Christina Barr*

NIAAA/NIH, Bethesda, Maryland

Background: Oxytocin is a neuropeptide that produces affiliative, amnesic and anxiolytic affects. Given its roles in some of these processes, regulation of this system may moderate risk for stress-related disorders. We wanted to examine effects of disruption of early maternal care in rhesus macaque infants on binding of an activating histone (H3K4me3) and mRNA expression levels in adult brain.
Methods: Brains ($N=14$) were archived from male macaques (*M. mulatta*) that were raised by their mothers (MR) or in peer-only groups (PR), an established model of early adversity. Hippocampal samples were dissected for DNA and RNA isolation. Chromatin Immunoprecipitation was performed with anti- H3K4me3, and ChIP- and RNA-SEQ were performed. Data previously generated by microarray was used to verify RNA-SEQ results.
Results: We observed H3K4me3 binding that spanned the first exon of the OXTR gene (Chr2: 52 233 300–52 234 100), with peak binding observed in a central 300 nucleotide region. Both overall H3K4 binding and within-peak binding were decreased in PR monkeys ($P=0.01$ and $P=0.005$). Oxytocin mRNA expression levels were also decreased ($P=0.008$).
Conclusions: Variation in maternal care as it relates to oxytocin system functioning has been demonstrated in rodents. Our results show that disrupted maternal care produces decreased binding of an activating histone and lower OXTR mRNA expression levels in adult macaque brain. Early environment-induced epigenetic regulation of OXTR may increase vulnerability to stress-related disorders, either alone of interactively with functional genetic variation. It could also produce long lasting effects on substance use, social cognition and affiliative behavior.
Keywords: oxytocin, stress, epigenetic, macaque, Chip-SEQ.
Disclosures: M. Baker, Nothing to Disclose; S. Lindell, Nothing to Disclose; Q. Yuan, Nothing to Disclose; Z. Zhou, Nothing to Disclose; J. Higley, Nothing to Disclose; S. Suomi, Nothing to Disclose; C. Barr, Nothing to Disclose.

T211. Consumption of a High-fructose Diet during Adolescence Alters HPA Axis Function, Increases Anxiety-like and Depressive-like Behaviors, and Remodels Hypothalamic Gene Expression

Gretchen Neigh*, Zachary Johnson, Constance Harrell

Emory University, Atlanta, Georgia

Background: The 26% increase in per capita fructose consumption over the past thirty years has been linked to

the obesity epidemic and Metabolic Syndrome, as a growing body of literature implicates fructose in altering lipid profiles and promoting insulin resistance. However, little is known about the impact of a high-fructose diet on behavior and the brain. Given that adolescents are the highest consumers of fructose and in a 'critical period' of brain development, this study sought to examine the effects of an adolescent high-fructose diet on affective-like behaviors, the HPA axis, and hypothalamic gene expression using whole transcriptome RNA-sequencing.
Methods: Male pups ($n = 29$) born to timed pregnant Wistar rats ($n = 17$) were culled on post-natal day (PND) 3 and weaned on PND23. Two days post-weaning (PND25), rats were pair-housed and assigned to either standard chow (Lab Rodent Diet 5001; $n = 14$) or a 55% fructose diet (Research diets D05111802; $n = 15$) and remained on their assigned diet through the conclusion of the study. Animal weight and fasting blood glucose was monitored weekly, while food consumption was monitored daily. Affective-like behavior was assessed in adulthood. A fasting glucose tolerance test was performed on PND80. In addition, trunk blood was collected, and plasma corticosterone was measured via ELISA. A separate cohort of male rats underwent the same procedures with the exception that the diet commenced in adulthood (after PND70), but total days of exposure were identical between the adolescent and adult cohorts. In order to assess the effects of a high-fructose diet on gene expression RNA was extracted from the hypothalamus of each individual in the adolescent groups and RNA-Seq performed generating $\sim$25 million reads per sample. Raw sequence reads was mapped to the most recent RAT assembly (RGSC5.0) using the STAR aligner and differential expression performed Cufflinks software suite.
Results: Consumption of a high-fructose diet during adolescence resulted in elevated baseline corticosterone concentrations and a blunted corticosterone response to an acute stressor as compared to age-matched controls fed a standard diet ($p < 0.05$). Assessment of behavior in the elevated plus maze demonstrated that a high-fructose diet throughout adolescence resulted in a reduction in open arm time as compared to age-matched controls fed a standard diet ($p < 0.05$). In addition, consumption of a high fructose diet throughout adolescence led to a reduced latency to immobility in the forced swim test and a greater amount of time spent immobile than controls fed a standard diet ($p < 0.05$). No changes in behavior or HPA axis function were observed in male rats that began the diet after adolescence. Consumption of the high-fructose diet during adolescence within these same subjects also resulted in significantly different expression pattern within the hypothalamus. In total 946 genes, or 5.4% of those tested, were differently expressed between the two groups.
Conclusions: The results of this analysis show a clear and significant effect of consuming a high-fructose diet during adolescence upon both behavioral and gene expression phenotypes. Furthermore, the effects of a high-fructose diet on behavior and HPA axis function appear to be specific to adolescent exposure. Consumption of a high-fructose diet starting in the peri-adolescent period and continuing into adulthood increased both anxiety-like and depressive-like behaviors as well as altered function of the HPA axis. With regards to expression, RNA-Seq analysis showed that 946 genes within the hypothalamus were differently expressed as a result of consuming a high-fructose diet. Of particular interest is the finding that mRNA levels of multiple genes within the dopaminergic system, such as dopamine transporter and tyrosine hydroxylase, were up-regulated by consumption of a high-fructose diet suggesting a potentially important link between diet, expression of genes within dopamine pathways, and depressive-like and anxiety-like behavior. Given the growing rates of consumption of high-fructose diets, particularly in the adolescent population, an understanding of the behavioral and brain implications of this environmental exposure are essential to anticipating and treating potential behavioral and brain disorders.
Keywords: fructose, adolescence, hypothalamus, HPA axis, mood disorder.
Disclosures: G. Neigh, Nothing to Disclose; Z. Johnson, Nothing to Disclose; C. Harrell, Nothing to Disclose.

T212. Use of Gonadotropin-releasing Hormone Agonist Experimental Model to Isolate Predictors of Depressive Symptoms in Menopause: Role of Nocturnal Hot Flashes and Sleep Disturbance

Hadine Joffe*, Semmie Kim, Sybil Crawford, Marlene P Freeman, Nicole Economou, David White, Lee S Cohen, Janet E Hall

Brigham and Women's Hospital, Boston, Massachusetts

Background: The menopause transition is a period of increased risk for depression in women. Epidemiologic studies have identified hot flashes, sleep disruption and fluctuating estradiol levels as risk factors for menopause-associated depression. However, because these patient-level characteristics are closely linked, it is difficult to disentangle them. We have previously shown that changes in depressive symptoms correlate with changes in perceived sleep quality in midlife women. Using a gonadotropin-releasing hormone agonist (GnRHa) experimental model, we have also demonstrated that depressive symptoms are more common after GnRHa administration among women who develop hot flashes in response to the GnRHa compared to those who remain free of hot flashes, although clinical depression is rare. Because nocturnal hot flashes are associated with sleep fragmentation, we hypothesized that sleep interruption would explain the association between hot flashes and depressive symptoms. A GnRHa experimental model enables the contributions of hot flashes and sleep disturbance to depressive symptoms to be disentangled because hot flashes are induced to varying degrees in two-thirds of women receiving GnRHa, and because pre-GnRHa sleep quality and depressive symptoms can be used to quantify changes in sleep and mood occurring after GnRHa administration. We isolated the effect of nighttime hot flashes on depressive symptoms from the effect of sleep disruption using a GnRHa model to induce hot flashes and simulate the gonadal steroid hormone changes of menopause.
Methods: Twenty-nine healthy premenopausal women without baseline hot flashes, sleep disorders, or psychiatric illness received the depot GnRHa leuprolide 3.75 mg during

the mid-luteal phase. Before and after 5 weeks on the GnRHa, we measured depressive symptoms, hot flashes, sleep disruption, and serum estradiol. Depressive symptoms were measured with the clinician-rated Montgomery-Asberg Depression Rating Scale (MADRS). Hot flashes were reported separately for daytime and nighttime symptoms on a daily diary. Perceived sleep quality was measured using the Pittsburgh Sleep Quality Index (PSQI). Subjective sleep fragmentation was assessed by the number of awakenings and wake-time after sleep-onset (WASO, minutes) reported on a 7-day sleep diary. Objective measures of sleep fragmentation (number of awakenings, WASO time) were obtained concurrently with the sleep diary using ambulatory sleep polysomnography (Safiro, Compumedics Ltd) for two nights at each time point. Within-woman change in depressive symptoms and each sleep parameter was calculated by subtracting symptom levels obtained prior to GnRHa from the corresponding data collected 5 weeks after GnRHa administration. Multivariate linear regression models were used to investigate the impact of the number of nighttime hot flashes and changes in each sleep parameter (PSQI score, subjective and objective number of awakenings and WASO time) on changes in depressive symptoms (MADRS scores).

Results: Of 29 women receiving GnRHa (age 27.3 ± 7.2 years), MADRS scores increased from before to after GnRHa (1.0 ± 1.1 to 4.1 ± 5.4; mean increase 2.6 ± 3.5, $p < 0.005$). Only one participant (3%) had a post-GnRHa MADRS score suggesting clinical depression. Twenty (69%) women reported developing hot flashes (median 3.7 per night, interquartile range [IQR] 2.0–7.0, and median 3.3 per daytime, IQR 2.2–15.0). Estradiol was suppressed to postmenopausal levels ($\leqslant 20$ pg/ml) in all participants. Increased depressive symptoms (MADRS scores) correlated with the reported number of nighttime hot flashes ($r = 0.50$, beta = 3.2, $p = 0.006$), but not daytime hot flashes ($r = 0.25$, $p = 0.18$). An increase in MADRS scores also correlated with worsening of perceived sleep quality (PSQI $r = 0.45$, $p = 0.01$) and with more WASO time reported on the sleep diary ($r = 0.34$, $p = 0.08$) but not with other sleep fragmentation parameters (objective awakenings, subjective awakenings, objective WASO time: all $r \leqslant 0.30$, all $p \geqslant 0.11$). After adjusting for the number of nighttime hot flashes and either the change in PSQI scores or WASO time reported, the number of nighttime hot flashes remained a significant predictor of increased depressive symptoms (beta range 2.8—3.1, $p \leqslant 0.02$) whereas only PSQI scores remained a significant predictor in the adjusted model (beta = 1.0, $p = 0.02$).

Conclusions: Using a GnRHa experimental model of menopause, we found that nighttime hot flashes are linked with depressive symptoms independent of their effect on sleep fragmentation. Depressive symptoms were associated with the development of nighttime, but not daytime, hot flashes. While reduction in perceived sleep quality and increased WASO time also correlate with an increase in depressive symptoms, these sleep changes do not explain the association between nighttime hot flashes and depressive symptoms. Results of this study suggest that nighttime hot flashes contribute to the vulnerability to depressive symptoms during the menopause transition and raise the possibility that hot flashes occurring at night specifically may be a marker of neural susceptibility to reproductive endocrine perturbations that manifest with depressive symptoms.

Keywords: depression; sleep; estrogen; menopause; hot flashes; women.

Disclosures: H. Joffe, **Part 1:** Noven: consultant/advisory board, Sunovion: unpaid consultant, **Part 2:** N/A, **Part 3:** N/A; S. Kim, Nothing to Disclose; S. Crawford, Nothing to Disclose; M. Freeman, **Part 1:** Research: Lilly; Forest; GSK, Consulting: PamLab, Advisory Boards: Otsuka; Lundbeck; Takeda; DSM Nutritionals: Med Editing, **Part 4:** Lilly; Forest; GSK; N. Economou, Nothing to Disclose; D. White, **Part 1:** Apnicure Inc: I am an employee and Chief Scientific Officer for the company., Philips Respironics: I was the Chief Medical Officer until 1/1/13 and am now a consultant., **Part 2:** As above., **Part 3:** As above.; L. Cohen, **Part 1:** Advisory/Consulting: Noven Pharmaceuticals; PamLab LLC, **Part 4:** Astra-Zeneca Pharmaceuticals; Bayer HealthCare Pharmaceuticals; Bristol-Myers Squibb; Cephalon, Inc.; Forest Laboratories, Inc.; GlaxoSmithKline; National Institute on Aging; National Institutes of Health; National Institute of Mental Health; Ortho-McNeil Janssen; Pfizer Inc.; Sunovion Pharmaceuticals, Inc.; J. Hall, Nothing to Disclose.

T213. Menopause and Metabolic Function: Interactive Influences on Depression Symptoms and Emotional Regulation

Alison Berent-Spillson*, Courtney Marsh, Carol Persad, Jon-Kar Zubieta, Yolanda Smith

University of Michigan, Ann Arbor, Michigan

Background: Depression, the most common psychiatric disorder, has a lifetime prevalence of nearly 20% in women. Aging women have an increased risk for developing depression, coinciding with fluctuating hormone levels during menopause. This risk may be exacerbated by metabolic disturbances that frequently accompany the menopause transition. The aging brain is sensitive to alterations in the metabolic environment, as demonstrated by the association between metabolic disorders and mood disturbances. There is clear evidence that reproductive hormones can influence neuronal function and emotion regulation; however, it is not yet established whether interactions between hormone levels and metabolism during normal aging influence emotion and mood state. Interpretation and inference from the aging literature is limited, in part due to the cross-sectional design of many studies. Changes in cerebral functioning often occur before noticeable age effects in behavior, raising the question of when these age-related changes are initiated. We evaluated the relationships between emotion regulation, metabolic function, and menopause status in women near the menopause transition. We hypothesized that the transition to menopause would be associated with changes in brain activation while viewing emotional images, reflecting the female aging process. We expected to find increased activation in limbic emotion regulation regions (amygdala, nucleus accumbens, cingulate gyrus, and prefrontal cortex). We further expected that women defined as insulin resistant

would demonstrate a shift in activation towards 'older' patterns, with corresponding increases in depression evaluation scores.
Methods: 64 women participated in this cross-sectional analysis, with a mean age of 52.0 ± 4.2 (SD) years. Women were classified as pre- or postmenopause based on hormonal and menstrual cycle criteria, and were classified as control or insulin resistant (IR) based on the homeostatic model assessment. All participants were otherwise healthy, not clinically depressed, and not taking hormones or psychoactive medication. Women underwent clinical evaluation including assessment of reproductive hormone levels and metabolic function, behavioral assessment of affect and mood symptoms, and fMRI to observe neural activation patterns while viewing emotionally neutral or unpleasant images. Data were analyzed using correlational and General Linear Model analyses comparing menopause status and insulin resistant groups, with age covariate. fMRI image data were analyzed using statistical parametric mapping in SPM8.
Results: Premenopausal women defined as insulin resistant had the highest scores on the Beck Depression Inventory (5.09 ± 3.2), which were similar to both postmenopausal groups (4.79 ± 4.6 control; 4.50 ± 3.7 IR), but significantly higher than control premenopausal women (2.90 ± 3.5, $p = 0.03$). During the negative emotional imagery task, all women had brain activity in the medial temporal cortex (MNI coordinates 54, −64, 0, family wise error corrected $p < 0.000$ right; −52, −72, 4, $p < 0.000$ left). All postmenopausal women and control premenopausal women had activation in the medial prefrontal cortex centering near −6, 52, 18 left ($p < 0.000$) and 6, 60, 12 right ($p < 0.000$). IR premenopausal women had activation in the left amygdala (−26, −4, −18, $p < 0.000$). Measures of metabolic function were associated with correct identification of images as emotionally unpleasant or neutral: correct identification of negative images was positively correlated with fasting glucose ($R = 2.75$, $p = 0.046$), but correct identification of emotionally neutral images was negatively correlated with both glucose ($R = -0.285$, $p = 0.038$) and hemoglobin A1c, a measure of long-term glucose load ($R = -0.334$, $p = 0.014$).
Conclusions: As has been demonstrated in previous studies, we found that menopause was associated with increased depression symptoms. However we found the relationship between insulin resistance and depression symptoms to be more complex, and appears to interact with menopause status: premenopausal IR women had significantly higher depression index scores than their control counterparts, and were similar to both control and IR postmenopausal women. This may suggest that menopause carries a greater risk for depression than metabolic function during the transition period. Depression has been associated with dysregulation of emotion response and regulation circuitry. Limbic and paralimbic brain regions, including the prefrontal cortex, cingulate, and amygdala, have roles in integration and regulation of emotionally relevant information, including salient, rewarding, and nonrewarding events, and are centrally implicated in the regulation of motivated behavior. The medial prefrontal cortex, in particular, is thought to be involved in cognitive regulation of emotional response. While premenopausal IR women had similar depression index scores to postmenopausal women, they exhibited notable differences in neural activation while viewing emotionally negative images, with less activation in the regulatory prefrontal regions, and more activation in the amygdala. A possible interpretation is that in premenopausal women, IR is associated with intensity of emotional response and decreased cognitive regulation of responses. Overall, results from our study suggest an interaction between hormonal and metabolic environments on mood symptoms and emotion regulation.
Keywords: metabolism, menopause, depression, emotion, fMRI.
Disclosures: A. Berent-Spillson, Nothing to Disclose; C. Marsh, Nothing to Disclose; C. Persad, Nothing to Disclose; J. Zubieta, **Part 1:** Eli-Lilly Speaker's Bureau, **Part 2:** Eli-Lilly Speaker's Bureau; Y. Smith, **Part 1:** Eli-Lilly Speaker's Bureau (husband), Up-to-Date Royalties, **Part 2:** Eli-Lilly Speaker's Bureau (husband), **Part 4:** Grant from SIMR, Inc.

T214. Effects of Intranasal CRF on Brain Response to Threat in Humans

Royce J Lee*, Jayant Pinto, Eryka Nosal, Vernon Towle

University of Chicago, Chicago, Illinois

Background: Corticotropin-releasing factor (CRF) is a centrally acting neuromodulator involved in the response to stress, but its role in human the human brain is not well understood. CRF was administered intranasally (IN) in order to test the hypothesis that brain CRF modulates neural processing of threat (angry and neutral Ekman faces). Neural processing was measured with frontal N100 response to rare neutral faces presented in the context of frequent happy Ekman faces. In Study 1, IN CRH was compared to IN placebo. In a Study 2, IN CRH was compared with intravenous CRH and a double-dummy placebo condition, in order to directly compare the IN *vs* IV route of administration.
Methods: All subjects provided written, informed consent. All procedures were approved by the IRB at The University of Chicago, Biological Sciences Division. In Study 1 (NARSAD), 100 mg CRF was administered intranasally in 19 adults (10 with past MDD, 9 healthy controls) in a randomized, placebo crossover study, followed 30 min later by the recording of EEG during presentation of rare Neutral and frequent Happy faces. Frontal N100 amplitude was computed and compared across sessions with RM-ANOVA, with the between subjects factor of Drug and within subjects factor of Group. In Study 2 (NIMH), the effect of 100 mg IN CRF on the N100 response to rare neutral faces was compared to that found following placebo and intravenously administered 100 mg CRF in 16 adults (8 with past MDD and 8 healthy controls). Frontal N100 response was compared using RM-ANOVA, with the between subjects factor of Drug and within subjects factor of Group.
Results: For Study 1, rare Neutral faces elicited a stronger N100 than frequent Happy faces ($F(1, 18) = 12.558$, $p = 0.002$). IN CRF was associated with increased frontal N100 to rare neutral faces relative to IN placebo ($F(1, 18) = 8.259$, $p = 0.01$). For Study 2, a significant main effect of *Drug* was found for N100 amplitude ($F 1, 28) = 4.466$, $p = 0.02$). Post-hoc *t*-tests revealed that IN

CRF but not IV CRF *increased* frontal N100 to Neutral faces relative to placebo ($t(1, 15) = -2.314$, $p = 0.04$). As expected, IV but neither IN CRH nor placebo resulted in elevated peripheral cortisol.

Conclusions: We prevent novel, replicating findings of an enhancement of early automatic cortical processing of threat (neutral faces) in response to IN CRH. No effect was found for IV CRH, consistent with the theory that IN administration leads to central absorption of CRH while IV administration does not. A role for CRF in the enhancement of early, automatic cortical response to threatening stimuli is consistent with animal models of the role of CRF in social and emotional behaviors. The EEG/ERP response to IN CRF represents a potentially useful biomarker of central CRF function for human research.

Keywords: CRF corticotropin-releasing factor stress EEG ERP intranasal.

Disclosures: R. Lee, Nothing to Disclose; J. Pinto, Nothing to Disclose; E. Nosal, Nothing to Disclose; V. Towle, Nothing to Disclose.

T215. Vasopressin and CRF Regulation of Hypothalamic-Pituitary-Adrenal Axis Responsivity in Healthy Volunteers and Drug Free Former Cocaine Dependent Participants

Brian Reed*, Elizabeth Ducat, Brenda Ray, Molly Deutsch-Feldman, Mary Jeanne Kreek

Rockefeller University, New York, New York

Background: Drugs of abuse have long been known to affect stress responsive systems in the body, and a dysregulated hypothalamic-pituitary-adrenal (HPA) axis is a hallmark of addictive diseases in general. We have recently found that in the case of cocaine addiction, the baseline levels of cortisol in drug free former cocaine dependent (DFFCD) persons (with 6 months or greater of abstinence) are significantly lower in comparison to both healthy volunteers as well as currently dependent cocaine users in acute abstinence. Additionally, the responsiveness of serum cortisol to both a moderate and a maximally stimulating challenge dose of corticotropin releasing factor (CRF) is lower in DFFCD subjects. The mechanism underlying reduced cortisol levels, and the implications, require further investigation. Vasopressin (AVP) and CRF are the two independent major regulators of HPA axis responsivity, released from hypothalamic neurons into the pituitary portal blood supply to activate the pituitary corticotrophs resulting in release of products of the propiomelanocortin peptide processing, with a principle product, adrenocortiotrophic hormone (ACTH), in turn activating release of cortisol from the adrenal glands. In these seminal and novel studies directly comparing AVP and CRF as a HPA challenge in normal volunteers and persons with addictive diseases (when possible in the same individuals), we investigated whether altered responsiveness to AVP might contribute to the lower cortisol. We also implemented novel adaptations for radioimmunoassay (RIA) quantification of AVP to determine whether the levels of AVP in the plasma after intramuscular injection correlate with the effects on ACTH and cortisol levels.

Methods: Subjects were recruited into our inpatient facility for the 3 day study. Subjects included in this study were either healthy volunteer, with no history of drug or alcohol dependence (normal volunteer, $n = 17$), as well as subjects who had a previous history of cocaine dependence, but who had remained abstinent from cocaine for 6 months or longer at the time of this study (DFFCD, $n = 8$). Subjects were administered, on successive days, with vehicle (saline, 0 mg/kg), low dose AVP(0.36 μg/kg), or high dose AVP (0.6 μg/kg), with intramuscular injections. Blood was drawn via an implanted catheter, at select time points prior to and for up to 6 h after injection, with serum obtained for cortisol (RIA) measurements, and plasma obtained for ACTH and AVP RIA measurements. Utilizing a cation exchange cartridge in addition to solid phase extraction reduced immunoreactive background material present in plasma for RIA quantification. Analysis of variance was used to examine the relationship of baseline cortisol and ACTH to drug dependence history, as well as dose response of cortisol and ACTH response to AVP, as reflected by area under the curve (AUC) from 0–120 min post injection. Linear regression was use to probe the relationship of elevations of plasma vasopressin to the effect on ACTH and cortisol. We also compared the responsiveness of cortisol and ACTH to AVP *vs* CRF.

Results: Analysis of variance (ANOVA) revealed a trend of lower cortisol baseline levels in DFFCD patients compared to NV, with no difference in ACTH baseline levels, consistent with our previous findings. ANOVA with repeated measures of AUC of cortisol and ACTH showed an effect of dose, but no effect of drug use history. Newman-Keuls posthoc tests revealed no difference between the high and low dose of AVP. Examining differences between AVP and CRF, we conducted a three way ANOVA of dose (high, low, and vehicle) × probe compound (AVP and CRF) × drug abuse history revealed a significant effect of both dose and probe compound on cortisol response, and an interaction between dose and drug abuse history. Newman-Keuls posthoc tests revealed a significant difference between DFFCD cortisol responses to AVP compared to CRF. No such effects were observed for ACTH AUC. Correlation analysis of peak plasma AVP levels determined via RIA with ACTH AUC in response to AVP revealed a trend ($R = 0.54$, $p = 0.11$, $n = 5$).

Conclusions: The comparison of HPA axis stimulation AVP and CRF in diseases involving perturbed HPA axis responsivity is important for the elucidation of the mechanism underlying the stress system dysfunction. The current study investigating cortisol and ACTH response to AVP in comparison with the CRF reveal differences in DFFCD subjects. The lower response of cortisol levels to CRF challenge in comparison to AVP challenge may indicate a relative reduction in sensitivity of the HPA axis to CRF in comparison to AVP. The higher AUC observed for both cortisol in response to AVP may reflect differences in route of administration and total molar dose, as indicated in part by the fact that peak levels of cortisol are similar whereas the higher cortisol AUC in response to AVP is largely due to a longer duration of cortisol rise. The improved methods for radioimmunoassay of AVP will allow for more detailed comparison of such questions related to dosing and pharmacokinetics in the future. This work was

supported by the Adelson Medical Research Foundation, and NIH-NIDA grants R21DA031990 (B.R.) and P50DA005130 (M.J.K.).
Keywords: neuroendocrine, cocaine, addiction, stress, radioimmunoassay.
Disclosures: B. Reed, Nothing to Disclose; E. Ducat, Nothing to Disclose; B. Ray, Nothing to Disclose; M. Deutsch-Feldman, Nothing to Disclose; M. Kreek, Nothing to Disclose.

T216. Real-time Functional MRI Feedback, Compared to Sham, Reduces Cue-induced Nicotine Craving in Smokers: Results from the First Clinical Trial

Colleen A Hanlon*, Karen Hartwell, Jeffrey J Borckardt, James J Prisciandaro, Melanie Canterberry, Xingbao Li, Max Owens, Todd LeMatty, Michael Saladin, Megan Moran-Santa Maria, Mark S George, Kathleen T Brady

Medical University of South Carolina, Charleston, South Carolina

Background: Realtime functional MRI feedback (rtfMRI) is an emerging and innovative technique which allows an individual to receive ongoing feedback about their own neural activity while they perform a given task. Here we present data from the first single-blind, sham controlled clinical trial for rtfMRI as a means of lowering cue-induced craving among smokers.
Methods: Forty nicotine dependent smokers, who stated that they were motivated to quit, were enrolled in the rtfMRI protocol which consisted of 3 rtfMRI sessions (1 h duration, 1 week apart), and 2 follow up visits (1 week, 1 month). Patients were randomized to either the real or sham feedback group. At each visit, a patient-tailored feedback region (craving ROI) was established through a 'crave' run in which the participants were instructed to crave when viewing smoking related cues. This ROI, in the region of the anterior cingulate or medial prefrontal cortex, was then 'fed back' to the individuals on 3 subsequent 'reduce' craving runs. During the reduce craving scans they were exposed to similar smoking and neutral cues while receiving visual feedback (a thermometer) of BOLD signal activity from the ROI. They were instructed to reduce their craving and the activity in the ROI. Smoking-cue reactivity was measured through psychophysiologic assessments and self-reported metrics before, during, and after each fMRI visit. The primary endpoint was a change in smoking cue-reactivity as measured by heart rate, skin conductance, and self-reported craving metrics.
Results: Individuals were unable to reliably identify if they were in the real or sham group, confirming that the integrity of the blind. The real and sham group did not differ in demographic or drug use variables (eg age, gender, smoking history, baseline carbon monoxide, FTND score). There was a significant effect of group for both psychophysiologic parameters (heart rate: $F = 14.13$, $p = 0.0002$; skin conductance: $F = 9.67$, $p = 0.0019$), with the 'real feedback' group having a lower physiologic response to cues. There was also a prominent difference among the self-reported craving metrics (Questionnaire of Smoking Urges Factor 1: $F = 4.52$, $p = 0.041$, peak craving: $F = 4.00$, $p = 0.053$) with the 'real feedback' group having a lower urge to smoke or peak craving than the sham group. Finally, there was a significant main effect of percent BOLD signal change in the craving ROI. That is, the real feedback group over time had a lower BOLD response in this region than did the sham feedback group.
Conclusions: These data demonstrate that smokers who are motivated to quit can modulate their cue-induced craving and regional brain activity by using three sessions of realtime feedback training from a patient-tailored ROI. Interestingly, these effects translate into a reduction of psychophysiologic arousal by the cues an hour after the scan as well as a lower self-reported craving during the scans. Further work is needed to determine if these exciting findings can be translated into some form of therapy for treatment seeking smokers, or those with other addictions.
Keywords: addiction, neuroimaging, neurofeedback, psychophysiology, nicotine.
Disclosures: C. Hanlon, Nothing to Disclose; K. Hartwell, Nothing to Disclose; J. Borckardt, Nothing to Disclose; J. Prisciandaro, Nothing to Disclose; M. Canterberry, Nothing to Disclose; X. Li, Nothing to Disclose; M. Owens, Nothing to Disclose; T. LeMatty, Nothing to Disclose; M. Saladin, Nothing to Disclose; M. Moran-Santa Maria, Nothing to Disclose; M. George, Nothing to Disclose; K. Brady, Nothing to Disclose.

T217. Selective Suppression of α-Synuclein in Monoaminergic Neurons of Mice by Intranasal Delivery of Targeted Small Interfering RNA or Antisense Oligonucleotides: Potential Therapy for Parkinson's Disease

Ariadna Recasens, Mireia Galofré, Iria Carballo-Carbajal, A Ferrés-Coy, Jordi Bové, Celine Perier, María del Carmen Carmona, M I Santos, S Baena, M Rosario Chica, Andrés P Montefeltro*, R Revilla, Analia Bortolozzi

nLife Therapeutics, Armilla, Spain

Background: α-Synuclein (α-Syn) appears to play a crucial role in the pathogenesis of several neurodegenerative disorders including Parkinson's disease (PD). The brains of Parkinson patients typically contain insoluble intracellular protein inclusions called Lewy bodies. Increased neuronal α-Syn levels represent a major component of Lewy bodies and therefore, the suppression of α-Syn expression provides a valid therapeutic target for PD. The goal of this study was to assess the ability of various small interfering RNA (siRNA) and antisense oligonucleotide (ASO) sequences directed against α-Syn to downregulate endogenous or overexpressed α-Syn mRNA levels in BE-M17 neuroblastoma cells. Moreover, we evaluated the feasibility of reducing α-Syn expression selectively in PD-vulnerable brain areas including substantia nigra pars compacta (SNc), ventral tegmental area (VTA), locus coeruleus (LC) and dorsal raphe nucleus (DR) of mice after the internalization of conjugated siRNA/ASO molecules into monoamine neurons following intranasal administration.
Methods: *In vitro characterization of siRNA/ASO molecules against α-Syn (α-Syn-siRNA/ASO).* Two α-Syn-siRNA and three α-Syn-ASO naked sequences were used. The studies included cell culture, α-Syn overexpression, siRNA/ASO

transfection conditions, and RNA analysis. Moreover, selected siRNA/ASO sequences were chemically modified to enhance their biostability and conjugated to a cell-specific ligand, indatraline (non-selective monoamine transporter inhibitor), to promote their selective delivery into monoamine neurons. *In vivo studies*. Wild-type C57Bl/6J adult male mice were intranasally (i.n) administered with conjugated siRNA/ASO molecules at 30 μg/day (2.1 nmol/day) for 4 consecutive days (d) and sacrificed 24 h, 3d and 7d after the last administration. Additionally, a group of mice received intracerebroventricular (i.c.v) or i.n. conjugated siRNA/ASO molecules labeled with Alexa488 and, the intracellular siRNA/ASO distribution in the brain was evaluated using confocal fluorescent microscopy. The efficacy and selectivity of α-Syn knockdown was assessed by *in situ* hybridization, immunohistochemistry and Western blot analyses. All animal procedures were conducted in accordance with the standard ethical guidelines (European Union regulations L35/118/12/1986) and approved by the local ethical committee of the School of Medicine, University of Barcelona.

Results: Based on *in vitro* assay, siRNA and ASO molecules able to produce a 75–85% downregulation of both endogenous and overexpressed α-Syn without affecting β- and γ-Syn were selected for further analyses. After i.c.v or i.n treatment, respectively, there was co-localization of conjugated siRNA/ASO molecules with tyrosine-hydroxylase-positive neurons in SNc/VTA and LC and, tryptophan-hydroxylase-positive neurons in DR, but not in other brain regions or in animals administered with fluorescent oligonucleotides without the targeting ligand. To further pursue the subcellular fate of the conjugated siRNA/ASO molecules, we examined their distribution as compared to well-known markers for several endomembrane compartments including Rab5 (early endosome marker) and Rab7 (late endosome marker). Moreover, i.n. administration of conjugated α-Syn-siRNA or α-Syn-ASO, both produced a cell-specific time-dependent reduction of endogenous α-Syn mRNA levels in SNc/VTA (α-Syn mRNA levels were 81.5 ± 2.2% and 84.1 ± 1.7% of PBS-treated mice, respectively), LC (76.6 ± 2.0% and 85.2 ± 3.9%, respectively) and DR (69.7 ± 0.7% and 70.6 ± 5.6%, respectively). In contrast, the mRNA expression of β- and γ-Syn as well as monoamine transporters was unchanged. The study of α-Syn protein levels by immunohistochemistry revealed a significant decrease in the number of α-Syn-positive cells in the SNc at 24 h after the last i.n administration of conjugated α-Syn-siRNA (78.8 ± 4.8% of PBS-treated animals) and a reduction of intracellular α-Syn protein levels in SNc neurons, measured by optical densitometry, at 3d after the last administration (77.4 ± 1.4% of PBS-treated animals). α-Syn protein levels in the SNpc were recovered by 7d post-treatment. These data were corroborated by Western blot analysis, where decreased α-Syn levels were detected in SN/VTA (49.5 ± 7.8% of PBS-treated animals) and striatum (52.1 ± 7.9% of PBS-treated animals) 24 h after last administration. This change was maintained only in SN/VTA (63.1 ± 6.2%) 3d post-treatment, but not in striatum. Knockdown of α-Syn in these animals was not associated with neurodegeneration in affected areas.

Conclusions: These results set the stage for the testing of these molecules as potential disease-modifying agents in neurotoxin-based and genetic models of PD linked to pathogenic increases in α-Syn. In this study we have characterized conjugated siRNA and ASO molecules that actively reduce endogenous α-Syn expression *in vivo* using the intranasal route to deliver directly siRNA/ASO into the brain.

Keywords: oligonucleotide, alpha-synuclein, Parkinson, intranasal-delivery, silencing.

Disclosures: A. Recasens, Nothing to Disclose; M. Galofré, Nothing to Disclose; I. Carballo-Carbajal, Nothing to Disclose; A. Ferrés-Coy, Nothing to Disclose; J. Bové, Nothing to Disclose; C. Perier, Nothing to Disclose; M. Carmona, **Part 4:** Spanish Ministry of Economy and Competitiveness (MINECO), INNPACTO Subprogram IPT-2012-1208-300000 co-financed by the European Regional Development Fund 'A way to build Europe', and Spanish Ministry of Science and Innovation ((MICINN)—CDTI, with the participation of the DENDRIA Consortium., **Part 5:** nLife Therapeutics, S.L.—Spanish company; M. Santos, **Part 4:** Spanish Ministry of Economy and Competitiveness (MINECO), INNPACTO Subprogram IPT-2012-1208-300000 co-financed by the European Regional Development Fund 'A way to build Europe', and Spanish Ministry of Science and Innovation ((MICINN)—CDTI, with the participation of the DENDRIA Consortium., **Part 5:** nLife Therapeutics, S.L.—Spanish company; S. Baena, **Part 4:** Spanish Ministry of Economy and Competitiveness (MINECO), INNPACTO Subprogram IPT-2012-1208-300000 co-financed by the European Regional Development Fund 'A way to build Europe', and Spanish Ministry of Science and Innovation ((MICINN)—CDTI, with the participation of the DENDRIA Consortium., **Part 5:** nLife Therapeutics, S.L.—Spanish company; M. Chica, **Part 4:** Spanish Ministry of Economy and Competitiveness (MINECO), INNPACTO Subprogram IPT-2012-1208-300000 co-financed by the European Regional Development Fund 'A way to build Europe', and Spanish Ministry of Science and Innovation ((MICINN)—CDTI, with the participation of the DENDRIA Consortium., **Part 5:** nLife Therapeutics, S.L.—Spanish company; A. Montefeltro, **Part 4:** Spanish Ministry of Economy and Competitiveness (MINECO), INNPACTO Subprogram IPT-2012-1208-300000 co-financed by the European Regional Development Fund 'A way to build Europe', and Spanish Ministry of Science and Innovation ((MICINN)—CDTI, with the participation of the DENDRIA Consortium., **Part 5:** nLife Therapeutics, S.L.—Spanish company.; R. Revilla, **Part 4:** Spanish Ministry of Economy and Competitiveness (MINECO), INNPACTO Subprogram IPT-2012-1208-300000 co-financed by the European Regional Development Fund 'A way to build Europe', and Spanish Ministry of Science and Innovation ((MICINN)—CDTI, with the participation of the DENDRIA Consortium., **Part 5:** nLife Therapeutics, S.L.—Spanish company; A. Bortolozzi, Nothing to Disclose.

T218. Autonomic Responses to Intraoperative Subcallosal Cingulate DBS

Patricio Riva Posse*, Cory Inman, Stephan Hamann, Steven Garlow, Robert Gross, Helen S Mayberg

Emory University, Atlanta, Georgia

Background: Subcallosal cingulate (SCC) deep brain stimulation (DBS) has shown long-term efficacy and safety for

treatment-resistant depression (TRD). Immediate changes in mood and behavior with intra-operative stimulation have been observed. Spontaneous self-reported effects include: 'feeling lighter, calmer', 'more connected', 'warm, flushed', 'aroused', 'more awake, aware, and reactive'; suggestive of acute autonomic activation. Notably, these behavioral responses are location-specific within each hemisphere. To test whether subjective responses to SCC DBS are accompanied by concurrent changes in sympathetic activity, heart rate (HR) and skin conductance (SCR) was measured during surgery. Based on the known anatomical connectivity of SCC to regions involved in regulation of sympathetic arousal, it was predicted that behaviorally salient stimulation would impact HR and SCR. These findings could be useful in defining optimal stimulation location and identification of neural networks involved in autonomic output.

Methods: Eight subjects in an ongoing study of SCC DBS for TRD had intraoperative testing. Seven had EKG data and 4 SCR data that were adequate. During surgery, individual contacts in each hemisphere were tested in a sham-control, double-blinded experiment (4 per hemisphere, 4 shams, randomly ordered). Stimulation parameters were constant (freq = 130 Hz, pulse width = 91 μsec, current = 6 mA). Each test lasted 6 min (3 min ON, 3 min OFF). During the stimulation phase, subjects were silent for one minute, reported subjective experiences for one minute and were silent for the final minute. Time-locked changes in HR and SCR were recorded during each stimulation cycle. HR was recorded with a lead I configuration. SCR was recorded from both hands. Post operatively the blinded rater categorized each stimulation period for presence or absence and magnitude of responses, specifically the most behaviorally salient one. Average HR was calculated for the 3-min stimulation periods and in 15-s windows across the stimulation periods. Change in HR was calculated by subtracting the average 15-s pre-stimulus baseline from each stimulation time window. Paired sample *t*-tests with bootstrapping were performed for all contrasts of interest. SCRs were defined as any phasic deviation in the skin conductance level that exceeded 0.1 micro Siemens (μS). Three measures were derived for SCR during each stimulation period: (1) maximum SCR amplitude, (2) proportion total number of SCRs, and (3) proportion cumulative SCR magnitude. The maximum SCR amplitude measure was defined as the maximal change in SCR over the stimulation period. The proportion total number of SCRs is indicative of SC reactivity to stimulation. The proportion cumulative SCR magnitude is a combined measure of the total number of SCRs and the magnitude of each of these non-specific SCRs.

Results: During intraoperative testing, relative to the pre-stimulation baseline, the most behaviorally salient contacts caused a significant increase in HR relative to non-effective contacts ($t(6) = 3.0$, $p = 0.03$) and sham ($t(6) = 3.9$, $p = 0.004$). Across each 3-min stimulation period the most behaviorally salient contacts produced an average increase in heart rate of 3.45 BPM ($SEM = 0.77$), less behaviorally salient contacts 1.22 BPM ($SEM = 0.56$), non-effective contacts 1.2 BPM ($SEM = 0.22$), and sham by 1.10 BPM ($SEM = 0.34$). In time window analyses, the most behaviorally effective contacts produced a significant increase in the initial 15 s relative to sham and from 60 to 150 s compared to non-behaviorally effective and sham stimulations (all $p < 0.05$). Similar to HR, the most behaviorally salient contacts generated an increased SCR relative to less behaviorally salient, non-effective, and sham stimulations for all 3 SCR measures. These results trend in parallel to HR changes, but are underpowered due to the small sample.

Conclusions: High frequency SCC DBS generates increases in sympathetic activity, as indicated by two measures of autonomic physiology. Autonomic changes are observed within seconds of initiating acute stimulation and prior to verbalization of subjective feelings. Importantly, these changes in sympathetic activity are correlated with the most behaviorally salient stimulation periods. These findings suggest that SCC DBS modulates brain regions involved in autonomic regulation consistent with known anatomical connectivity of SCC to brainstem and dorsal cingulate. As such, these physiological findings may help to optimize contact selection and location within specific neural networks. Further, changes in sympathetic activity with SCC DBS provide a novel strategy for examining the interactions of affective experience and the autonomic nervous system in TRD.

Keywords: deep brain stimulation; psychophysiology; skin conductance response; heart rate; autonomic nervous system.

Disclosures: P. Riva Posse, Nothing to Disclose; C. Inman, Nothing to Disclose; S. Hamann, Nothing to Disclose; S. Garlow, Nothing to Disclose; R. Gross, **Part 1:** Medtronic, **Part 3:** Medtronic, **Part 4:** Medtronic; H. Mayberg, **Part 1:** St Jude Medical, Inc., **Part 2:** St Jude Medical, Inc., **Part 3:** none, **Part 4:** none.

T219. A Two-site Pilot Study Suggests that Three Days (9 Sessions) of High Dose Left Prefrontal Repetitive Transcranial Magnetic Stimulation (rTMS) is Feasible, Safe, and Reduces Suicidal Thinking in Suicidal Inpatients

Mark S George*, Rema Raman, Sonia Jain, David M Benedek, Christopher G Pelic, Geoffrey G Grammer, Karen Stokes, Matthew Schmidt, Chad Spiegel, Nancy DeAlmeida, Kathryn Beaver, Jeffrey J Borckardt, Xiaoying Sun, Murray B Stein

Medical University of South Carolina, Charleston, South Carolina

Background: Suicide attempts and completed suicides are common, yet there are no proven acute medication or device treatments for treating a suicidal crisis. Repeated daily left prefrontal repetitive transcranial magnetic stimulation (rTMS) for 4–6 weeks is a new FDA-approved treatment for acute depression. Some open-label studies have found rapid reductions in suicidality with rTMS.This study tests whether a very intense dose of rTMS to suicidal inpatients is feasible and safe, and also whether this might rapidly improve suicidal thinking.

Methods: Design: This prospective, 2-site, randomized, active sham-controlled (1 : 1 randomization) design incorporated 9 sessions of rTMS over 3 days as adjunctive to usual inpatient treatment for suicidality. Patients came from

two inpatient military hospital wards (one VA, the other DOD). Research staff screened approximately 377 inpatients, yielding 41 adults admitted for suicidal crisis, all of whom also had either post-traumatic stress disorder (17), mild traumatic brain injury (1), or both (23).Methods: Repetitive TMS (rTMS) was delivered to the left prefrontal cortex with a figure-eight solid core coil at 120% motor threshold, 10 hertz (Hz), 5 s (s) train duration, 10 s intertrain interval for 30 min (6000 pulses) 3 times daily for 3 days (total 9 sessions;54 000 stimuli). Sham rTMS used a similar coil that contained a metal insert blocking the magnetic field and utilized electrodes on the scalp, which delivered a matched somatosensory sensation. Main Outcome Measure: Primary outcomes were the daily change in severity of suicidal thinking as measured by the Beck Scale of Suicidal Ideation (SSI) administered at baseline and then daily, as well as subjective visual analog scale measures before and after each TMS session. Mixed model repeated measures (MMRM) analysis was performed on modified intent to treat (mITT) (37) and completer (27) populations. **Results:** This intense schedule of rTMS with suicidal inpatients was feasible and safe (About 6 times greater than the FDA currently approved daily dose). Minimal side effects occurred, none differing by arm, and the retention rate was 88% throughout the 3 treatment days. From the mITT analyses, SSI scores declined rapidly over the 3 days for both groups (sham change −15.3 points, active change −15.6 points), with a trend for more rapid decline on the first day with active rTMS (sham change −6.3 points, active −10.7 points, $p = 0.12$). This decline was more pronounced in the completers analysis [sham change −5.9 (95% CI: −10.1, −1.7), active −13 points (95% CI: −18.7, −7.4); $p = 0.054$]. Subjective ratings of 'being bothered by thoughts of suicide' declined non-significantly more with active rTMS than with sham at the end of 9 sessions of treatment in the mITT analysis [sham change −31.9 (95% CI: −41.7, −22.0), active change −42.5 (95% CI: −53.8, −31.2); $p = 0.17$] and significantly more in the completers sample [sham change −24.9 (95% CI: −34.4, −15.3), active change −43.8 (95% CI: −57.2, −30.3); $p = 0.028$].
Conclusions: Delivering high doses of left prefrontal rTMS over three days (54 000 stimuli) to suicidal inpatients is possible and safe, with few side effects and no worsening of suicidal thinking. The suggestions of a rapid anti-suicide effect (day 1 SSI data, Visual Analogue Scale data over the 3 days) need to be tested for replication in a larger sample. If confirmed, high dose TMS may become a new rapid treatment for acute suicidality by theoretically restoring cortical connectivity and control.
Keywords: PTSD, TBI, depression, suicide, TMS.
Disclosures: M. George, **Part 1:** Consultant—Tal Medical, **Part 4:** Neuronetics: equipment lent for this study, a DOD PTSD study, a maintenance of effect study and a depression in adolescents study., Brainsway: clinical site for a unipolar depression study and a bipolar depression study. Magventure: equipment support for VA CSP #556, Cervel: clinical trial in unipolar depression, Neosync: clinical trial in unipolar depression; R. Raman, Nothing to Disclose; S. Jain, Nothing to Disclose; D. Benedek, Nothing to Disclose; C. Pelic, Nothing to Disclose; G. Grammer, Nothing to Disclose; K. Stokes, Nothing to Disclose; M. Schmidt, Nothing to Disclose; C. Spiegel, Nothing to Disclose; N. DeAlmeida, Nothing to Disclose; K. Beaver, Nothing to Disclose; J. Borckardt, Nothing to Disclose; X. Sun, Nothing to Disclose; M. Stein, Nothing to Disclose.

T220. Anxiety Moderates Response to Psychosocial Treatment for Depression in Bipolar Disorder: Results from Systematic Treatment Enhancement Program for Bipolar Disorder

Thilo Deckersbach*, Amy Peters, Navneet Kaur, Andrew K Corse, Amanda R Arulpragasam, Louisa Sylvia, Pedro Vieira da Silva, Michael Otto, Ellen Frank, David Miklowitz, Michael Berk, Darin Dougherty, Andrew A Nierenberg

Massachusetts General Hospital, Boston, Massachusetts

Background: Bipolar disorder, characterized by episodes of depression and/or mania, is a chronic and debilitating illness. Pharmacotherapy is the first line of treatment but often fails to bring patients with bipolar disorder to sustained clinical and functional remission. Several adjunctive psychosocial interventions have been developed to treat bipolar disorder. This includes cognitive-behavioral therapies (CBT), family-focused treatment (FFT), and interpersonal and social rhythm therapy (IPSRT). One of the largest randomized controlled treatment trials investigating the efficacy of psychotherapy for depression in bipolar disorder was conducted in the context of the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD). This study showed that FFT, IPSRT, and CBT did not differ in their efficacy of decreasing the length of time until recovery from depressive episodes (Miklowitz *et al*, 2007). Between 20 and 50% of individuals with bipolar disorder also have a lifetime anxiety disorder. Relative to bipolar patients without an anxiety disorder, individuals with both bipolar disorder and a co-morbid anxiety disorder experience a worse course of illness. We investigated whether co-morbid anxiety moderates the response to intensive psychotherapy (CBT, FFT, or IPSRT) in the STEP-BD randomized controlled trial of psychotherapy for bipolar depression.
Methods: STEP-BD was a large NIMH funded, multisite, study conducted in bipolar disorder, enrolling 4361 participants across 21 sites in the US. Embedded within STEP-BD was a randomized controlled trial of psychotherapy for bipolar depression. The study randomly assigned participants to receive an intensive psychosocial treatment (CBT, FFT or IPSRT) over 9 months or a minimal psychosocial intervention, collaborative care, consisting of 3 sessions in 6 weeks. Of the 293 patients with DSM-IV bipolar disorder who participated in the randomized controlled trial, 269 had diagnostic information available regarding the presence or absence of a DSM-IV lifetime co-morbid anxiety disorder.
Results: Patients, who received psychotherapy had significantly higher year-end recovery rates ($\chi^2(1, n = 269) = 3.83$, $p = 0.05$), than patients in collaborative care. Sixty-six percent ($n = 177$) of the 269 bipolar patients had a co-morbid lifetime anxiety disorder, whereas 40% ($n = 92$) did not. For patients with co-morbid lifetime anxiety, 66% ($n = 65$) of participants recovered with psychotherapy,

whereas only 49% ($n = 38$) recovered with collaborative care. This corresponded to a small to medium effect size (Number needed to Treat [NNT] = 5.88). That is, among patients with co-morbid anxiety disorders, one would need to treat approximately 6 patients with intensive psychotherapy compared to collaborative care to see one additional patient recover with psychotherapy. For patients without a lifetime anxiety disorder, there was no difference between rates of recovery for participants randomized to psychotherapy *vs* collaborative care (psychotherapy: 64% [$n = 32$] recovered; collaborative care: 62% [$n = 26$]). This corresponded to a very small effect size (NNT = 50).In terms of specific anxiety disorders, 60% ($n = 25$) of individuals with lifetime generalized anxiety disorder recovered with psychotherapy, whereas 27% ($n = 7$) of individuals with lifetime generalized anxiety disorder recovered in collaborative care (medium to large NNT = 3.03). Sixty-three percent ($n = 28$) of individuals with lifetime PTSD responded to psychotherapy, whereas 44% ($n = 8$) recovered in collaborative care (small to medium NNT = 5.56). There were only small differences in recovery rates to psychotherapy *vs* collaborative for participants meeting lifetime criteria for panic disorder (NNT = 7.69 [small–medium]), social phobia (NNT = 7.69 [small–medium]), and OCD (NNT = 10 [small]. Effect sizes for current anxiety disorders resembled those for lifetime anxiety disorders.

Conclusions: Depressed patients with bipolar disorder and co-morbid anxiety may be in particular need for additional psychotherapy for treating acute depression. These results need to be replicated in studies that stratify bipolar patients to treatments based on their anxiety co-morbidity status.

Keywords: anxiety, psychosocial treatment, depression, bipolar disorder, STEP-BD.

Disclosures: T. Deckersbach, **Part 1:** MGH Psychiatry Academy, BrainCells Inc., Systems Research and Applications Corporation, Boston University, the Catalan, Tufts University, **Part 4:** K-23 NIMH Career Award 1K23MH074895-01A2, NARSAD, TSA, OCF; A. Peters, Nothing to Disclose; N. Kaur, Nothing to Disclose; A. Corse, Nothing to Disclose; A. Arulpragasam, Nothing to Disclose; L. Sylvia, **Part 1:** Employed by Massachusetts General Hospital, served as a Consultant for Bracket Global and Clintara, received research support from NIMH, a former stockholder in Concordant Rater Systems, and have received support from New Harbinger Publishers.; P. Vieira da Silva, Nothing to Disclose; M. Otto, **Part 1:** Have served as a consultant for MicroTransponder, Inc., receives research support from NIMH, and royalties from Oxford University Press and Routledge.; E. Frank, **Part 1:** Served as a consultant for Servier International, and have received other financial or material support from Guilford Press and the American Psychological Association Press. ; D. Miklowitz, **Part 1:** Received research support or honoraria from NIMH, Brain and Behavior Research Foundation, Danny Alberts Foundation, and Attias Family Foundation; received other financial or material support from Guilford Press, and John Wiley and Sons. ; M. Berk, **Part 1:** An employee of Barwon Health and Deakin University; received research support from NIH, NHMRC, CRC, Rotary, Beyond Blue, Stanley Medical Research Institute and the Simons Foundation; he has received honoraria from Lundbeck, Astrazeneca, Servier, Lilly, Janssen, Pfizer, and Merck; served as a speaker or on the advisory board for Astrazeneca, Lundbeck, Lilly, and Janssen; and have received financial or material support from Allen & Unwin and Cambridge University Press. ; D. Dougherty, Nothing to Disclose; A. Nierenberg, **Part 1:** Received honoraria or travel expenses including CME activities from APSARD, Belvoir Publishing, Boston Center for the Arts, University of Texas Southwestern Dallas, Hillside Hospital, American Drug Utilization Review, American Society for Clinical Psychopharmacology, Bayamon Region Psychiatric Society, San Juan, PR, Baystate Medical Center, Canadian Psychiatric Association, Columbia University, Douglas Hospital/McGill University, IMEDEX, International Society for Bipolar Disorders, Israel Society for Biological Psychiatry, John Hopkins University, MJ Consulting, New York State, Massachusetts Association of College Counselors, Medscape, MBL Publishing, Physicians Postgraduate Press, Ryan Licht Sang Foundation, Slack Publishing, SUNY Buffalo, University of Florida, University of Miami, University of Wisconsin, University of Pisa, and SciMed. Presenter for the Massachusetts General Hospital Psychiatry Academy (MGHPA). The education programs conducted by the MGHPA were supported through Independent Medical Education (IME) grants from the following pharmaceutical companies in 2008: Astra Zeneca, Eli Lilly, and Janssen Pharmaceuticals; in 2009 Astra Zeneca, Eli Lilly, and Bristol-Myers Squibb. No speaker bureaus or boards since 2003., Own stock options in Appliance Computing, Inc. and Brain Cells, Inc. Additional income is possible from Infomedic.com depending on overall revenues of the company but no revenue has been received to date. Through MGH, Andrew Nierenberg is named for copyrights to the Clinical Positive Affect Scale and the MGH Structured Clinical Interview for the Montgomery Asberg Depression Scale exclusively licensed to the MGH Clinical Trials Network and Institute (CTNI). Have received grant/research support from AHRQ, Cephalon, Forest, Mylin, NIMH, PamLabs, Pfizer Pharmaceuticals, Takeda, and Shire. In the next 2 years, it is possible to receive grants from Dey Pharmaceuticals, Sunovion, and Targacept.

T221. Addition of a Comprehensive, Individualized, Person Centered Management Program, to Memantine Alone Produces a 900% Increment in a Pivotal Trial Global Measure over Medication Treatment Alone in Advanced Alzheimer's Disease

Barry Reisberg*, Sunnie Kenowsky, Sloane Heller, Istvan Boksay, James Golomb, Santosh Ghimire, Carol Torossian, Iryna Lobach

New York University School of Medicine, New York, New York

Background: A decade ago, the senior author and colleagues published a multicenter study which demonstrated the efficacy of memantine in the treatment of moderate to severe Alzheimer's disease (AD) (Reisberg, *et al*, *N Engl J Med*., 2003). This study served as a pivotal trial which supported the US and EU approvals of memantine as the first treatment for advanced AD. The advent of pharmacologic treatment of advanced AD served to highlight the continuing needs of these persons. Therefore, we simulta-

neously developed a science of AD management (Reisberg, *et al*, *Int Psychogeriatr*, 1999; Reisberg, *et al*, *Am J Alzheimers Dis Other Demen*, 2002). After the U.S. approval of memantine treatment we embarked upon a study comparing a Comprehensive, Individualized, Person Centered Management Program (CI-PCM) in persons receiving memantine treatment, with memantine treatment alone. The inclusion criteria, outcome measures and study design were based on our 2003 NEJM memantine study. Subjects were randomized to CI-PCM plus memantine treatment ($n = 10$), or memantine treatment alone (controls, $n = 10$). A primary pivotal, outcome measure was the NYU CIBIC-Plus, a global primary outcome used in the 2003 pivotal memantine trial. We recently reported that the CI-PCM + memantine treatment subjects showed significant improvement over the controls on this global outcome measure at all time periods examined (ie, 4, 12 and 28 weeks, $p < 0.01$) (Reisberg, *et al*, *Alzheimer's & Dementia*, in press). Herein we describe the source and the meaning of the observed differences.

Methods: The NYU CIBIC-Plus (Clinician's Interview Based Impression of Change, Plus Caregiver Input) assessment is comprised of 2 parts: Part 1. A subject interview, and Part 2: A caregiver interview. Part 1 has a cognitive component, a behavioral component total score, and a behavioral global score. Part 2 has a functional disability stage, a behavioral component total score, and a behavioral global score. Differences between the CI-PCM treatment group and the control group on the 6 CIBIC-Plus elements were examined at week 28.

Results: There were no differences on any of the subject interview measures. Significant differences were found in all of the caregiver interview elements: functional component, $p = 0.012$; behavioral total score, $p = 0.007$; and behavioral global rating, $p = 0.009$. The overall magnitude of the difference on the NYU CIBIC-Plus between the CI-PCM and the control group, with both receiving memantine was 2.7.

Conclusions: The magnitude of the effect seen on the NYU CIBIC-Plus assessment with the CI-PCM program, 2.7 points, can be compared with the magnitude of the effect observed on the NYU CIBIC-Plus with memantine treatment *vs* placebo in the 2003 NEJM pivotal trial, 0.3 points. Hence, a 900% improvement in comparison with memantine treatment alone was observed. This difference is based on point differences between two different studies using the same measure. This effect of the CI-PCM program seems enormous in comparison with the size of the effect conventionally seen with AD medication treatments. The effects were due to changes in subject functioning and behavioral disturbances. No effects of the CI-PCM program were seen on cognition. At this stage of AD (stage 6 on the Functional Assessment Staging Scale [FAST]), persons with AD have difficulties with daily life activities such as dressing, bathing, and subsequently with toileting and continence. At this stage functional change and improvements in behavior may be more important than modest improvements in cognitive tests. One question which arises from these results is why behavioral improvements were not observed in the direct subject interview. We believe this may be in part because many control subjects had such difficult behavior that the CI-PCM behavior strategies had to be used in order to enable the subjects to be examined at the research center. In conclusion, the CI-PCM program appears to have been extraordinarily effective in mitigating functional and behavioral disturbances in moderately severely impaired AD persons with overall global effects on AD, 900% over and above the effects of medication (memantine) alone.

Keywords: Alzheimer's disease, management program, advanced dementia, behavioral disturbances, functioning.

Disclosures: B. Reisberg, **Part 1:** Forest Research Institute (grant support 2007–2013), Medivation Incorporated (grant support 2009–2012), **Part 2:** New York University School of Medicine, Personal practice of geriatric psychiatry, **Part 4:** Forest Research Institute (grant support 2007–2013), Medivation Incorporated (grant support 2009–2012), ; S. Kenowsky, **Part 1:** Forest Research Institute (research grant support, 2005–2013), **Part 2:** New York University Langone Medical Center, Personal practice assisting caregivers, **Part 4:** Forest Research Institute (research grant support, 2005–2013), ; S. Heller, Nothing to Disclose; I. Boksay, Nothing to Disclose; J. Golomb, Nothing to Disclose; S. Ghimire, Nothing to Disclose; C. Torossian, Nothing to Disclose; I. Lobach, Nothing to Disclose.

T222. Neuropeptide-S Microinfusion into Basolateral Amygdala Rescues Behavior in a Rat Model of Posttraumatic Stress Disorder by Increasing Expression of BDNF and Neuropeptide Y-Y1 Receptor

Aleksander Mathé*, Joseph Zohar, Hagit Cohen

Karolinska Institutet, Stockholm, Sweden

Background: NeuropeptideS (NPS) mainly localized in clusters of cells around Locus coeruleus and with a wide receptor (NPSR1) distribution in the limbic areas, is an anxiolytic and arousal peptide in rodents while in humans it is associated withpanic disorders. In an ongoing series of experiments to further elucidate neurobiological correlates of behavior in PTSD and dissect anxiolytic *vs* antidepressant actions of NPY (conceivably involving amygdala, respectively hippocampus and frontal cortex) instead of injecting NPY (Cohen *et al*, 2011) into the dorsal hippocampus, we injected the anxiolytic peptide NPS into basolateral amygdala (BLA).

Methods: All experiments were approved by the Committee for Protection of Animals. This study assessed the long-term effects of NPS(1.0 nmol/5 μl) bilaterally microinjected into BLA one hour after predator scent stress exposure (PSS), in a controlled prospectively-designed animal model for PTSD (Cohen *et al*, 2011). Acoustic startle response (ASR) and elevated plus maze (EPM) tests were carried out 7 days later. One day after the behavioral tests the animals were sacrificed, the brains harvested and stored deep-frozen until laboratory analyses. Immunohistochemical technique was used to detect the expression of the NPY, NPY-Y1 receptor,and brain derived neurotrophic factor (BDNF) Corticosterone in serum was measured by ELISA. The behavioral effects of NPS-R1 antagonist (SHA68 [D-Cys(tBu)5]NPS) (10 nmol/5 μl) (Camarda *et al* 2009), NPY-Y1 receptor antagonist (BIBO3304 ((R)-N-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-N2-(diphenylacetyl)-argininamidetrifluoroacetate)) (20 μg), or both, administered centrally one hour after PSS were evaluated in the same manner.

Results: PSS exposure significantly increased open arms entries and time spent in open arms ($p < 0.008$ and < 0.02, respectively). Post-PSS exposure treatment with NPS had

marked protective effect; no animal treated with NPS displayed extreme behavioral responses (EBR). Interestingly, NPS significantly decreased ASR in both exposed and unexposed animals ($p<0.005$ and <0.05, respectively). Consistently with previous experiments, PSS exposure significantly reduced NPY as well as NPYY1R and BDNF expression in the hippocampal subregions (CA1: $p<0.02$, <0.025 and <0.0006, respectively; CA3: $p<0.05$, <0.006, and <0.015, respectively; DG: $p<0.045$, <0.003 and <0.0025, respectively). NPS microinfusion into BLA significantly increased NPYY1R and BDNF (CA1: $p<0.0055$, and <0.0002; CA3: $p<0.0001$, and <0.0006; DG: $p<0.0001$, and <0.0008, respectively) but had no effect on NPY in the hippocampus. Serum corticosterone was significantly increased ($p<0.04$) following PSS. Moreover, in line with its arousal properties, NPS further elevated corticosterone levels 40 min after the injection. A single dose of NPY-Y1 receptor antagonist or NPSR1 antagonist microinfused into the BLA 30 min before microinfusion of NPS preceding resulted in a significant reduction of behaviors representing anxiety and avoidance responses (time open: $p<0.01$, startle amplitude: $p<0.001$). In contrast, the same drug treatment, that is microinfusion of NPY-Y1 receptor antagonist together with NPS antagonist associated with administration of NPS, but administered one hour after stress-exposure, was associated with significantly poorer long-term outcome than exposed vehicle-control or even unexposed vehicle-control.

Conclusions: Present experiments demonstrate the high reproducibility of PSS as an animal model of PTSD. In similarity to the previous experiment, PSS reduced expression of BDNF, NPY and NPYY1 receptor in hippocampus. The anxiolytic peptide NPS completely abolished the extreme behavioral response to PSS. Moreover, it restored the decreased expression of BDNF and, unexpectedly, NPYY1 receptor. Since NPS did not affect the decreased expression of NPY, it is conceivable that NPS in addition to acting on its receptor also acts, directly or indirectly, by so far unknown mechanism, on the NPYY1 receptor.

References.

Cohen H, Liu T, Kozlovsky N, Kaplan Z, Zohar J, Mathé AA (2012). *Neuropsychopharmacology* **37**: 350–363.

Camarda V, Rizzi A, Ruzza C, Zucchini S, Marzola G, Marzola E, Guerrini R, Salvadori S, Reinscheid RK, Regoli D, Calo G (2009). *J Pharmacol Exp Ther* **328**: 549–555.

Keywords: PTSD, predator scent stress, neuropeptide S, neuropeptide Y, neuropeptide S antagonist.

Disclosures: A. Mathé, Nothing to Disclose; J. Zohar, Nothing to Disclose; H. Cohen, Nothing to Disclose.

T223. Monitoring Depression Severity Using the Patient Health Questionnaire During a Treatment Course of Transcranial Magnetic Stimulation

Umut Ozbek, Jonathan Cohen, Rebecca Gordon, Marc J Dubin*

Weill Cornell Medical College, New York, New York

Background: Observer-administered rating scales of major depression have been used to monitor response to Transcranial Magnetic Stimulation (TMS). These scales, which include the 17-item Hamilton Depression Rating Scale (HAMD-17) and Montgomery–Asberg Depression Rating Scale (MADRS), are time consuming to administer and subject to inter-rater variability. To assess safety and guide treatment, depressive symptoms must be monitored efficiently and frequently in busy TMS treatment settings. The Patient Health Questionnaire-9 (PHQ-9), an easy to complete self-report rating scale of depression, is well validated in many treatment settings. We asked whether PHQ-9 correlates with the HAMD-17 during a course of TMS for medication resistant depression. We hypothesized that PHQ-9 and HAMD-17 correlate in TMS-responders.

Methods: We measured the HAMD-17 and PHQ-9 at weekly intervals (at baseline and through the course of treatment) during a 5-week treatment course of 10 Hz rTMS over the left dorsolateral prefrontal cortex (DLPFC). We also considered the HAMD-6, consisting of the sum of items # 1, 2, 7, 8, 10 and 13 from the HAMD-17. We analyzed data from 38 individuals, all of whom entered the study during a current episode of major depression and who failed two antidepressant trials of adequate dose and duration during the current episode. HAMD-17 was measured by one individual (RG) throughout the study. First, absolute changes in HAMD-17 and PHQ-9 were determined. Second, the relationship between HAMD-17 and PHQ-9 at baseline, as well as the relationship between the change in each during the course of treatment, were measured. Finally, factor analyses of HAMD-17, HAMD-6, and PHQ-9 were conducted to gain insight into specific ranges of symptoms they characterize.

Results: The means of the three scales all decline over the course of TMS: HAMD-17 (21%), HAMD-6 (24%), and PHQ-9 (36%). Using standard definitions of response and remission, the PHQ-9 is the most sensitive to response. Across all subjects and all time points within the 5 week treatment course, HamD-17 ($r=0.80$, $p<0.0001$) and HamD-6 ($r=0.76$, $p<0.0001$) both correlate with PHQ-9. Although there were examples of subjects in whom HAMD-17 correlated with PHQ-9 and HAMD-6 correlated with PHQ-9 throughout the treatment course, there were also counterexamples. HAMD-17 and PHQ-9 correlated more strongly in subjects who responded to TMS (as measured by $a>50\%$ reduction in HAMD-17). The same was true of HAMD-6 and PHQ-9, with treatment response measured by $a>50\%$ reduction in HAMD-6. Factor Analysis of Week 5 HAMD-6 scores produced one factor (principal component) with all items weighing on this factor except 'Retardation.' Factor Analysis of Week 5 PHQ-9 scores produced two factors, with all items weighing on factor #1 except 'Suicide.' Factor #2 consisted only of the item 'Suicide.'.

Conclusions: The PHQ-9 is easy to administer, correlates with the HAMD-17 and HAMD-6 and is sensitive to TMS response. Within individuals, correlations between PHQ-9 and HAMD-17 and HAMD-6 are greatest in TMS responders. Although the PHQ-9 correlates with the HAMD-17 at the sample level, there are many counterexamples in individual subjects. This disagreement may result from greater heterogeneity of the items comprising the HAMD-17 (Bagby, 2004), which includes items for insomnia, anxiety, and insight. In contrast, the items of the PHQ-9 are more specific for MDD, perhaps leading to 8 of the 9 items of the PHQ-9 loading on one factor. Factor #2 of the PHQ-9, with unique item loading of the 'suicide' item, requires further

investigation. This factor may have the potential to identify patients who are at high risk despite a normal PHQ-9. It may also be a predictive factor of TMS response or non-response.
Keywords: TMS, treatment resistant depression, rating scales, factor analysis.
Disclosures: U. Ozbek, Nothing to Disclose; J. Cohen, Nothing to Disclose; R. Gordon, Nothing to Disclose; M. Dubin, Nothing to Disclose.

T224. Electroconvulsive Therapy Pre-treatment with Low Dose Propofol: Comparison with Unmodified Treatment

Adarsh Tripathi, Nathan Winek, Kapil Goel, Douglas D'Agati, Jesus Gallegos, Geetha Jayaram, Thai Nguyen, Punit Vaidya, Peter Zandi, Jitendra Trivedi, Irving M Reti*

Johns Hopkins University, Baltimore, Maryland

Background: Electroconvulsive therapy (ECT) has been an established treatment for major mental illness since the 1930's and has been administered under anesthesia since the development of muscle relaxants in the late 1940's. However, in many developing countries it continues to be administered completely unmodified to acutely mentally ill patients who are poor and cannot afford anesthesia services. Surveys of psychiatrists indicate that tens of thousands of patients receive completely unmodified ECT around the world every year. Although there have been calls for the banning of this practice, including from the World Health Organization, for many patients with severe mental illness, no alternative exists.
Methods: To address this issue, we have evaluated whether administration of intravenous propofol 0.5 mg/kg for sedation by the ECT psychiatrist just prior to treatment improved acceptance of and reduced anxiety surrounding the treatment. The study was an open label, controlled trial with the comparison group receiving treatment as usual ie completely unmodified ECT. As far as we are aware, this is the only controlled trial that has evaluated an alternative to completely unmodified ECT. The trial was conducted at The King George's Medical University in Lucknow, India. Forty-nine patients received propofol and 50 patients unmodified ECT. Anxiety was monitored 5–10 min before ECT using the State-Trait Anxiety Inventory (STAI). Patient attitude towards ECT was evaluated by an established questionnaire (Freeman and Kendell, 1980). We monitored post-ictal delirium with the Confusion Assessment Method for the Intensive Care Unit (CAM-ICU) and cognitive side-effects using the Mini Mental State Exam (MMSE).
Results: The two cohorts had comparable socio-demographic profiles, diagnoses and clinical responses. Patients who received propofol experienced less anxiety monitored by the STAI just prior to ECT ($p < 0.001$), and had a more favorable attitude towards treatment. Propofol patients were less likely to experience post-ictal delirium monitored by the CAM-ICU ($p = 0.015$) and had fewer cognitive side-effects on the MMSE ($p = 0.004$). Importantly, there were no adverse events associated with propofol administration.
Conclusions: This study demonstrates low dose propofol can be safely administered by the ECT psychiatrist to sedate patients pre-treatment. It was associated with reduced anxiety and a more positive attitude towards ECT, without compromise of efficacy. However, a randomized double blind controlled study is necessary to confirm these benefits.
Keywords: cliincal trial, safety, severe mental illness, cognitive side-effects, delirium.
Disclosures: A. Tripathi, Nothing to Disclose; N. Winek, Nothing to Disclose; K. Goel, Nothing to Disclose; D. D'Agati, Nothing to Disclose; J. Gallegos, Nothing to Disclose; G. Jayaram, Nothing to Disclose; T. Nguyen, Nothing to Disclose; P. Vaidya, **Part 4:** DrVaidya has received research support from NIH, the Hope for Depression Research Foundation, Neuronetics Inc. and Brainsway Inc; P. Zandi, **Part 4:** Dr Zandi has received grant support from NIH; J. Trivedi, Nothing to Disclose; I. Reti, **Part 4:** The work described in the abstract was supported by a Johns Hopkins Center for Global Health Award to Dr Reti. Also, DrReti has received research support from NIH, the Hope for Depression Research Foundation, Neuronetics Inc. and Brainsway Inc.

T225. The Effects of an Index Course of Magnetic Seizure Therapy and Electroconvulsive Therapy on Verbal Learning and Memory

Shawn M McClintock*, Mustafa Husain, C Munro Cullum, Angel V Peterchev, Ira Bernstein, Louis Stool, Bruce Luber, Paul Croarkin, Elisabeth Bernhardt, Kenneth Trevino, Sarah Lisanby

Duke University School of Medicine, Durham, North Carolina

Background: Magnetic seizure therapy (MST) uses magnetic pulses to induce a focal seizure for the treatment of depression. This strategy capitalizes upon the established safety of transcranial magnetic stimulation and the robust antidepressant efficacy of seizures as induced with electroconvulsive therapy (ECT). MST is hypothesized to be a safe and effective antidepressant strategy for patients with major affective disorders. Preclinical and clinical evidence has substantiated that MST produces little to no neurocognitive adverse effects. Also, clinical evidence has found MST produces antidepressant effects. Presently, there are limited data regarding the safety of MST when it is administered at high doses (eg, 1000 pulses). Such information is needed, as evidence from ECT investigations have found that higher doses result in increased adverse neurocognitive effects, particularly in the neurocognitive domain of verbal learning and memory. Thus, the purpose of this study was to compare the effects of an index course of high-dose MST and standard ECT on verbal learning and memory in patients with a current major depressive episode.
Methods: This was a three-center, between-subject, randomized, double-masked controlled clinical trial that compared the neurocognitive effects of high dose MST and ultra-brief pulse RUL ECT. All participants provided written informed consent for this IRB approved investigation before completing study procedures. The study was conducted

under a US FDA IDE. Adults with a major depressive episode in the context of unipolar or bipolar depression, based on the SCID-I, were randomly assigned to treatment with MST or ECT. For MST, a Magstim Theta device with a round coil positioned on the vertex was used to administer the stimulus. Seizure threshold was titrated at the first session by increasing the train duration, and subsequent treatments were provided at maximal device output (100% maximal pulse amplitude, 100 Hz pulse frequency, 10 s train duration). For ECT, treatments were provided via standard RUL electrode configuration, 800 mA pulse amplitude, and ultra-brief pulse width (0.3 ms). Seizure threshold was titrated at the first session by increasing the train duration and frequency. Subsequent treatments were provided at 6′ the seizure threshold. Patients were treated until they achieved remission.

Results: In terms of change from baseline to end, patients who received MST relative to those who received ECT showed better delayed recall ($F(1, 59) = 4.40$, $p = 0.04$), retention ($F(1, 59) = 4.50$, $p = 0.04$), as well as recognition ($F(1, 57) = 5.03$, $p = 0.03$) of learned words. Indeed, patients in the ECT group showed significant declines from average to mild/moderate impaired performance after ECT, and patients in the MST cohort showed stability of average performance from baseline to end. There were no differences between MST and ECT on total words learned across five learning trials ($F(1, 59) = 2.66$, $p = 0.11$), interference with a distractor list ($F(1, 58) = 0.96$, $p = 0.33$), or immediate recall of learned words ($F(1, 59) = 0.96$, $p = 0.33$).

Conclusions: This study provides evidence that MST has neurocognitive advantages relative to ECT, particularly with regard to delayed recall, retention, and recognition of verbally presented information. Specifically, patients in the ECT cohort significantly declined from average performance at baseline to mild to moderate impaired performance on the RAVLT after the index course. Patients in the MST group maintained their average performance on the RAVLT from baseline to end of the index course. These findings are consistent with prior research suggesting MST to have benign or no neurocognitive adverse effects. Research has found that recall worsens after acute treatment with ECT, which is associated with select ECT parameters including bitemporal electrode placement, sine wave pulse width, and increased dosage. Importantly, adverse neurocognitive effects have been associated with poor clinical and functional outcome, and increased risk of relapse. The continued development of low stimulus intensity seizure induction paradigms such as MST could provide for a safer new treatment option for patients with depression. Future research is warranted to assess the effects of MST on other memory systems including memory for visual and autobiographical information.

Keywords: magnetic seizure therapy, electroconvulsive therapy, neuropsychology, memory, depression.

Disclosures: S. McClintock, **Part 1:** Consultant to Shire Development.; M. Husain, **Part 4:** Research grant support from the NIH, Stanley Medical Research Institute, Cyberonics, Neuronetics, Brainsway, NeoSync, St. Jude Medical (ANS), and Magstim, Inc.; C. Cullum, Nothing to Disclose; A. Peterchev, **Part 4:** Dr Peterchev is an inventor on patents and patent applications on TMS/MST technology assigned to Columbia and Duke, including technology licensed to Rogue Research; was a principal investigator on a research grant to Duke from Rogue Research and equipment donations to Columbia and Duke by Magstim, MagVenture, and ANS/St Jude Medical; and has received patent royalties from Rogue Research through Columbia for TMS technology; I. Bernstein, **Part 4:** Dr Bernstein has received a grant from the National Council of Licensure Examination for Registered Nurses (NCLEX-RN/National Council Licensure Examination for Practical Nurses (NCLEX-PN), is a member of the NCLEX-PN Board of Advisors with regard to their test construction, and receives textbook royalties from Sage publications.; L. Stool, Nothing to Disclose; B. Luber, Nothing to Disclose; P. Croarkin, **Part 4:** Dr Croarkin reports research support from Pfizer Inc, New York, New York. He has served as a site subprincipal or principal investigator (without additional compensation) for Eli Lilly and Co, Indianapolis, Indiana; Forest Laboratories, Inc, New York, New York; Merck and Co, Inc, Whitehouse Station, New Jersey; and Pfizer Inc.; E. Bernhardt, Nothing to Disclose; K. Trevino, Nothing to Disclose; S. Lisanby, **Part 4:** Dr Lisanby has been Principal Investigator on research grants to Duke from Brainsway, Neosync, and St Jude Medical and equipment support from Magstim and MagVenture.

T226. Rapid Reduction in Suicide Risk in Depressed Elderly Treated with Electroconvulsive Therapy (ECT): Data from Phase I of the PRIDE Study

Charles Kellner, Georgios Petrides*, Rebecca Knapp, W Vaughn McCall, Mustafa Husain, Robert Young, Sarah Lisanby

The Zucker Hillside Hospital, Northsore-LIJ Health System, New York, New York

Background: Electroconvulsive Therapy (ECT) is regarded as the fastest antidepressant treatment with remission rates of 60–70% after 2–3 weeks (6–9 ECT). Among symptoms that resolve quickly are suicidal thoughts and behaviors. We report change in suicide ratings after ECT from the Prolonging Remission in Depressed Elderly (PRIDE) study.

Methods: PRIDE is an NIMH-supported, multi-site study in which unipolar depressed geriatric patients receive ultra-brief pulse (UBP) right unilateral (RUL) ECT, three times a week, augmented by venlafaxine. Ratings are made at baseline and before each ECT, using item #3 of the Hamilton Rating Scale for Depression 24-item ($HRSD_{24}$),which assesses suicidal thoughts/behaviors on a scale of 0–4. Complete resolution of suicidality occurred when Item #3 decreased to 0 and remained 0 for all subsequent ratings, including *at least two* terminal ratings.

Results: As of December 2012, 152 patients had entered PRIDE. Of these, 30 had $HRSD_{24}$ item #3 ratings of 3 (active suicidal thoughts) or 4 (active suicidal attempt) at baseline, indicating high suicide risk. After the course of treatment, 76.7% (23/30) of patients had complete resolution of suicidality. 40% (12/30) of patients had complete resolution of suicidality after 3 or fewer ECT, within 1 week of treatment. Of the 23 patients whose suicidality resolved completely, 61% had resolved after 3 or fewer ECT. 83.3% of patients (25/30) had a final $HRSD_{24}$ item #3 score of 0. At

baseline, the mean $HRSD_{24}$ item #3 score was 3.3 (SD = 0.47), which decreased to 1.3 (SD = 1.0) after only one ECT.
Conclusions: ECT works quickly and reliably to decrease suicide risk in depressed elderly. Ultra-brief RUL ECT, has potent anti-suicide effects.
Keywords: electroconvulsive therapy, major depression, suicide risk.
Disclosures: C. Kellner, Nothing to Disclose; G. Petrides, Nothing to Disclose; R. Knapp, Nothing to Disclose; W. McCall, Nothing to Disclose; M. Husain, Nothing to Disclose; R. Young, Nothing to Disclose; S. Lisanby, Nothing to Disclose.

T227. Transcendental Meditation for the Treatment of PTSD in Veterans

Kelvin O Lim*, Amy Moran, Michael Kuskowski, Gregory Lamberty

University of Minnesota Medical School, Minneapolis, Minnesota

Background: Post Traumatic Stress disorder remains a major clinical problem for veterans. The Department of Veterans Affairs estimates that upwards of 30% of Vietnam veterans, 20% of Iraqi veterans and 11% of Afghanistan veterans suffer from PTSD. Non-pharmacological, evidence based interventions for PTSD include prolonged exposure, Eye Movement Desensitization and Reprocessing and Cognitive Processing Therapy. All of these approaches involve some form of re-experiencing of the trauma, which a substantial proportion of veterans are not willing to undertake. The Department of Veterans Affairs has been interested in examining other non-pharmacological interventions for PTSD that do not involve re-experiencing of trauma, such as meditation. There are three main types of meditation each with their own distinctive EEG signatures and cognitive processes: focused attention techniques, open monitoring techniques and automatic self-transcending techniques. Transcendental Meditation, which falls in the later category, is a simple, easy to learn mental technique that is practiced twice a day for twenty minutes and produces a state of deep relaxation and reduced mental activity. The purpose of this study was to examine the acceptability and effectiveness of Transcendental Meditation for the treatment of veterans with PTSD.
Methods: Subjects were recruited through fliers and announcements in the VA. Subjects had to be on stable medication for 2 months, clinically stable and without active substance abuse, a psychotic disorder or bipolar disorder. They met criteria for PTSD based on the CAPS. This was an open label study in which all subjects were taught Transcendental Meditation. Instruction in TM was performed by certified TM teachers. Instruction consisted of 1.5 h sessions on 4 consecutive days followed by 6 1.5 h group sessions over 7 weeks. Subjects were assessed at Baseline before instruction and 8 weeks after Baseline. Assessments included the PTSD Check List—Civilian (PCL). A paired sample *t*-test was used to examine within subject change on the PCL.
Results: Twenty-one subjects were enrolled in the study and began instruction. Two subjects withdrew, one subject was removed due to active illicit drug use and one subject withdrew due to a life threatening illness. Seventeen subjects completed the full instruction period and baseline and 8 week followup. Mean age of the subjects was 61.5 ± 10.6 years. There was one woman. Mean PCL at baseline was 64 and fell to 54 at eight weeks ($t = 3.715$, $df = 16$, p-value = 0.0018).
Conclusions: Instruction in Transcendental Meditation was well tolerated by a high proportion of the veterans. There was a significant reduction in symptoms based on the PCL. Future work should include further study of TM for PTSD using a randomized clinical trial design and examining what factors may be good predictors of treatment response.
Keywords: PTSD transcendental meditation veterans.
Disclosures: K. Lim, Nothing to Disclose; A. Moran, Nothing to Disclose; M. Kuskowski, Nothing to Disclose; G. Lamberty, Nothing to Disclose.

T228. Modifiable Risk Factors and Reports of Depression in Young, Middle-aged, and Older Adults

Prabha Siddarth, Aaron Kaufman, David A Merrill, Cyrus A Raji, Fernando Torres-Gil, Gary W Small*

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

Background: Previous research has linked modifiable risk factors, such as physical inactivity, unhealthy diet, and smoking with a greater risk for depressive illness. However, most studies to date involve relatively small convenience samples, address only one or two risk factors simultaneously, and focus on samples with a limited age range. To address this knowledge gap, we assessed rates of reports of depression in Gallup Poll telephone surveys of a random sample of adults aged 18–99 years to determine relationships between such modifiable risk factors and self-reported depression.
Methods: A daily telephone survey (Gallup-Healthways Well-Being Index) of U.S. residents yielded a random sample of 37 921 respondents ranging in age from 18 to 99 years, including 13 885 younger (age 18–39 years), 13 744 middle-aged (40–59 years), and 10 292 older (60–99 years) adults. The survey included demographic information as well as questions on smoking, eating habits, body weight, and frequency of exercise, and whether the respondents had been diagnosed with depression. Weighted logistic regressions and chi-square tests were used in the analyses.
Results: Nearly seventeen percent (16.9%) of respondents surveyed reported having been diagnosed with depression. Reports of depression varied across different age groups (15.2% of younger, 18.7% of middle-aged, and 16.7% of older adults). Controlling for sex, ethnicity and educational and income levels, modifiable risk factors including smoking, obesity, not exercising, and not eating healthy were associated with greater frequency of depression in all age groups. Smoking was the most important risk factor for depression for all age groups, and the effect of smoking was greatest for the young adult age group. In the young adult age group, the odds of being diagnosed with depression was

nearly three times greater for smokers compared to non-smokers (odds ratio, OR = 2.70, 95% CI 2.4–3.0); while the odds ratio was less than two for middle-aged (OR = 1.80, 95% CI 1.6–2.0) and older (OR = 1.83, 95% CI 1.5–2.2) adults. The second most important risk factor was obesity, with a 65%, 54%, and 67% increase in the odds of reporting depression for an obese younger, middle-aged, and older adult compared to a non-obese person from each age group (younger: OR = 1.65, 95% CI 1.5–1.9; middle-aged: OR = 1.54, 95% CI 1.4–1.7; older: OR = 1.67, 95% CI 1.5–1.9). Self-reports of less frequent exercise were associated with a greater frequency of depression regardless of age (younger: OR = 1.28, 95% CI 1.1–1.4; middle-aged: OR = 1.48, 95% CI 1.3–1.6; older: OR = 1.61, 95% CI 1.4–1.8), while not eating healthy was associated with higher frequency of depression only in middle-aged and older adults (middle-aged: OR = 1.21, 95% CI 1.1–1.3; older: OR = 1.22, 95% CI 1.1–1.4).

Conclusions: These findings are consistent with previous studies confirming a relationship between reports of unhealthy behavior habits and risk for depression. Although respondents were not examined directly and the results do not prove a causal relationship (ie, confirm that unhealthy lifestyles raise risk for depression or that depression raises risk for unhealthy behavior habits), the findings support previous results from smaller samples indicating the contribution of such modifiable risk factors to depressive illness. To our knowledge, this is the only large-scale survey of such variables in a sample representative of the U.S. population and spanning a wide age range. These results point to the importance of future clinical trials to determine the effect size of behavioral interventions on preventing and reducing symptoms of depression.

Keywords: depression, nutrition, physical activity, smoking, obesity.

Disclosures: P. Siddarth, Nothing to Disclose; A. Kaufman, Nothing to Disclose; D. Merrill, Nothing to Disclose; C. Raji, Nothing to Disclose; F. Torres-Gil, Nothing to Disclose; G. Small, **Part 1:** Equity ownership of TauMark, LLC, Speaker, Advisor for Lilly, Novartis, Pfizer, **Part 2:** Novartis, **Part 4:** POM Wonderful, Ahmanson Foundation,.

T229. Electroconvulsive Therapy Response and Resting State Functional Connectivity in Older Patients with Major Depressive Disorder

Chris Abbott*, Thomas Jones, Nicholas T Lemke, Shruti Gopal

University of New Mexico, Albuquerque, New Mexico

Background: Cross-sectional resting state functional magnetic resonance imaging (fMRI) investigations have shown increased connectivity in major depressive disorder (Sheline 2010). The dorsal medial prefrontal cortex (DMPFC), specifically Brodmann areas 9 and 32, may be an important 'nexus' or hub of connectivity among regions implicated in depressive episodes. Recent longitudinal investigations have shown reduced connectivity in these regions associated with electroconvulsive therapy (ECT) response (Perrin 2012). However, cognitive and limbic connectivity changes within the DMPFC have yet to be assessed in a longitudinal investigation. Based on previous resting state fMRI investigations in major depression, we hypothesize that ECT response will be associated with normalization of aberrant connectivity within the DMPFC.

Methods: This investigation utilized a case-control design. Depressed subjects met the following inclusion criteria: (1) DSM-IV TR diagnosis of MDD; (2) the clinical indications for ECT; and (3) a Hamilton Depression Rating Scale–24 item (HDRS–24) > 21. Depressed subjects were on psychotropic medications, but medication changes were limited to dosage changes between the two imaging assessments. Demographically matched healthy comparison subjects (HC) were scanned at one time interval. A Thymatron System IV delivered a right unilateral ($n = 17$) or bitemporal ($n = 2$) stimulus delivery. Seizure threshold obtained during the first session with a dose titration method guided subsequent stimulus dosage ($6 \times$ threshold for right unilateral, $2 \times$ threshold for bitemporal). Treatments occurred thrice weekly until adequate clinical response or clinical decision to stop treatment for those patients that were not responding. With respect to the fMRI data, the first four volumes were dropped from the smoothed (10 mm FWHM), warped, and realigned imaging data. Voxels with high temporal standard deviation (> 0.98) associated with physiological noise were removed with *tCompCor*, a component based noise correction method (Behzadi 2007). The data was bandpass filtered (0.008–0.09 Hz) and despiked with AFNI. Average time courses for seeds in the dorsal lateral prefrontal cortex (DLPFC, ±36, 27, 29), precuneus (Prec, ±7, −60, 21), and the subcallosal cingulate gyrus (SCC, ±10, 35, −2) (Sheline 2010) generated r-to-z transformed connectivity maps. We used SPM8 (threshold, $P_{\text{uncorr}} < 0.01$) to assess longitudinal differences in connectivity associated with two (left, right) voxel cubes (3375 mm^3) in the DMPFC (−24, 35, 28; 18, 34, 29) for each connectivity map.

Results: The average age for the depressed subjects ($n = 19$) was 65.3 years ±8.0 (7 males, 12 females; 7 psychotic, 12 non-psychotic depression). The HC ($n = 21$) were matched for age (65.5 years, $P = 0.94$) and gender (12 females, 9 males, $P = 0.46$). The post-ECT HDRS-24 confirmed clinical response from a pre-ECT assessment of 32.6 ± 8.5 to a post-ECT assessment of 8.4 ± 8.6. The pre/post ECT analysis revealed significantly increased left DLPFC connectivity to the right DMPFC region of interest ($T = 3.11$, $P = 0.003$). The pre-ECT/HC revealed decreased left DLPFC connectivity to the right DMPFC in the pre-ECT group ($T = 2.67$, $P = 0.006$). The post-ECT/HC contrast had no significant differences in connectivity in the right DMPFC. In a post-hoc analysis, (1) correlations between connectivity changes in our regions of interest with changes in symptoms (HDRS) and (2) group differences in connectivity changes between psychotic and non-psychotic depression were not significant ($P > 0.05$).

Conclusions: ECT response is associated with increased left DLPFC connectivity in the right DMPFC. Prior to ECT, depressed participants had reduced connectivity in the left DLFPC relative to HC. Treatment response increased connectivity and 'normalized' the left DLPFC connectivity differences in the post-ECT participants relative to the HC. Our study did not reveal any connectivity changes within the DMPFC and the other connectivity maps (right DLPFC,

Prec, SCC). Larger investigations are needed to confirm the specificity of the relationship between ECT response and the left DLPFC. Unlike studies in mid-life, which show increased connectivity in depression, later life depression may be associated with reduced connectivity in attentional networks (Alexopoulos 2012). We have extended these findings by showing normalization of aberrant, state-related DLPFC connectivity in the context of ECT response.
Keywords: major depressive disorder, resting state fMRI, electroconvulsive therapy.
Disclosures: C. Abbott, Nothing to Disclose; T. Jones, Nothing to Disclose; N. Lemke, Nothing to Disclose; S. Gopal, Nothing to Disclose.

T230. Clinical Experience of Seven DBS for OCD Patients at an Academic Medical Center

Laurie M McCormick*, James Beeghly, Jeremy Greenlee

University of Iowa Carver College of Medicine, Iowa City, Iowa

Background: The FDA approved a Humanitarian Device Exemption for the use of Deep brain stimulation (DBS) for obsessive-compulsive disorder (OCD) in 2009. This approval was based on a relatively small sample of patients with OCD and more research is needed.
Methods: Over the past three years, our psychiatry and neurosurgery team has implanted and/or completed the initial programming for DBS of the anterior-limb of the internal capsule/nucleus accumbens of seven patients with severe and treatment resistant OCD. Pre and post-treatment assessments were completed with the Global Assessment of Functioning (GAF), Yale-Brown Obsessive-Compulsive Symptoms Scale (YBOCS), and the Hamilton Depression Rating Scale (HDRS).
Results: One of these patients also had comorbid anorexia nervosa, two had comorbid psychosis, two had comorbid depression, and two no other psychiatric comorbidities other than OCD. The average age of these seven patients was 39.8 (range 19.4–64.9) and including 4 men and 3 women. The first six of these patients had clinically significant improvement in their GAF and had at least a minor to significant reduction of obsessive-compulsive symptoms as measured by the YBOCS. The two patients with comorbid depression were on maintenance ECT prior to implantation and had a significant reduction in depressive symptoms as measured by the HDRS. One of the two patients who had comorbid psychosis had significant reduction in OCD symptoms and also had a significant reduction in psychotic symptoms. The most recently implanted patient (less than one month) had a few weeks of improvement but has relapsed to baseline levels of severe rituals and behaviors, though alterations in programming are still being attempted. The only surgical complication in the series was one patient who required replacement of both extension wires after abnormal impedances were noted during routine depleted pulse generator replacement. Presented are the lead configuration and stimulation parameters for each patient and their individual responses over the course of up to two years.
Conclusions: DBS for OCD should still be considered a treatment of last-resort, but can lead to clinically meaningful alleviation of incapacitating psychiatric symptoms and improved functioning.
Keywords: DBS, OCD, major depression, outcome.
Disclosures: L. McCormick, Nothing to Disclose; J. Beeghly, Nothing to Disclose; J. Greenlee, Nothing to Disclose.

T231. Hyperthermia and the Improvement of ASD Symptoms

Casara J Ferretti*, Bonnie P Taylor, Rachel Noone, Emma Racine, Jonathan Kirsch, Eric Hollander

Montefiore Medical Center, Bronx, New York

Background: Current literature in autism spectrum disorders (ASD) supports a link between central nervous system (CNS) dysfunction, neuroinflammation and a dysregulated immune response. Our group has been interested in the association between ASD and immune dysfunction, as the involvement of inflammatory mechanisms suggests that immunomodulatory intervention may be an experimental therapeutic option for individuals with ASD. In particular, the observation that some ASD patients manifest clinical improvement in response to fever suggests that ASD symptoms may be modulated by immune-inflammatory factors. The febrile hypothesis of ASD stems from this observation, and could be due to one of three possible causes (1) the direct effect of temperature; (2) a resulting change in the immune inflammatory system function associated with the infection of fever; and/or (3) an increase in the functionality of a previously dysfunctional locus coeruleus-noradrenergic (LC-NA) system. Although parental reports have demonstrated improved social cognition and language skills and decreased disruptive behaviors during febrile episodes of their child with ASD, little has been done to explore the potential direct effect an increased body temperature may have on autism symptomatology.
Methods: We are completing a double blind crossover study with 15 children with ASD between the ages of 5 and 17 years old. Five children with ASD (confirmed by DSM-IV criteria) without fever response acted as controls prior to the completion of the protocol on those with ASD and history of fever response, to ensure the appropriate parameters were in place. The five control subjects were place in a HydroWorx acquatic therapy pool at the single temperature of 102°F (hyperthermia condition) for one hour and 15 min, on a single day. They were monitored before, during and after the visit by a staff member with medical back up. Safety measures (vital signs, temperature, urine pregnancy test, BMI) were collected in addition to a baseline and endpoint Social Responsiveness Scale (SRS) completed by the parent. Once data was collected on the 5 control subjects, 10 patients with ASD (confirmed by DSM-IV criteria supported by ADOS) and history of fever response were screened and enrolled. At screening patient assessments (IQ test, pupillometry, Social Language Test), parent assessments (ABC, SRS, SCQ, RBS-R) and clinician assessments (CGI-S, CGI-I) were completed in addition to medical history, concomitant medications and vital signs. Patients with ASD and a history of fever response received both treatment conditions at the Hydroworx aquatic

therapy pool, 102°F (hyperthermia condition) and 98°F (control condition). Per information collected from the control subjects, ASD patients with fever response were kept in the pool until their temperature reached approximately 102°F and maintained at that temperature for approximately 30 min. Vital signs, temperature monitoring and clinical observations were completed throughout their time in the pool. Parents observed the patients throughout their time in the pool and completed the SRS, ABC and RBS-R once the child was at the higher temperature for approximately 30 min. Clinicians completed the Social Language Test and pupillometry when the child exited the pool, with the temperature still heightened. Additionally, in order to observe potential epigenetic effects of the hyperthermia treatment buccal swabs were collected pre and post pool entry for DNA and RNA extraction.

Results: Five control subjects without a history of fever completed the hyperthermia condition at 102°F, and demonstrated the feasibility of increasing a child's temperature to 102°F using the Hydroworx aquatic therapy pool; the child's ability to tolerate spending periods of time at the hyperthermia condition; and the ability to complete needed safety measures and behavioral assessments. Ten subjects with ASD and a history of fever response were entered into the study and completed the hyperthermia condition (102°F) and control condition (98°F) at the aquatic therapy pool. Social and behavioral changes were observed by comparing baseline to endpoint in the hyperthermia and control conditions on parent assessments (SRS, ABC, RBS-R, SCQ), clinical biomarkers (pupillometry, social language test) and genetic biomarkers.

Conclusions: This study demonstrates the feasibility of observing the direct effect of temperature in children with ASD, both with and without a fever response, and preliminary data on the relationship between body temperature and changes in social/behavioral measures, biomarkers and epigenetic expression. This method explores the direct effects of temperature on ASD symptoms, and offers a window into understanding mechanisms involved in improvement in ASD symptoms during fever episodes. Future analyses will help to determine the genetic pathways involved in fever response, the role of the locus coeruleus/noradrenergic system, and other immune-inflammatory pathways in mediating ASD symptoms. Funding provided by the Simons Foundation.

Keywords: autism, fever, immune, inflammation, biomarkers.

Disclosures: C. Ferretti, Nothing to Disclose; B. Taylor, Nothing to Disclose; R. Noone, Nothing to Disclose; E. Racine, Nothing to Disclose; J. Kirsch, Nothing to Disclose; E. Hollander, **Part 4:** Funding provided by The Simons Foundation.

T232. Substrate-selective COX-2 Inhibition Decreases Anxiety via Endocannabinoid Activation

Daniel Hermanson, Nolan Hartley, Joyonna Gamble-George, Lawrence Marnett, Sachin Patel*

Vanderbilt University School of Medicine, Nashville, Tennessee

Background: Augmentation of endogenous cannabinoid (eCB) signaling represents an emerging approach to the treatment of affective disorders. Cyclooxygenase-2 (COX-2) oxygenates arachidonic acid to form prostaglandins, but also inactivates eCBs *in vitro*. However, the viability of COX-2 as a therapeutic target for *in vivo* eCB augmentation has not been explored.

Methods: Here we utilized medicinal chemistry and *in vivo* analytical and behavioral pharmacological approaches to demonstrate a key role for COX-2 in the regulation of endocannabinoid (eCB) levels *in vivo*. A novel pharmacological strategy involving 'substrate-selective' inhibition of COX-2 was developed used to augment eCB signaling without affecting related non-eCB lipids or prostaglandin synthesis.

Results: Administration of the substrate-selective inhibitor LM-4131 increased brain and peripheral anandamide levels without affecting prostaglandin levels or levels or other related non-endocannabinoid lipids. Behaviorally, LM-4131 reduced anxiety-like behaviors in a variety of pre-clinical models. These effects were mediated via activation of CB1 type cannabinoid receptors. LM-4131 also reduced stress-indcued anxiety states in animals.

Conclusions: These data indicate that substrate-selective COX-2 inhibition represents a viable approach to enhance eCB signaling for the treatment of affective disorders. These data also highlight a key role for COX-2 in the regulation of central eCB signaling and validate COX-2 as a new molecular target for psychiatric drug discovery.

Keywords: endocannabinoid, COX-2, anxiety, depression, drug discovery.

Disclosures: D. Hermanson, **Part 1:** DJH, LJM and SP have submitted a patent application entitled 'Compositions and Methods for Substrate-Selective Inhibition of Endocannabinoid Oxygenation,' which includes the compound LM-4131.; N. Hartley, Nothing to Disclose; J. Gamble-George, Nothing to Disclose; L. Marnett, **Part 1:** DJH, LJM and SP have submitted a patent application entitled 'Compositions and Methods for Substrate-Selective Inhibition of Endocannabinoid Oxygenation,' which includes the compound LM-4131.; S. Patel, **Part 1:** DJH, LJM and SP have submitted a patent application entitled 'Compositions and Methods for Substrate-Selective Inhibition of Endocannabinoid Oxygenation,' which includes the compound LM-4131.

T233. Oxytocin and Facial Expressivity in Patients with Schizophrenia and Healthy Participants

Josh Woolley*, Chris Fussell, Wanda Lai, Olivia Lam, Brandon Chuang, Bruno Biagianti, Dan Fulford, Daniel H Mathalon, Sophia Vinogradov

University of California San Francisco, San Francisco, California

Background: Restricted expression of affect, including both reduced frequency and intensity of facial emotional expression, is a common negative symptom of schizophrenia that is correlated with worse functional outcomes and quality of life. Furthermore, there are currently no available treatments for these deficits. The neuropeptide oxytocin (OT) has multiple prosocial effects when administered intranasally in humans and offers a potential remedy for these expressivity deficits. OT has been implicated in

bonding and has shown promise in enhancing social cognition in schizophrenia. However, the effects of oxytocin administration on facial expressivity have not been investigated in any healthy or patient population. Therefore, we investigated the effects of oxytocin on emotional expression in patients with schizophrenia and age-matched healthy controls while they observed emotionally provocative photos.

Methods: Twenty-five male individuals with SCID-confirmed schizophrenia and twenty-seven male, age-matched, healthy participants participated in the study. Testing was performed in a randomized, double-blind, cross-over, placebo-controlled, within-subject design, with the two testing days separated by one week. On each test day, 40 IU of oxytocin or placebo (PCB) was self-administered intranasally. Participants were video recorded while they performed a facial trustworthiness assessment task. During this task, participants were shown 49 faces, and 49 positive (eg, sports), 49 negative (eg, snakes), and 49 neutral (eg, household objects) affective photos from the International Affective Picture System (IAPS). Positive, neutral, and negative photos were chosen from the IAPS based on published ratings of arousal and valence. Effectiveness of the photos to produce pleasant and unpleasant feelings in patients with schizophrenia was validated in a previous study. Participants' facial expressions were coded from the videos independently by two raters, who were blind to condition, using the Facial Expression Coding System (FACES). FACES is a behavioral coding system validated for use in patients with schizophrenia based on a dimensional model of emotion, in which each expression is coded for valence (positive/negative) and intensity (weak/strong). FACES ratings converge with ratings made using Ekman's Facial Action Coding System and with data from facial muscle activity, psychophysiology, and subjective report. Inter-rater agreement was excellent (correlation coefficients: 0.94–0.96). Given the preponderance of zeroes in the data (particularly for the schizophrenia group; ie, lack of facial affect), we conducted non-parametric tests where possible.

Results: Healthy controls (HC) and individuals with schizophrenia (SZ) were well matched on age (Mean (SD) SZ: 43.2 (11), HC: 42 (13.7) $p = 0.5$). Mann-Whitney U tests revealed that, on the PCB day, individuals with SZ showed significantly lower number (SZ: 1.1 (2.4) *vs* HC: 5.8 (7.6)) and intensity of facial expressions (SZ: 0.5 (0.9) *vs* HC: 1.0 (0.7)) than HCs (all p's < 0.01) consistent with previous studies. To test the effects of intranasal OT administration on facial expressivity we performed a repeated-measures ANOVA with one within-subject factor, Drug (OT and PCB), and one between-subject factor, Group(SZ and HC), for number and intensity of facial expressions. For number of expressions, we found a significant Drug effect (SZ: PCB: 1.1 (2.4) *vs* OT: 2.7 (6.5); HC: PCB: 5.8 (7.6) *vs* OT: 8.4 (9.8); $F(1) = 6.6$, $p = 0.01$) but no significant Drug X Group interaction ($F(1) = 0.4$, $p = 0.5$). Looking separately at positive and negative expressions revealed a significant Drug effect for negative (SZ: PCB: 0.4 (1.4) *vs* OT: 1.7 (4.9); HC: PCB: 3.0 (3.9) *vs* OT 4.6 (5.6); ($F(1) = 6.7$, $p = 0.01$) but not positive (SZ: PCB: 0.7 (1.6) *vs* OT: 1.0 (2.6); HC: PCB: 2.8 (5.1) *vs* OT: 3.8 (7.9); ($F(1) = 1.4$, $p = 0.2$) expressions. We found no significant Drug or Drug X Group effects for intensity of facial affect (p's > 0.05). Looking separately by group, related samples Wilcoxon Signed Rank tests revealed that OT increased the total number of facial expressions significantly in SZ ($p = 0.01$); and non-significantly in HC ($p = 0.1$) but had no effect on the intensity of facial expressions in either group.

Conclusions: Our results suggest that administration of a single dose of oxytocin increases facial expressivity in SZ and HC during viewing of emotionally provocative photos. While OT appeared to selectively increase negative expressions this was likely due to our stimuli being more effective at inducing negative expressions. The mechanism of OT's effect on facial expressivity is unknown. OT may increase facial expressivity by increasing participants' psychological or physiological response to the emotional cues. Alternatively, facial expressivity is modulated by parasympathetic tone and OT is known to affect parasympathetic tone in humans and rodents. Therefore, OT administration may increase facial expressivity by directly altering parasympathetic regulation to facial musculature without affecting other responses to the emotional cues. Further research is necessary to explore these various hypotheses. In sum, the present study provides support for using OT as a pharmacological agent to remediate the facial expressivity deficits in SZ. Larger studies focused on patients with schizophrenia who have significant baseline negative symptoms are needed to confirm and extend our findings.

Keywords: schizophrenia, oxytocin, social cognition, facial affect, negative symptoms.

Disclosures: J. Woolley, Nothing to Disclose; C. Fussell, Nothing to Disclose; W. Lai, Nothing to Disclose; O. Lam, Nothing to Disclose; B. Chuang, Nothing to Disclose; B. Biagianti, Nothing to Disclose; D. Fulford, Nothing to Disclose; D. Mathalon, **Part 1:** Consultant to BristolMyersSquibb Inc.; S. Vinogradov, **Part 1:** consultant to BrainPlasticity Institute.

T235. Endothelial Function in Schizophrenia

Bernard A Fischer*, William R Keller, Robert P McMahon, Walter Meyer, Michael Miller, Robert W Buchanan

Maryland Psychiatric Research Center, Columbia, Maryland

Background: Chronic inflammation can lead to endothelial dysfunction resulting in a prothrombotic state, promotion of leukocyte recruitment, release of pro-inflammatory cytokines, and decreased capacity for arterial dilation. People with schizophrenia have increased levels of pro-inflammatory cytokines compared to healthy controls. This population also has a significantly elevated risk for vascular disorders and associated premature death. This pilot study used flow-mediated vasodilation (FMD) to assess endothelial function in people with schizophrenia (Sz) compared to healthy controls (HC). In FMD, the diameter of an artery is measured using ultrasound before and after occluding the vessel for 5 min. In response to the occlusion, the endothelium will mediate a vasodilation response. The percent change after occlusion indicates the responsivity of the endothelium. More poorly functioning endothelium will result in a 'stiffer' artery and less vasodilation in response to

occlusion. It was hypothesized the Sz group would show significantly less vasodilation in response to vascular occlusion than the HCs.
Methods: Sz participants were recruited from the Maryland Psychiatric Research Center (MPRC) and from a community mental health center. HCs were recruited from a volunteer pool maintained at the MPRC. Presence or absence of psychiatric diagnoses was confirmed by the Structured Clinical Interview for DSM-IV. FMD was completed in the morning after an 8 h fast (no decongestants, caffeine, tobacco, or alcohol for 12 h). Female participants completed the study during the follicular phase of their menstrual cycle to standardize hormonal effects on arterial reactivity. Brachial artery diameter was measured by ultrasound before and 1 min after a 5-min occlusion by inflated blood pressure cuff. Group differences were tested with the Kruskal-Wallis Test. FMD is reported as percent increase from baseline diameter.
Results: Fifteen Sz participants (3 females/12 males) and 15 HC participants (1 female/14 males) completed the study. There was no significant difference in mean age between the Sz group (36.6 + 10.6) and the HC group (31.5 + 10.0; $X^2 = 1.61$, $p = 0.20$). There were no group differences in mean systolic (Sz = 122 + 12, HC = 122 + 14; $X^2 = 0.002$, $p = 0.97$) or diastolic (Sz = 76 + 10, HC = 69 + 8; $X^2 = 3.34$, $p = 0.07$) blood pressure. The FMD change was not significant between the groups (Sz = 6.0% + 6, HC = 6.7% + 6; $X^2 = 0.12$, $p = 0.76$).
Conclusions: Clinically meaningful differences in FMD are cited as 1–2%. The observed difference in FMD between the Sz and HC groups was near this magnitude and in the hypothesized direction, but was not significant. This probably represents type II error given the small sample size in this pilot study. Future directions for this work are to complete larger studies and to examine the impact of medications, including antipsychotics, on endothelial reactivity.
Keywords: schizophrenia, endothelium, vascular system, metabolic health, inflammation.
Disclosures: B. Fischer, Nothing to Disclose; W. Keller, Nothing to Disclose; R. McMahon, Nothing to Disclose; W. Meyer, Nothing to Disclose; M. Miller, Nothing to Disclose; R. Buchanan, Nothing to Disclose.

T236. New Insight Into How Ventral Tegmental Area Neurons Encode Action Sequence and Outcome Associations

Jesse Wood*, Nicholas Simon, Frederick S Koerner, Robert E Kass, Bita Moghaddam

Center for Neuroscience at the University of Pittsburgh, Pittsburgh, Pennsylvania

Background: Dopamine neurons in the ventral tegmental area (VTA) encode information about predicted outcomes. This information is theoretically used to select behavioral policies, with the goal of maximizing the value of the expected outcome. This model, along with physiological data, has created a powerful framework for understanding VTA neuronal activity in the contexts of associative learning and action selection. While it is generally agreed that VTA neurons encode information about action-outcome associations, most physiological data from the VTA comes from Pavlovian learning and simple instrumental paradigms. Thus, in the context of complex sequences of behavior there is only a limited understanding of what information is encoded by VTA neurons, or when during behavior this information is encoded. We are interested in addressing a fundamental question about how VTA neurons contribute to learning and performing instrumental behaviors: How are action-outcome relationships, which require multiple actions to be executed in order to receive a reinforcer, represented by VTA neurons?.
Methods: We recorded VTA single units in rats learning to perform an instrumental behavior over multiple sessions. In each trial, following the onset of a cue light (start cue), rats could nose poke (actions) to earn a sugar pellet reinforcer (outcomes). When an action was reinforced, the cue light was immediately terminated, and 500 ms later, outcomes were delivered into a reward trough. To earn outcomes, animals repeated an unknown and random number of the same action in each trial. This ensured no coarse differences in behavior and minimized the likelihood that rewards could be anticipated during an action. We trained rats on a fixed ratio 1 reinforcement schedule, meaning each action was reinforced, for a single session. In sessions 2–4, each action was reinforced with a probability of 0.2. In sessions 5–7, we decreased this probability further to 0.1. Spike counts around events of interest (trial start cues, actions and outcome delivery) were calculated in 25 ms bins, smoothed, and averaged to yield population activity. To examine how neuronal activity was affected by sequences of actions, we used a non-linear regression model of firing rate as a function of the action number.
Results: As expected, animals learned to execute random numbers of actions to earn outcomes in each trial. Performance of the action sequence reached stable group averaged rates (approximately 1.5 actions/sec) when random reinforcement schedules were introduced. As the reinforcement schedule was switched from a fixed to random ratio, fewer VTA units had significant start cue evoked increases in firing rate. We also found that with greater experience with the random ratio reinforcement schedules, VTA population activity was increasingly elevated during the delay between the final action and outcome delivery. Thus, as animals learned to execute sequences of actions, the VTA population responses evoked by salient events were highly plastic. We also examined how VTA neurons represented information just before, during, and just following the execution of an action. Consistent with previous reports, when firing rates during peri-action periods were averaged together, decreased firing rates were seldom observed and increased firing rates were the most common response. However, when peri-action neuronal responses were examined according to the action number within a trial, an unexpected pattern emerged. When activity was averaged across successive actions, different subpopulations of units represented subsets of the actions. We detected units responsive to lower numbered actions (eg 2–4), higher numbered actions (eg 7–10), and many actions (eg 1–12). These patterns were observed even amongst simultaneously recorded units.
Conclusions: It is critical that an animal keep track of information related to action sequences, including approximately how many actions have been executed in the current

sequence. In contrast to the notion that VTA neurons may represent sequences of actions homogenously, we found that VTA neurons represent action number in a non-linear and heterogeneous fashion. These data suggest that downstream neurons may decode this information from the activity of VTA neurons, via a weighting algorithm or through more complex ensembles codes. Psychiatric illnesses, such as schizophrenia and drug abuse, are characterized by deficits in sequencing behaviors or selecting optimal behavioral policies. Thus, from both a basic and clinical perspective, it is essential to investigate how the VTA supports these fundamental aspects of behavior. Additionally, VTA representations of stimuli predicting outcome availability (start cue) or outcome delivery were highly plastic as animals gained experience with the action-outcome relationships. These data provide clues as to how the functional connectivity of VTA networks is reconfigured as animals learn instrumental associations, and collectively offer new insights into how information necessary for basic aspects of behavior is encoded.

Keywords: learning, dopamine, VTA, electrophysiology, actions.

Disclosures: J. Wood, Nothing to Disclose; N. Simon, Nothing to Disclose; F. Koerner, Nothing to Disclose; R. Kass, Nothing to Disclose; B. Moghaddam, Nothing to Disclose.

T237. Adolescent Ventral Tegmental Area Neurons Maintain Cue Evoked Responding After Extinction: A Mechanism for Adolescent Behavioral Flexibility?

Nicholas Simon*, Yunbok Kim, Jesse Wood, Bita Moghaddam

University of Pittsburgh, Pittsburgh, Pennsylvania

Background: Adolescence is associated with risky behavior and the symptomatic onset of major psychiatric disorders. During adolescence, the brain undergoes substantial remodeling to regions implicated in reward processing, manifested behaviorally as patterns of responding to reward-related stimuli that differ from in adulthood. Dopamine neurons in the ventral tegmental area (VTA) are strongly implicated in adolescent behavioral and psychiatric vulnerabilities, but little is known about how adolescent VTA neurons process reward and other behaviorally relevant information. Here, we assessed neuronal activity in VTA during acquisition and extinction of instrumental behavior in adolescent and adult rats. We then probed a separate cohort of adolescent and adult rats for differences in reward-related behavior.

Methods: We recorded single unit activity from VTA of adolescent (PND 35–45) and adult rats during a reward-related instrumental task. Rats were trained to perform a nose-poke into a port during presentation of a cue to receive a sucrose pellet reward. After 6 days of training, the following session began with nose-pokes reinforced with reward as previously, then shifted to extinction after 30 completed trials. Finally, rats were given another day of extinction in which the reward was never available. Following data collection, we quantified population activity evoked by the cue, nose-poke, and reward delivery, then compared these neuronal responses between adolescents and adults throughout training.

Results: Both age groups similarly learned the task, and quickly adapted their behavior to lack of reward availability during extinction. There were no age-related differences in baseline firing rate or phasic responses to task-related events in VTA during learning and maintenance of the task. However, during extinction, evoked responding to a cue predictive of reward availability rapidly diminished in adult VTA neurons, whereas adolescent VTA neurons displayed persistent phasic activation to cue presentation. Thus, adolescent VTA maintained information about available outcomes even after responding for outcomes had decreased. Next we ran separate groups of adolescent rats in experiments testing cue- and reward-driven behavior. We found no difference between adolescent and adult rats in extinction or outcome-driven reinstatement. However, when a learned instrumental cue was shifted in modality to a Pavlovian cue, adolescent rats acquired an approach response (goal-tracking) to this cue more readily than adults. Finally, a cue initially acquired as a response inhibitor was shifted to a Pavlovian predictor of reward. Adolescent rats again learned to goal-track in response to the cue following the shift in value more rapidly than adults. Importantly, changes in cue-outcome relationships only produced age-related behavioral differences when the cue had previously acquired task-related salience. In a traditional reversal learning task in which a cue with no predictive value became associated with reward, both groups acquired the reversal equally.

Conclusions: The data presented here suggest that the adolescent VTA encodes events related to instrumental behavior similarly to adults during learning and maintenance, but processes reward-related cues differently than in adults during extinction. Behavioral extinction or outcome driven-reinstatement did not differ between adults and adolescents, indicating that prolonged cue-evoked activity in adolescents may not be related to perseverative behavior or the re-establishment of prepotent responding. Instead, adolescents more effectively adjusted their responding to changes in cue-outcome relationships compared to adults. Thus, the adolescent reward system may facilitate the ability of previously salient stimuli to rapidly acquire changes in value.

Keywords: adolescence, VTA, learning, dopamine, flexibility.

Disclosures: N. Simon, Nothing to Disclose; Y. Kim, Nothing to Disclose; J. Wood, Nothing to Disclose; B. Moghaddam, Nothing to Disclose.